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Polymorphisms of candidate genes determining the clinical and hemostasiological characteristics of endocarditis of various etiology

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ABSTRACT

Aim. To investigate polymorphisms of 18 genes as possible molecular genetic markers of predisposition or resistance to development of non-infective (NE) or infective endocarditis (IE).

Materials and methods. The study encompassed 81 patients with NE and 94 patients with IE. The control group included 225 conditionally healthy people. Polymorphisms of 18 genes were tested using polymerase chain reaction (PCR).

Results. For the first time, a statistically significant relationship was identified between gene polymorphisms and valvular vegetations: for genes in the hemostatic system – rs6025 (1691 G > A) of the *F5* gene (AG genotype), rs1126643 (807 C > T) of the *ITGA2* gene (TT genotype); for folate pathway genes – rs1805087 (2756 A > G) of the *MTR* gene (AG genotype) and rs11697325 (*–8202* A/G) of the *MMP9* gene (AA genotype) and rs2476601 (C1858T) of the *PTPN22* gene (TT genotype). The protective effect of gene polymorphisms was revealed: for the *NOS3* gene (4b / 4b genotype) and G (–572) C of the *IL6* gene (CC genotype). For two polymorphisms, an association with thromboembolic complications in NE was revealed: rs1126643 (807 C > T) of the *ITGA2* gene and rs1799889 (–675 5G > 4G) of the *PAI* (*SERPINE1*) gene. In IE, such an association was detected for the polymorphism rs11697325 (–8202 A/G) of the *MMP-9* gene.

Conclusion. The polymorphisms of candidate genes were revealed, that are associated with the clinical and hemostasiological characteristics of IE and NE. In NE, for the first time, the association with thromboembolic complications was identified for two polymorphisms: rs1126643 (807 C > T) of the *ITGA2* gene and rs1799889 (– 675 5G > 4G) of the *PAI-1* (*SERPINE1*) gene. In IE, such a relationship was detected for one polymorphism – rs11697325 (8202 A/G) of the *MMP-9* gene.

Keywords: endocarditis, candidate genes, single nucleotide polymorphism, hemostasis, thromboembolic complications

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study.

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The study was approved by the Ethics Committee at the Research Institute of Therapy and Preventive Medicine (Protocol No. 16 of 03.06.2014).

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Полиморфизмы генов-кандидатов, связанные с клинико-гемостазиологическими характеристиками эндокардитов разной этиологии

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РЕЗЮМЕ

Цель. Изучить полиморфизмы 18 генов как возможных молекулярно-генетических маркеров предрасположенности или резистентности к развитию неинфекционного (НЭ) и инфекционного (ИЭ) эндокардитов.

Материалы и методы. Пациенты с неинфекционным (81 человек) и инфекционным (94) эндокардитами; контрольная группа 225 условно здоровых человек. Полиморфизмы 18 генов изучали с помощью полимеразной цепной реакции (ПЦР).

Результаты. Впервые для полиморфизмов генов установлена статистически значимая ассоциация с синдромом вегетаций на клапанах сердца: для генов системы гемостаза – rs6025 (1691 G > A) гена *F5* (AG), rs1126643 (807 C > T) гена *ITGA2* (TT), гена фолатного цикла – rs1805087 (2756 A > G) гена *MTR* (AG), а также rs11697325 (–8202 A/G) гена *MMP9* (генотип AA) и rs2476601 (C1858T) гена *PTPN22* (TT). Выявлена «протективная» роль полиморфизмов: гена *NOS3* (4b/4b) и G (–572) C гена *IL6* (CC). Для двух полиморфизмов обнаружена ассоциация с тромбоэмболическими осложнениями при НЭ – rs1126643 (807 C > T) гена *ITGA2* и rs1799889 (– 675 5G > 4G) гена *PAI1* (*SERPINE1*) и для одного – при ИЭ – rs11697325 (–8202 A/G) гена *MMP-9*.

Заключение. Выявлены полиморфизмы генов-кандидатов, ассоциированные с клинико-гемостазиологическими характеристиками неинфекционного и инфекционного эндокардитов. Впервые при неинфекционном эндокардите для двух полиморфизмов обнаружена ассоциация с тромбоэмболическими осложнениями – rs1126643 (807 C > T) гена *ITGA2* и rs1799889 (–675 5G > 4G) гена *PAI1* (*SERPINE1*) и для одного полиморфизма – при инфекционном эндокардите – rs11697325 (–8202 A/G) гена *MMP-9*.

Ключевые слова: эндокардиты, гены-кандидаты, однонуклеотидный полиморфизм, гемостаз, тромбоэмболические осложнения

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Endocarditis is characterized by altered microcirculation (microthrombosis, vascular remodeling, platelet aggregation, fragmented blood flow) and blood rheology with development of disseminated intravascular coagulation [1]. The study of hemostatic system parameters is one of the promising directions for forming a panel of markers for risks of thromboembolic complications in endocarditis [2, 3]. Hyperfibrinogenemia is an independent factor leading to hypercoagulation [4]. Changes in platelet – leukocyte aggregate formation and thrombocytosis are the most important disorders of the hemostasis determining the development of hyperaggregation [5].

Genetic predisposition to endocarditis has been of great interest for scientists and clinicians [6–8]. There are data on the association of single nucleotide polymorphisms in the genes of the hemostatic system and folate pathway with vegetations on the cardiac valvular apparatus [9]. In the sample of 123 patients with IE, an association of a higher risk of IE with the TT genotype of the rs1205 polymorphism in the *CRP* gene was identified, while a decrease in the IE risk was correlated with the GA genotype of the rs1143634 polymorphism in the *IL1B* gene, the GT genotype of the rs3212227 polymorphism in the *IL12B* gene, the AC genotype of the rs1130864 polymorphism in the *CRP* gene, and the G allele of the rs1801197 polymorphism in the *CALCR* gene [10].

The aim of the study was to investigate polymorphisms of 18 genes as possible molecular and genetic markers of predisposition or resistance to development of non-infective (NE) and infective endocarditis (IE).

MATERIALS AND METHODS

The NE group included 81 patients aged 43.0 ± ± 13.9 years: 40 men and 41 women. The IE group consisted of 94 patients: 76 men and 18 women aged 41.0 ± 16.0 years. The control group included 225 conditionally healthy people of the same age.

An inclusion criterion was the presence of endocarditis. The diagnosis of NE was established after IE exclusion according to the Duke criteria [11]: negative blood culture for IE, normal body temperature, and the absence of sonographic signs of infection in the valves. In NE, only thickening and compaction of the valve flaps and minimal regurgitation were detected. In addition, there was no progression of heart disease and other complications of IE, such as tears, ruptures, and perforations of the valve flaps, as well as abscesses. Exclusion criteria encompassed pregnancy and recent acute conditions (trauma, polychemotherapy, surgery).

The informed consent was signed by all the patients in accordance with the ethical principles of the Declaration of Helsinki developed by the World Medical Association “Ethical Principles of Conducting Scientific Medical Research with Human Participation”. The study was approved by the Ethics Committee at the Research Institute of Therapy and Preventive Medicine (Protocol No. 16 of 03.06.2014).

Genomic DNA was isolated from 6–10 ml of venous blood, DNA extraction was performed using phenol – chloroform extraction. Polymorphisms of the genes of the hemostatic system and folate pathway were tested using real-time PCR on test systems manufactured by DNA-Technology (Russian Federation): rs6046 (10976 G > A) of the *F7* gene, rs5985 (103 G > T) of the *F13* gene, rs1800790 (- 455 G > A) of the *FGB* gene, rs1799963 (20210 G > A) of the *F2* gene, rs6025 (1691 G > A) of the *F5* gene, rs1126643 (807 C > T) of the *ITGA2* gene, rs5918 (1565 T > C) of the *ITGB3* gene, rs1799889 (-675 5G > 4G) of the *PAI-1* (*SERPINE1*) gene, rs1801131 (1298 A > C) of the *MTHFR* gene, rs1801133 (677 C > T) of the *MTHFR* gene, rs1805087 (2756 A > G) of the *MTR* gene, rs1801394 (66 A > G) of the *MTRR* gene. The polymorphisms of the *NOS3* (4a / 4b) and *ACE* (rs1799752) genes were tested us-

ing PCR with flanking sequences. The polymorphisms of the *CTLA4* (rs231775), *MMP-9* (matrix metalloproteinase-9) (rs11697325), *PTPN22* (rs2476601), and *IL6* (rs1800795) genes were tested using PCR – restriction fragment length polymorphism (RFLP). Genotyping was performed at the Research Institute of Therapy and Preventive Medicine – a branch of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences.

Statistical analysis was performed using SPSS, version 21.0. The distribution of genotype frequencies of all polymorphisms in the control group complied with the Hardy – Weinberg equilibrium. The odds ratio (OR) was calculated using the Woolf – Haldane analysis, which allows for calculations for a 2 x 2 table for cases when at least one of the table cells has a zero value. Statistical significance of differences in the frequencies of the studied variables in the alternative groups was determined by the $\chi 2$ test with the Yates correction for continuity and by the two-sided Fisher's exact test for 2 x 2 tables. To assess the development of thromboembolic complications, a bivariate logistic regression analysis of its association with gender, diagnosis, and hemostatic system parameters, including standardization by age, was performed. The differences were considered statistically significant at $p < 0.05$.

RESULTS

The average age of the patients did not differ significantly. The majority of patients were young and middle-aged people. In the NE group, the percentage gender distribution was equal; in the IE group, men significantly prevailed, $p = 0.001$. According to the ultrasound findings, the degree of heart valve disorders varied from minor disorders (slight regurgitation, thickening of the valve flaps), which is typical of NE, to severe defects (heart valve stenosis or heart valve disease) in IE.

According to the genotyping findings, at the first stage, the genotype frequencies of the studied polymorphisms were determined in the groups of patients with endocarditis and the control group; then compliance of the genotype frequency distribution with the Hardy – Weinberg equilibrium was evaluated in the control group (using the $\chi 2$ test). In all the studied polymorphisms, the genotype frequency distribution complied with the Hardy – Weinberg equilibrium.

When studying the polymorphisms of 18 candidate genes for endocarditis, we revealed an association with five markers (Table 1). Carriers of the CT and CC genotypes of the rs1126643 polymorphism (807 C > T) in the *ITGA2* gene were identified in 88 % of NE patients and in 73 % of IE patients. For carriers of the TT genotype, the probability of vegetation formation in IE was 2 times higher than in the control group (OR = 2.36, 95% confidence interval (CI): 1.1–5.8, $p = 0.04$). In addition, carrying the A allele in the *MTR* gene doubled the OR for developing both NE and IE (OR = 2.02, 95 % CI: 1.05–3.92, $p = 0.04$), and carrying the AA genotype increased the OR for developing valvular vegetations by more than 2 times (OR = 2.35, 95% CI: 1.35–4.11, $p = 0.02$).

Table 1

Statistically significant parameters of polymorphisms of candidate genes for endocarditis (compared with the control group)

Non-infective endocarditis	Endocarditis	Infective endocarditis
For the *MMP-9* rs11697325 (-8202 A/G) AA genotype		
$p > 0.05$	OR = 1.95; 95% CI: 1.10–3.48, $p = 0.03$	OR = 2.31; 95% CI: 1.11–4.81, *p = 0.03*
For the *PTPN22* rs2476601 (C1858T) TT genotype		
$p > 0.05$	OR = 8.49; 95% CI: 1.67–43.20, $p = 0.006$	OR = 18.56; 95% CI: 3.59–96.01, $p = 0.0002$
Polymorphisms of genes of the hemostatic system		
For the *F5* rs6025 *(1691 G>A)* AG genotype		
occurrence frequency 8.9%, $p = 0.04$	$p > 0.05$	not detected
For the *ITGA2* rs1126643 *(807 C>T)* TT genotype		
$p > 0.05$	$p > 0.05$	OR = 2.36; 95% CI: 1.10–5.80, $p = 0.04$
MTR rs1805087 *(2756 A>G)* polymorphisms of the *MTR* gene of the folate pathway		
For the AG genotype		
OR = 0.47; 95% CI: 0.23–0.98, $p = 0.05$	OR = 0.44; 95% CI: 0.24–0.79, $p = 0.006$	OR = 0.4; 95% CI: 0.18–0.90, $p = 0.027$
For the AA genotype		
OR = 2.22; 95% CI: 1.11–4.45, $p = 0.03$	OR = 2.35; 95% CI: 1.35–4.11, $p = 0.03$	OR = 2.52; 95% CI: 1.19–5.32, $p = 0.02$
G allele		
$p > 0.05$	OR = 2.02, 95% CI: 1.05–3.92, $p = 0.04$	$p > 0.05$

The factor V Leiden mutation in IE was not detected. At the same time, the number of carriers of the factor V Leiden mutation (AG polymorphism) was significantly larger in NE (8.9 % vs. 3.6 % in the control group, $p = 0.04$).

A statistically significant increase in the frequency of carrying the AA genotype of the rs11697325 (-8202 A/G) polymorphism in the *MMP-9* gene was revealed in the group of patients with endocarditis (in 40 % of cases) compared with the control group (in 22% of cases), $p = 0.03$. Therefore, carrying the AA genotype doubled the risk of developing endocarditis compared with the control group, OR = 1.95, 95% CI: 1.1–3.48, $p = 0.03$. When comparing the IE group with the control group, carrying the AA genotype of the rs11697325 (-8202 A / G) polymorphism in the *MMP-9* gene increased the risk of developing endocarditis by more than 2 times, OR = 2.31, 95% CI 1.11–4.81, $p = 0.04$ (Table 1).

PTPN22 expressed on lymphocytes through formation of a complex with C-terminal Src kinase (CSK) suppresses subsequent mediators of T-cell receptor signaling. Substitution of the arginine amino acid residue with a tryptophan one (R620W) excludes the possibility of interaction between PTPN22 and CSK, which leads to impaired feedback regulation of activated lymphocytes [12]. The OR for developing IE in carriers of the TT genotype of the rs2476601 polymorphism (C1858T) in the *PTPN22* gene is significantly higher compared with carriers of the other two genotypes (C / T + C / C), OR = 18.56, 95% CI: 3.59–96.01, $p = 0.0002$ (Table 1).

Excessive production of prothrombin is a risk factor for myocardial infarction and thrombosis, including pulmonary embolism, which often has a fatal outcome [13]. Polymorphism rs1799963, caused by substitution of guanine (G) with adenine (A) at position 20210 of the *F2* gene, leads to its increased expression with a risk of arterial and venous thrombosis [14]. Among our patients with endocarditis, no carriers of the AG polymorphism in the *F2* gene were registered, so the contribution of this polymorphism to the development of vegetations on the cardiac valvular apparatus and thromboembolic complications could not be studied (Table 2).

Table 2

Statistically significant parameters of gene polymorphisms that prevent from development of endocarditis (compared with the control group)

Non-infective endocarditis	Endocarditis	Infective endocarditis
For the *NOS3* 4b / 4b genotype		
OR = 0.44; 95 % CI: 0.22–0.9, $p = 0.03$	OR = 0.47; 95 % CI: 0.27–0.81, $p = 0.006$	$p > 0.05$
For the *IL6* G (- 572) C CC genotype		
OR = 0.28, 95% CI: 0.08–0.96, $p = 0.03$	$p > 0.05$	$p > 0.05$

The *IL6* gene encodes the interleukin-6 (IL-6) protein, which takes part in the development of the immune response, initiating its own phase of reproduction. In the NE group, a decrease in the frequency of the SS genotype was detected, OR = 0.28, 95% CI: 0.08–0.96, $p = 0.03$ (Table 2).

When examining the polymorphisms in the nitric oxide synthase 3 (*NOS3*) gene, the frequency of the 4b / 4b genotype in the *NOS3* gene in the group of healthy individuals was higher than in patients with endocardia, so the presence of the 4b / 4b genotype in the *NOS3* gene reduced the probability of developing NE, OR = 0.44, 95 % CI: 0.22–0.9, $p = 0.03$ (Table 2). According to the data [15], a decrease in the content of nitric oxide disrupts normal vascular function and increases vascular tone and thrombus formation, and a decrease in the NOS3 activity has a protective effect.

According to the logistic regression analysis, the development of thromboembolic complications was revealed 1.5 times more often in IE than in NE, 95% CI: 1.06–3.94, $p = 0.04$. Associations of polymorphisms were revealed: rs1126643 (807 s > T) of the *ITGA2* gene, OR = 2.09, 95% CI: 1.14–3.85, $p = 0.02$ and rs1799889 (- 675 and 5G > 4G) of the *PAI-1* (*SERPINE1*) gene with the development of thromboembolic complications in NE, OR= 4.12, 95% CI: 1.25–13.63, $p = 0.02$; in IE, the rs11697325 (-8202 A / G) polymorphism of the *MMP-9* gene, OR = 3.43, 95% CI: 1.15–12.11, $p = 0.04$.

DISCUSSION

The most significant thromboembolic events in patients with endocarditis in our study were acute stroke and myocardial infarction. Possible associations between the development of thromboembolic complica-

tions and polymorphisms of 18 candidate genes were studied.

Disruption of the folate pathway leads to hyperhomocysteinemia, hypercoagulation, and heart valve thrombosis [16, 17]. Thromboembolic complications were detected 1.5 times more often in IE than in NE, $p = 0.04$. Associations of rs1126643 polymorphism (807 C > T) of the *ITGA2* gene and rs1799889 (-675 5G > 4G) polymorphism of the *PAI-1 (SERPINE1)* gene with the development of thromboembolic complications in NE were revealed; and in IE, such an association was identified for the rs11697325 (-8202 A / G) polymorphism of the *MMP-9* gene.

Studying polymorphisms of candidate genes for endocarditis allowed to identify 5 unfavorable prognostic markers. Platelet receptor genes are likely to be important factors of thrombotic risk upregulation.

In carriers of certain alleles and genotypes of the collagen receptor gene (*ITGA2* 807 C > T), adhesion of platelets to one another and to the vascular endothelium increases, which leads to increased thrombosis [18, 19]. For carriers of the TT genotype, the probability of vegetation formation in IE is twice as high as in the control group.

Disruption of the folate pathway contributes to hyperhomocysteinemia, which can lead to hypercoagulation and heart valve thrombosis [20, 21]. The *MTR* gene encodes the amino acid sequence of methionine synthase, one of the key enzymes of methionine metabolism, which catalyzes production of methionine from homocysteine via its remethylation. We found that carrying the A allele doubled the risk of developing both NE and IE (OR = 2.02, 95% CI: 1.05–3.92, $p = 0.04$), and carrying the AA genotype increased the risk of developing vegetations by more than 2 times.

It is known that in the presence of factor V Leiden mutation at position 1691 in the gene encoding coagulation factor V, adenine is replaced with guanine, so factor V is not cleaved by protein C, a natural physiological anticoagulant, as it happens under normal conditions, but becomes resistant to its action, which leads to an increase in the concentration of factor V in the blood serum. The factor V Leiden mutation has a modifying effect on the *PAI-1* polymorphism in terms of the risk of recurrent thromboembolic complications and increases the risk of venous thromboembolism by 4 times [16]. In the NE group, the number of factor V Leiden mutation carriers was significantly larger (8.9 % vs. 3.6 % in the control group, $p = 0.04$), while in the IE group, the factor V Leiden mutation was not detected.

Some proteins expressed in endocarditis may be used as favorable prognostic biomarkers. The study of protective gene variants revealed associations for two polymorphisms. Prothrombin is a precursor of thrombin and plays an important role in fibrin formation. Excessive production of prothrombin is a risk factor for myocardial infarction and various thromboses, including pulmonary embolism, which often has a fatal outcome [14]. The rs1799963 polymorphism, due to the replacement of guanine (G) with adenine (A) at position 20210 in the *F2* gene, leads to increased gene expression in the A variant. Patients carrying one copy of this allele have a 5-fold increased risk of thrombus formation; and among those with two copies of the 20210A allele, the risk increases by 50 times. Under such conditions, the carriers become prone to earlier or more severe arterial and venous thrombosis, especially if there is a family history of such events [13]. There were no carriers of the A allele among our patients with endocarditis.

The protective effect of the SS minor allele genotype of interleukin-6 (IL-6) in the development of acute coronary syndrome was shown [23]: compared with SS homozygotes, the SG genotype is characterized by a 2.2-fold increase in the risk of developing the disease. In our study, in the NE group, the presence of a protective SS genotype was identified compared with the IE group ($p = 0.03$).

A decrease in the content of nitric oxide disrupts normal vascular function and increases vascular tone and thrombus formation, while an increase in the activity of this enzyme has a protective effect [15]. In the study of *NOS3* gene polymorphisms, the frequency of carrying the 4b / 4b genotype of the *NOS3* gene in the group of healthy individuals was higher than in patients with endocarditis. Therefore, the presence of the 4b / 4b genotype of the *NOS3* gene statistically significantly ($p = 0.006$) reduces the risk of developing IE and NE.

CONCLUSION

We identified polymorphisms of candidate genes associated with clinical and hemostasiological characteristics of NE and IE, which can be used to assess the risk of developing these diseases, as well as for differential diagnosis. For 5 out of the 18 studied gene polymorphisms, an association with the risk of developing endocarditis, namely, with valvular vegetations, was revealed: for proinflammatory genes – rs11697325 (-8202 A / G) of the *MMP-9* gene (AA genotype) and rs2476601 (C1858T) of the *PTPN22* gene (TT

genotype); for genes of the hemostatic system – rs6025 (1691 G > A) of the *F5* gene (AG genotype) and rs1126643 (807 C > T) of the *ITGA2* gene (TT genotype); for the gene of the folate pathway – rs1805087 (2756 A > G) of the *MTR* gene (AG genotype).

For the first time, the protective effect of two gene polymorphisms for developing endocarditis was established: proinflammatory gene polymorphisms of the *NOS3* gene (4b / 4b genotype), G (- 572) C of the *IL6* gene (CC genotype).

In terms of differential diagnosis, for the first time, an association with thromboembolic complications was revealed for two polymorphisms in NE – rs1126643 (807 C > T) of the *ITGA2* gene and rs1799889 (-675 5G > 4G) of the *PAI-1* (*SERPINE1*) gene. In IE, an association with thromboembolic complications was revealed for one polymorphism – rs11697325 (-8202 A / G) of the *MMP-9* gene.

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Authors contribution

Bakhareva Y.S. – examination and treatment of patients, collection and preparation of biomaterial, review of literature, statistical processing of the research results and their interpretation, drafting of the manuscript. Maksimov V.N. – design of the study, provision of material and technical facilities for laboratory tests, interpretation of the results. Ivanova A.A. – preparation of samples with biomaterial, carrying out of PCR. Chapaeva N.N. – differential diagnosis of endocarditis, consultations on research planning. Aidagulova S.V. – review of literature, editing of the manuscript. Voevoda M.I. – consultations on interpretation of the results, design of the study.

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Study of the effectiveness of ventriculosubarachnoid drainage in neonatal hydrocephalus according to the data of the Republic of Crimea for the period 2000–2018

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ABSTRACT

Despite the achieved success in the treatment of neonatal hydrocephalus, the task of restoring circulation, outflow, and absorption of cerebrospinal fluid (CSF) remains urgent.

The aim of the study was to investigate the effectiveness of ventriculosubarachnoid drainage in compensating hydrocephalus without shunt implantation.

Materials and methods. We collected and studied clinical material for the period from 2000 to 2018 according to the data of the Republic of Crimea. We identified groups of premature (n = 184) and full-term (n = 107) infants who underwent standard treatment with lumbar puncture, subgaleal drainage, and ventriculoperitoneal shunting (VPS). In case of ventricular occlusion in 143 premature and 46 full-term infants, at the initial stage of treatment, the option of coronary – lambdoid subarachnoid ventriculostomy (RF Patent No. 2715535) in combination with lumbar punctures was included. With progression of hydrocephalus, ventriculosubarachnoid stenting (RF Patent No. 2721455) with subgaleal drainage was considered as an option.

Results. The inclusion of the proposed options made it possible to increase the rate of hydrocephalus compensation without VPS to 75.5% in premature infants and to 80.4% in full-term infants versus 28.3% and 20.6%, respectively, according to the standard protocol ($p < 0.001$). In other cases, the imbalance between CSF production and absorption persisted, which required integration of a stent with a peritoneal part of the shunt, without replacing the system.

Conclusion. The obtained result allows to consider the inclusion of the proposed options in the modern treatment algorithm for neonatal hydrocephalus.

Keywords: ventriculosubarachnoid drainage, hydrocephalus, infants

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Изучение эффективности опции вентрикуло-субарахноидального дренирования при неонатальной гидроцефалии по данным Республики Крым за период 2000–2018 гг.

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РЕЗЮМЕ

Несмотря на достигнутые успехи в лечении гидроцефалии неонатального периода, остается актуальной задача восстановления ликвородинамики, оттока и всасывания ликвора.

Цель – изучение эффективности опций вентрикуло-субарахноидального дренирования в компенсации гидроцефалии без имплантации шунтов.

Материалы и методы. Собран и изучен клинический материал за период 2000–2018 гг. по данным Республики Крым. Выделены группы недоношенных (n = 184) и доношенных (n = 107) детей, которым выполнялось стандартное лечение с люмбальными пункциями, субгалеальное дренирование и вентрикуло-перитонеальное шунтирование (ВПШ). При окклюзии желудочков у 143 недоношенных и 46 доношенных детей на начальном этапе лечения включалась опция коронаро-транслябдовидной субарахно-вентрикулостомии (патент РФ № 2715535) в комплексе с люмбальными пункциями, а при прогрессировании гидроцефалии – вентрикуло-субарахноидальное стентирование (патент РФ № 2721455) с субгалеальным дренированием.

Результаты. Включение предложенных опций позволило повысить процент компенсации гидроцефалии без ВПШ до 75,5% у недоношенных детей и 80,4% у доношенных против 28,3 и 20,6% соответственно при стандартном протоколе (p < 0,001). В остальных случаях сохранялся дисбаланс продукции – всасывания ликвора, что потребовало интеграции стента с перитонеальным сегментом шунта без замены системы.

Заключение. Полученный результат позволяет рассматривать включение предложенных опций в современный лечебный алгоритм при неонатальной гидроцефалии.

Ключевые слова: вентрикуло-субарахноидальное дренирование, гидроцефалия, дети

Конфликт интересов. Авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Все законные представители пациентов подписали добровольное информированное согласие на участие в исследовании. Исследование одобрено этическим комитетом КФУ им. В.И. Вернадского (протокол № 53 от 06.12.2018).

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INTRODUCTION

Current publications on pediatric neurosurgery and neurology describe the principles of treatment for posthemorrhagic hydrocephalus (PHH) in newborns with normalization of the increased intracranial pressure, including lumbar punctures (LP) and ventricular punctures (VP), external and internal ventricular drainage, and artificial ventriculoperitoneal shunts (VPS) [1–14]. A sequence of treatment options according to the "LVV protocol" is considered as guidelines for PHH therapy: LP and VP, ventriculosubgaleal drainage (VSGD), and if they are ineffective – VPS [1–6].

The relevance of the issue is determined by the absence of a generally accepted opinion regarding treatment for decompensated PHH with subarachnoid space (SAS) block and impaired resorption [7–10]. There is a need to personalize treatment tactics [2] aimed at restoring CSF circulation and reducing the frequency of VPS surgery [11–14]. Solution of these issues becomes interdisciplinary in the joint practice of a pediatric neurosurgeon, neonatologist, and neurologist.

The aim of the study was to investigate the effectiveness of ventriculosubarachnoid drainage options for PHH compensation in newborns.

MATERIALS AND METHODS

We have collected, studied, and analyzed clinical data on the treatment of PHH in 480 newborns in the Republic of Crimea for the period from 2000 to 2018. The study was approved by the Ethics Committee at V.I. Vernadsky Crimean Federal University (Protocol No. 53 of 06.12.2018). All legal representatives of the patients signed an informed consent to participate in the study.

327 children were preterm (group 1) and 153 were full-term infants (group 2). 184 children in group 1 and 107 children in group 2 received standard treatment according to the "LVV protocol".

In case of ventricular occlusion with SAS block in 143 preterm infants (group 1) and 46 full-term infants (group 2), the standard treatment protocol at the initial stage included coronary – lambdoid subarachnoid ventriculostomy (CLSV) [15] and in case of PHH progression – ventricular drainage in the SAS using ventriculosubarachnoid stenting system (VSS) [16].

The inclusion criterion for the proposed treatment options was decompensated PHH. In case of restoration of CSF circulation with compensation of PHH, further stages of correction were excluded. Tables 1 and 2 describe the amount of care provided for premature and full-term infants with the inclusion of the CLSV and VSS options.

Table 1

The amount of neurosurgical care in premature infants (group 1)

PHH correction stages	*n* (%)
According to the "LVV protocol"	
LP and VP with 20–22G needles	184 (100)
VSGD	151 (82.1)
VPS	132 (71.7)
inclusion of the proposed options in case of decompensated PHH	
CLSV (using 14 G needles) in combination with LP	143 (100)
Ventricular drainage in SAS in combination with VSGD	94 (65.7)
VSS system integration in the peritoneal part of the shunt	35 (24.5)

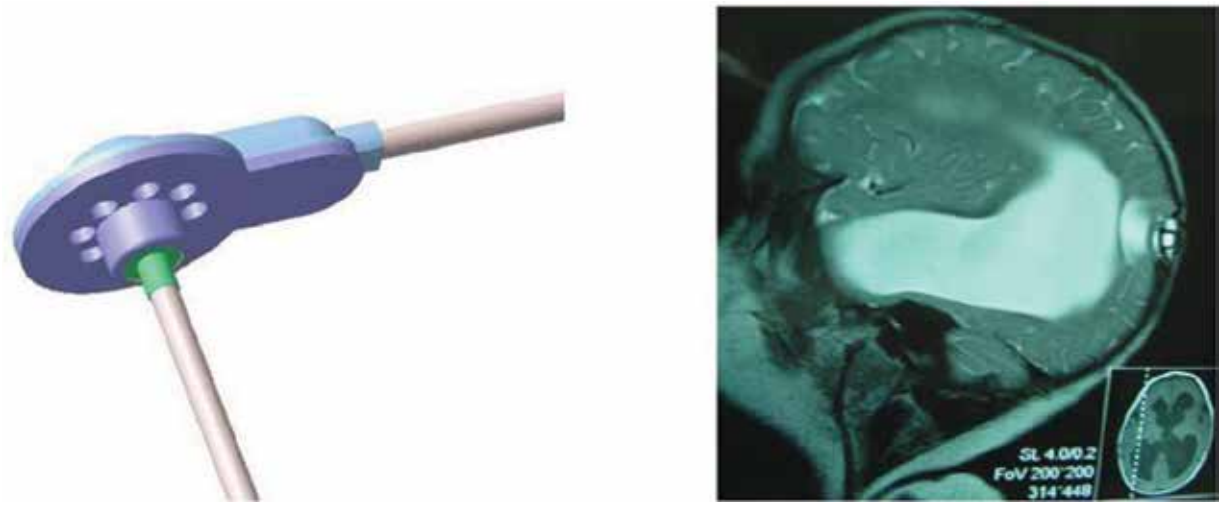
Table 2

The amount of neurosurgical care in full-term infants (group 2)

PHH correction stages	*n* (%)
According to the "LVV protocol"	
LP and VP with 20–22G needles	107 (100)
VSGD	90 (84.1)
VPS	85 (79.4)
inclusion of the proposed options in case of decompensated PHH	
CLSV (using 14 G needles) in combination with LP	46 (100)
Ventricular drainage in SAS in combination with VSGD	22 (47.8)
VSS system integration in the peritoneal part of the shunt	9 (19.6)

There were no complications and mortality associated with surgical trauma. CLSV was performed using a two-point puncture of the anterior and occipital horns of the lateral ventricles through coronary and lambdoid sutures using 14G needles, with evacuation of blood and CSF and SAS decompression. Irrigation of the ventricles was performed with normal saline with encephalolysis when the needles reached the SAS. Drainage canals were formed between the ventricles and SAS with collateral outflow of CSF and elimination of occlusion. We repeated the procedure 3 times with a 4-day interval, alternating it with irrigation of the craniospinal CSF pathways using LP, until CSF circulation was normalized.

The advantages of the method include ease of application (a child in a humidicrib), the effectiveness of evacuation of blood clots from the ventricles with minimization of brain injury during hemorrhagic tamponade, and reduction of irrigation time for the craniospinal CSF spaces. The VSS system developed by the authors provided CSF drainage in the SAS through a ventricular drain and a pump base perforation (Figure).



a *b*

Figure. VSS system: *a* – general view of the pump, *b* – magnetic resonance imaging (MRI) after surgery

For this purpose, after insertion of the ventricular drain into the ventricle with the control of CSF flow, the pump was installed in the burr hole with a diameter of up to 10 mm with the fixing cuff expanded in the SAS and fixed by sutures along the edges of the trepanned area. In addition, we exercised temporary CSF outflow from the pump through the distal drain into the subgaleal pocket (SP), which made it possible to alleviate intracranial pressure drops in the postoperative period with evacuation and irrigation of the CSF pathways. The dome of the pump was punctured and normal saline was injected with control of its outflow into the SAS and ventricles. Re-infusion of normal saline through the pump with vigorous irrigation of the SAS and ventricles and passive evacuation of CSF through SP in combination with LP was performed on days 3–5, 7, 10, 14, and at the end of week 3, 4, 5, and 6 after surgery. While maintaining the imbalance between the increasing age-related volume of CSF production and its absorption after 6 weeks, the VSS system was integrated through the connector of the distal drain with the peritoneal part of the shunt (Codman, Medtronic, USA), including a chamber with a medium-pressure valve, regulating the discharge of CSF into the abdominal cavity.

The data were processed using the Statistica 10.0 software (StatSoft Inc., USA). Fisher's exact test (FET) was applied to compare the efficiency rates of PHH compensation in premature and full-term infants under the standard LVV protocol and after the inclusion of the options for ventriculosubarachnoid drainage in the treatment regimen. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Compensation of hydrocephalus at the initial stage of treatment according to the LVV protocol after LP was observed in 33 of 184 premature infants, which made it possible to avoid drainage in 17.9% of cases (Table 3).

Table 3

Comparative analysis of compensation of hydrocephalus in premature infants

Compensation of hydrocephalus	*n* (%)	*p**
After LP and VP (according to the LVV protocol)	33 (17.9)	0.046
After the inclusion of CLSV (in combination with LP)	49 (34.3)	
After VSGD (according to the LVV protocol)	19 (12.6)	< 0.001
After the inclusion of VSS (in combination with VSGD and and LP)	59 (62.8)	

*according to FET – here and in Table 4.

When the CLSV option was included in the hydrocephalus treatment protocol, PHH compensation was achieved in 49 out of 143 children, which allowed to exclude drainage in 34.3% of cases ($p = 0.046$). A decrease in drainage manipulations after the inclusion of CLSV resulted from effective evacuation of blood clots and CSF from the ventricles using 14G needles, irrigation of the ventricles and SAS with normal saline, elimination of the occlusion, and reduction of the irrigation time for the CSF pathways.

Compensation of hydrocephalus at the stage of surgical management after VSGD was observed in 19 of 151 children (12.6%), after inclusion of VSS, it was noted in 59 of 94 children, which made it possible to

exclude VPS in 62.8% cases ($p < 0.001$). The cumulative positive outcome with compensated hydrocephalus without VPS in preterm infants after the inclusion of the CLSV and VSS in the treatment regimen was achieved in 75.5% of cases vs. 28.3% with the standard protocol ($p < 0.001$).

Clinical case. Child K. was delivered by a nulliparous woman with an aggravated obstetric history at the Perinatal Center at 26 weeks of gestation, with an extremely low body weight of 650 g. The Apgar score was 1–2 points. Grade 2 cerebral ischemia was detected, and mechanical ventilation was performed. After birth, grade 4 periventricular and intraventricular hemorrhage with hemorrhagic tamponade of both lateral and third ventricles was diagnosed against the background of morphofunctional immaturity with respiratory and cardiovascular insufficiency. Treatment at the initial stage with CLSV in combination with LP made it possible to stabilize CSF circulation. After 30 weeks, PHH progression, compression and blockage of SAS were noted. Surgical treatment was performed with indirect ventricular drainage in the SAS using VSS system according to the described method, in combination with VSGD and LP. A decrease in the protein level in the CSF from 4.6 to 2.4 g / l on day 10, and to 0.8 g / l according to the last test, was revealed. By week 37, compensated PHH was noted according to the clinical data and CT-guided neurosonography findings at week 40. Follow-up during the first year of life did not show progression of intracranial hypertension; no seizures were noted.

The revealed differences were also noted in full-term infants (Table 4).

Table 4

Comparative analysis of hydrocephalus compensation in full-term infants

Compensation of hydrocephalus	*n* (%)	*p*
After LP and VP (according to the LVV protocol)	17 (15.9)	0.027
After the inclusion of CLSV (in combination with LP)	24 (52.2)	
After VSGD (according to the LVV protocol)	5 (5.6)	< 0.001
After the inclusion of VSS (in combination with VSGD and LP)	13 (59.1)	

According to the LVV protocol, compensation of PHH after LP was observed in 17 out of 107 full-term infants, which made it possible to exclude drainage in 15.9% of cases. When the CLSV was included in the treatment regimen, PHH compensation was achieved in 24 out of 46 children, which allowed to exclude drainage in 52.2% of cases ($p = 0.027$).

PHH compensation after VSGD was observed only in 5 out of 90 children (5.6%). After inclusion of VSS in the treatment regimen, compensated PHH was noted in 13 out of 22 full-term infants, which allowed to avoid VPS in 59.1% cases ($p < 0.001$). The cumulative positive outcome with compensated PHH in full-term infants after the inclusion of CLSV and VSS in the treatment regimen was achieved in 80.4% of cases vs. 20.6% under the standard protocol ($p < 0.001$).

In case of integration of the VSS system with the peritoneal part of the shunt, irrigation of the ventricles and SAS was performed with normal saline, which allowed to eliminate dysfunction of the ventricular segment and the pump. No signs of overdrainage were noted. To improve the efficiency of the VSS system, a hermetic self-expanding ventriculosubarachnoid stent was proposed [17] with an additional sealing cuff to place the stent into the burr hole without suturing and to eliminate the risk of liquorrhea.

DISCUSSION

Treatment of PHH in newborns involves LP at the initial stage. At the same time, this does not ensure the evacuation of blood clots from the ventricular system and requires long-term irrigation of the craniospinal CSF spaces; and a blood clot in the third ventricle and other types of occlusion are contraindications for LP [1–8].

Ventricular punctures using 20–22G needles do not provide effective evacuation of blood clots from the ventricles with a high risk of damage to the brain matter during vigorous aspiration. During long-term external drainage required for clot lysis, the risk of infection increases and occlusion of CSF pathways is not eliminated [1–8].

An increase in the effectiveness of treatment with the inclusion of CLSV results from the use of brain needles with a larger diameter and larger areas of access points with elimination of occlusion, formation of a ventriculosbarachnoid anastomosis, evacuation of blood clots from the ventricles, as well as evacuation of its degradation products from the craniospinal CSF spaces with a decrease in the irrigation time and the risk of adhesions.

VSGD provides long-term evacuation of blood from the ventricles with the elimination of occlusion of CSF pathways [1–8]. An increase in the effectiveness of treatment with VSS is achieved by restoring intracranial circulation and CSF absorption at the

stage of long-term irrigation of the craniospinal CSF spaces with normal saline in combination with VSGD and LP. The need for long-term irrigation with exclusion of blood release into the basal cisterns is a limiting factor for endoscopic ventriculostomy in the neonatal period [9].

VPS artificially drains CSF to the abdominal cavity. Shunt dependence and high rates of dysfunction and complications are noted [9–14]. Integration of the VSS into the peritoneal part of the shunt provided CSF drainage to the SAS and redirected excess CSF to the abdominal cavity with adaptation of the resorptive capacity to the increasing volume of CSF in the first year of life. Besides, it reduced the risk of system dysfunction without its replacement and reinstallation.

CONCLUSION

PHH compensation indicates the need for ventriculosubarachnoid drainage using CLSV and VSS to eliminate occlusion, ensure effective irrigation of the CSF spaces, and restore circulation and absorption of CSF. The inclusion of the CLSV and VSS options in the treatment algorithm led to an increase in the compensated PHH to 75.5% in preterm infants and to 80.4% in full-term infants vs. 28.3% and 20.6%, respectively, under the standard LVV protocol ($p < 0.001$). Integration of the VSS system with the peritoneal part of the shunt, while maintaining the imbalance between CSF production and absorption, allows to expand the potential scope of using shunt systems for the treatment of PHH in newborns.

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Authors contribution

Volkodav O.V. — conception and design; analysis and interpretation of data. Zinchenko S.A. — critical revision for important intellectual content. Khachatryan V.A. – final approval of the manuscript for publication.

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Serum level of laminin in rats fed with a high-fat diet with sulodexide administration

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ABSTRACT

Background. Increased consumption of animal fat with food contributes to the accumulation of lipids both in the blood and in individual cell structures. Excess fat initiates oxidative stress reactions, which may result in a violation of the structural and functional integrity of cells, in particular, hepatocytes and endotheliocytes. Cytolysis may release specific liver enzymes and activate synthesis of extracellular matrix components, one of the markers of which is a non-collagen glycoprotein laminin.

The drug sulodexide, having a pronounced angioprotective, hypolipidemic, and fibrinolytic effects, contributes to restoration of a number of metabolic disorders.

Aim. To study the content of lipid metabolism parameters, major enzymes of hepatic cytolysis, and laminin in the blood of rats fed with a high-fat diet against the background of sulodexide administration.

Materials and methods. For the study, outbred rats were selected, which were divided into three groups – two experimental groups and one control group. The rats of the first and second experimental groups were fed with a diet with a high content of animal fat (44% of the daily calorie content) for 35 days. In addition, the rats of the second experimental group were daily subcutaneously injected with sulodexide at a dose of 8.5 LRU/kg in terms of the animal's body weight for 35 days. Starting from day 36 of the experiment, the rats of the control group, as well as the rats of the two experimental groups were fed with a standard diet of the vivarium. The animals were decapitated and blood was taken on day 21, 35, and 60 of the experiment. In the blood serum, the levels of the main lipid metabolism parameters, specific liver enzymes, and laminin were determined.

Results. An increase in the body weight of animals and the level of the studied lipid metabolism parameters in the blood serum was revealed. It is likely that the structural integrity of hepatocytes was affected with the release of liver enzymes into the bloodstream and an increase in their content in the blood of rats. In addition, synthesis of extracellular matrix components was activated with an increase in the serum level of laminin, which performs important structural and regulatory functions.

Conclusion. The use of sulodexide had a favorable effect on the studied metabolic disorders caused by a high-fat diet. It resulted in the normalization of the synthesis of laminin, one of the major non-collagen proteins of the extracellular matrix.

Keywords: high-fat diet, lipid metabolism, liver enzymes, laminin

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Содержание ламинина в сыворотке крови крыс в условиях высокожировой диеты при коррекции сулодексидом

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РЕЗЮМЕ

Повышенное поступление животного жира с пищей способствует накоплению липидов как в крови, так и в отдельных клеточных структурах. Излишек жиров инициирует реакции окислительного стресса, следствием которых может явиться нарушение структурной и функциональной целостности клеток, в частности гепатоцитов и эндотелиоцитов. В результате цитолитических процессов возможно высвобождение специфических ферментов печени и активация синтеза компонентов межклеточного матрикса, одним из маркеров которого является неколлагеновый гликопротеин ламинин.

Препарат «Сулодексид», обладая выраженным ангиопротекторным, гиполипидемическим, фибринолитическим действием, участвует в восстановлении ряда обменных нарушений.

Цель. Изучение содержания показателей липидного обмена, основных ферментов цитолиза гепатоцитов и ламинина в крови крыс при высокожировой диете на фоне коррекции сулодексидом.

Материалы и методы. Для исследования были отобраны беспородные крысы, которых разделили на три группы – две опытных и одну контрольную. Крысам первой и второй опытных групп была назначена диета с повышенным содержанием животного жира (44% от суточной калорийности) в течение 35 сут. Крысам второй опытной группы ежедневно в течение 35 сут подкожно вводился сулодексид в дозировке 8,5 ЛЕ/кг в перерасчете на массу тела животного. Крысы всех групп с 36-х сут опыта находились на стандартном рационе вивария. Декапитацию животных и забор крови проводили на 21, 35 и 60-е сут опыта. В сыворотке крови определяли содержание основных показателей липидного обмена, специфических ферментов печени и ламинина.

Результаты. Наблюдался рост массы тела животных, увеличение содержания изучаемых показателей липидного обмена в сыворотке крови. Вероятно, затрагивалась структурная целостность гепатоцитов с вымыванием печеньспецифических ферментов в кровяное русло и повышением их содержания в крови крыс. Активировался синтез компонентов внеклеточного матрикса с возрастанием содержания в крови исследуемого неколлагенового белка – ламинина, выполняющего значительную структурную и регуляторную функцию в его организации.

Заключение. Применение сулодексида благоприятно повлияло на исследуемые метаболические нарушения, вызванные приемом пищи с повышенным содержанием жира, в том числе привело к нормализации синтеза одного из основных неколлагеновых белков межклеточного матрикса – ламинина.

Ключевые слова: высокожировая диета, липидный обмен, ферменты печени, ламинин

Конфликт интересов. Авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Obesity and metabolic syndrome have reached epidemic proportions in the 21st century. The urgency of the problem of weight gain is associated with an increased risk of development and progression of atherogenic dyslipidemia, cardiovascular diseases, and nonalcoholic fatty liver disease (NAFLD) [1]. NAFLD is characterized by low-grade inflammation in hepatocytes, which ultimately can lead to activation of connective tissue growth and formation of liver fibrosis [1].

A number of previous studies have shown a correlation between the accumulation of fat in hepatocytes and the stage of liver fibrosis [2]. Increased consumption of fat contributes to an increase in the supply of free fatty acids (FFA) to liver cells, a decrease in the rate of beta-oxidation of FFA, as well as an increase in the synthesis or secretion of very-low-density lipoproteins (VLDL) in the liver, and then low-density lipoproteins, formed from VLDL following hydrolysis of triglycerides under the action of lipoprotein lipase located on the capillary endothelium.

A significant contribution to fibrogenesis is made by oxidative stress following lipid peroxidation with the formation of highly reactive toxic compounds and activation of inflammatory reactions with the release of proinflammatory cytokines. The emerging oxidative stress and excess of triglycerides in hepatocytes can cause their cytolysis with the release of liver enzymes and, as a consequence, active synthesis of the extracellular matrix. High blood level of triglycerides in the composition of low-density lipoproteins can have a damaging effect on endothelial cells. Laminin, a non-collagen protein of connective tissue, can be one of the markers of these processes.

Laminins are a family of large glycoproteins that bind to one other and form about 15 heterotrimeric macromolecules. The molecular weight of laminin ranges from 400 to 900 kDa. Currently, 16 laminin isoforms have been identified in mammals [3]. Laminins are some of the components of the basement membranes in various tissues, mediating their interaction with cells. They contribute to the formation of an integral structure between cellular receptors and the basement membrane due to their polymerization and act as key molecules in the formation of unique tissue structures. Together with other non-protein components of the extracellular matrix, they participate in cell adhesion, tissue reconstruction, and maturation of collagen fibers.

The drug sulodexide (Vessel Due F®) is an anticoagulant that has antiplatelet, antithrombotic, angioprotective, hypolipidemic, and fibrinolytic effects. The active ingredient is an extract from the mucous membrane of the small intestine of animals; it is a natural mixture of a low-molecular-weight heparin (80%) and dermatan sulfate (20%) [4]. The mechanism of the angioprotective effect is associated with restoration of the structural and functional integrity of vascular endothelial cells. It normalizes blood rheology by lowering the level of triglycerides and reducing blood viscosity [4].

The aim of the research was to study the content of lipid metabolism parameters, major enzymes of hepatic cytolysis, and laminin in the blood of rats fed with a high-fat diet against the background of sulodexide administration.

MATERIALS AND METHODS

The study was carried out on white outbred male rats with an initial body weight of 200–250 g. Work with rodents was carried out in accordance with the Order of the Ministry of Health of the Russian Federation No. 199n of 01.04.2016 “On the approval of the rules of good laboratory practice”. The study was approved by the local Ethics Committee at Izhevsk State Medical Academy (Protocol No. 652 of 23.04.2019).

The animals were kept in cages at 23 ± 2°C with 12h / 12h light – dark regime and had free access to food and water. All animals were divided into three groups – a control group (15 rats) and two experimental groups (15 animals each). The rats of the control group were fed with a standard vivarium diet. For 35 days, the animals of the two experimental groups received a high-fat diet – 44% of lard and 9% of vegetable oil from the daily diet [5]. In addition, for 35 days, the rats in the second experimental group received daily subcutaneous injections with Vessel Due F® at a dose of 8.5 LRU / kg in terms of the animal’s body weight [6]. From day 36 to day 60 of the experiment, all the rats were fed with a standard diet of the vivarium. On day 60 of the experiment, changes in the studied parameters were assessed in the long run [7].

The body weight of the animals was determined before the start of the experiment and at each time interval studied. Fasted animals were removed from the experiment on days 21, 35, and 60 by decapitation under short-term ether anesthesia. The material for the research was blood taken from the cervical vein. Blood serum was obtained from blood samples by centrifugation (3,000 rpm for 15 minutes) to determine biochemical parameters and laminin. An

automatic chemistry analyzer AU-480 (Beckman Coulter, USA) with appropriate test systems was used to determine the concentration of the main lipid metabolism parameters (total cholesterol (TC), high-density lipoproteins (HDL) and low-density lipoproteins (LDL), triglycerides (TG)), as well as the activity of the major liver enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH). The laminin concentration was determined by the enzyme-linked immunosorbent assay (ELISA) using the Laminin ELISA Kit (USA).

Statistical processing of the results was carried out using the SPSS Statistics software. Descriptive statistics are presented as the median and the interquartile range *Me* [Q_1; Q_3]. The data obtained did not follow a normal distribution; therefore, the statistical significance of the differences was calculated using the nonparametric Mann – Whitney test. The value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The body weight of the animals from the experimental group 1 fed with a high-fat diet increased by day 35 of the experiment and continued to increase till the end of the experiment. The body weight of the animals from the experimental group 2 who received a high-fat diet and injections of sulodexide did not differ significantly from the control values (Table 1).

After consumption of high-fat food, the following changes in the lipid profile were observed in the blood serum of the rats in the experimental group 1: an increase in the concentration of TC by 165.3%, LDL – by 458.8%, TG – by 522.5%, and HDL – by 58.6% compared with the control by day 60 of the experiment (Table 2).

Table 1

Change in body weight of the experimental rats compared with the control, *n* = 5

Conditions of the experiment	Experiment time, days		
	Day 21	Day 35	Day 60
Diet	15.2; $p = 0.089$	25.9; $p = 0.041$	17.6; $p = 0.039$
Correction	0.1; $p = 0.405$	7.4; $p = 0.076$	15.2; $p = 0.118$

Table 2

Biochemical parameters in the blood serum of rats fed with a high-fat diet, mmol / l, *Me* [Q_1; Q_3]

Parameter	Experiment time, days					
	Day 21		Day 35		Day 60	
	Control, *n* = 5	Experiment, *n* = 5	Control, *n* = 5	Experiment, *n* = 5	Control, *n* = 5	Experiment, *n* = 5
TC	0.78 [0.75; 0.92]	1.69 [1.69; 1.81] $p = 0.009$	0.85 [0.81; 0.91]	1.98 [1.89; 2.01] $p = 0.007$	0.89 [0.84; 0.96]	2.07 [1.85; 2.11] $p = 0.009$
HDL	0.46 [0.41; 0.47]	0.74 [0.73; 0.79] $p = 0.012$	0.52 [0.50; 0.59]	0.69 [0.67; 0.7] $p = 0.025$	0.55 [0.51; 0.59]	0.73 [0.71; 0.79] $p = 0.008$
LDL	0.17 [0.17; 0.23]	0.46 [0.45; 0.49] $p = 0.009$	0.19 [0.17; 0.22]	0.56 [0.47; 0.57] $p = 0.009$	0.21 [0.14; 0.33]	0.95 [0.56; 1.2] $p = 0.009$
TG	0.40 [0.4; 0.47]	1.77 [1.48; 2.89] $p = 0.009$	0.46 [0.41; 0.57]	1.90 [1.87; 1.91] $p = 0.011$	0.52 [0.49; 0.56]	2.49 [1.58; 2.7] $p = 0.016$

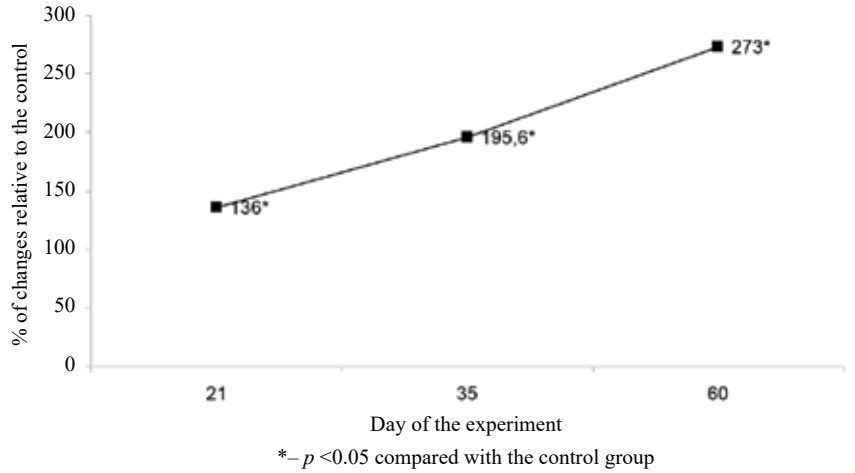
It is reasonable to expect these changes from the standpoint of assimilation of exogenous lipids due to their increased absorption and enhanced synthesis of VLDL in the liver, and then LDL in the vascular endothelium. Excess of TG is deposited in the form of fatty vacuoles in hepatocytes, which leads to the formation of fatty liver disease – steatosis [8]. As a result, lipid peroxidation processes are activated, proinflammatory cytokines are synthesized, and oxidative stress develops.

These processes may result in hepatic cytolysis, leading to the release of intracellular enzymes, such as ALT, AST, ALP, and LDH, and their accumulation in the blood. In our experiment, a statistically significant increase in ALT activity by 104.4%, 184.1%, 219.3% ($p < 0.05$) on day 21, 35, and 60 of the experiment, respectively, and a tendency toward elevation of AST, ALP, and LDH with a statistically significant increase by 27.8%, 204.7%, and 42.6%, respectively, by day 60 of the experiment were noted.

In the case of persistent damage, slowdown in regeneration and replacement of hepatocytes with an excess amount of extracellular matrix proteins were observed [2]. In addition, the excess of TG and TC following free radical oxidation might have a destructive effect on the vascular endothelium. Laminin, the basement membrane glycoprotein, may be one of the markers of ongoing pathological changes.

Laminin is a structural non-collagen glycoprotein of the basement membrane consisting of three short arms with globules and one long arm [9]. Each laminin chain consists of several domains on which active centers of interaction with various biologically active substances, such as type IV collagen, fibronectin, and nidogen, are located. Nidogen occupies a prominent place in the structure of the cellular matrix having a covalent bond to collagen and forming an insoluble, non-covalently bound complex with laminin. This complex is fixed with cells, which determines the main function of laminin as an adhesive protein of various epithelial and mesenchymal cells providing tissue resistance to stretching and affects cellular growth, morphology, differentiation, and mobility [10]. Therefore, when the cells are exposed to a damaging factor, the release of extracellular matrix components into the bloodstream is likely.

In the course of the experiment, an increase in the laminin content was observed already on day 21 of the experiment and by the end of the experiment (Fig. 1).



*– p <0.05 compared with the control group

Fig. 1. Changes in the concentration of laminin against the background of a high-fat diet relative to the control, %

The study of biochemical liver parameters against the background of sulodexide administration and a high-fat diet showed that the activity of the major enzymes of hepatic cytolysis decreased at the indicated points of the experiment, with the highest value on day 60 compared with similar parameters in the first group of animals (Fig. 2).

The hepatoprotective effect of the drug may also be associated with its hypolipidemic effect, an increase in the activity of lipoprotein lipase, and, therefore, with increased breakdown of TG in the composition of lipoproteins, and, as a consequence, with a decrease in the concentration of products of their peroxidation and restoration of hepatocytes. According to the results of the experiment, after administration of sulodexide, a tendency toward a decrease in the concentration of the studied lipid profile parameters was noted: TC decreased by 26%, HDL – by 1.4%, LDL – by 52%, and TG – by 26% on day 21 of the experiment. An even greater decrease was recorded on day 35 of the experiment: TC reduced by 37%, HDL – by 30%, LDL – by 18%, and TG – by 64% ($p < 0.05$) with a pronounced decrease by day 60 of the experiment (Fig. 3).

A decrease in the difference in these parameters against the background of sulodexide administration compared with the control values by the end of the experiment is worth noting (Fig. 4). The angioprotective effect of sulodexide associated with restoration of the structural and functional integrity of the vascular endothelium, normalization of negative electric charge density, and decreased basement membrane thickness and extracellular matrix production explains a decrease in the laminin concentration observed against the background of the diet and after administration of the drug by 26.3%, 22.6%, and 37.4% on day 21, 35, and 60 of the experiment, respectively (Fig. 5).

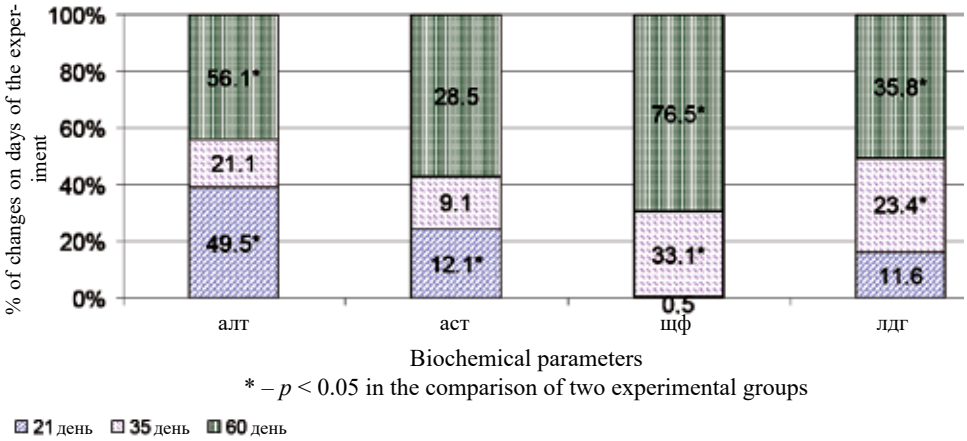


Fig. 2. Changes in the activity of enzymes involved in hepatic cytolysis under the conditions of a high-fat diet and sulodexide injections by days of the experiment, %

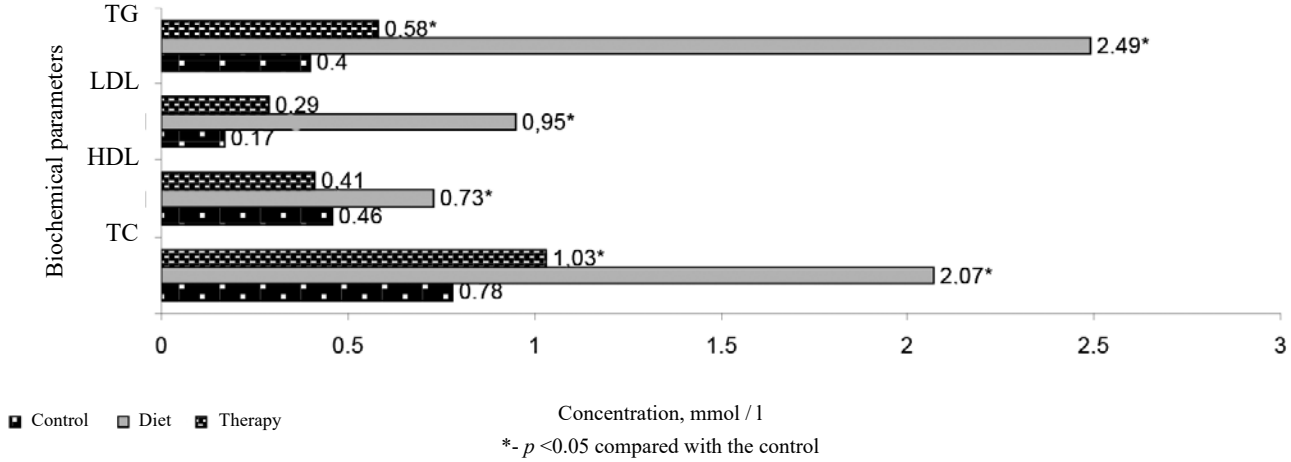


Fig. 3. Parameters of lipid metabolism under conditions of a high-fat diet and against the background of sulodexide administration by the end of the experiment

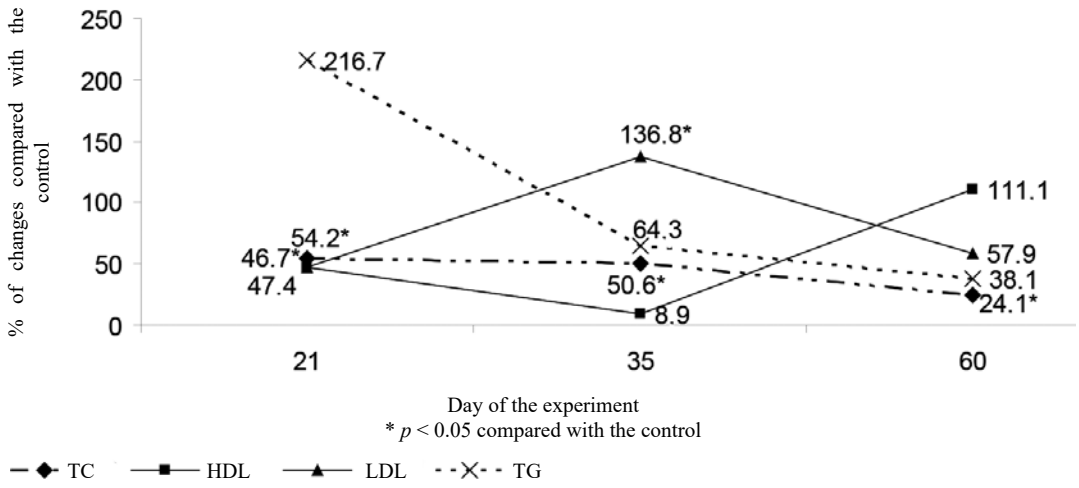


Fig. 4. Dynamics of the lipid profile parameters against the background of sulodexide administration relative to the control

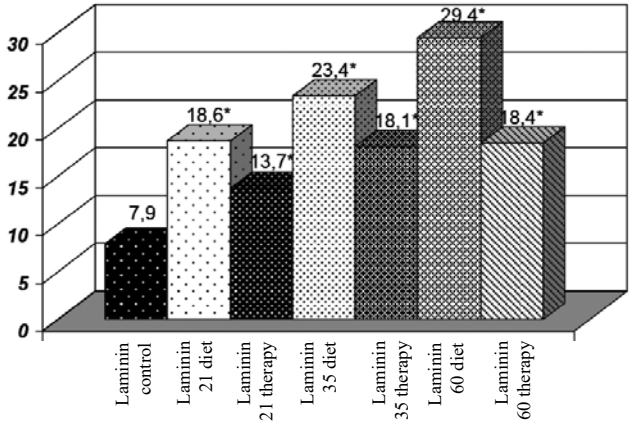


Fig. 5. Concentrations of laminin against the background of the diet and sulodexide administration: the abscissa is the time of the experiment, days; the axis of ordinates is the laminin concentration in the blood serum, ng / ml. * – $p < 0.05$ compared with the control. Due to a lack of statistically significant differences in the control values, the mean value for the control was calculated for all days of the experiment

The analysis of the data obtained allows to conclude that the administration of sulodexide may be reasonable for managing steatosis and liver fibrosis and restoring the integrity of the vascular endothelium, which is typical of a high-fat diet.

CONCLUSION

The results of the experiment indicate that a high-fat diet leads to a change in the concentration of lipid metabolism parameters in the blood serum of rats. This can initiate destructive processes in the liver cell membrane with the release of the main biochemical markers of hepatic cytolysis (ALT, AST, ALP, LDH) into the bloodstream, as well as endothelial dysfunction with an increase in the laminin level in the blood of animals.

The studied drug sulodexide has a rather pronounced hypolipidemic and angioprotective effect. It is able to reduce the degree of hepatic cytolysis due to the activation of lipolysis and is likely to restore the structure of the basement membrane in the vascular endothelium. This is one of the reasons for applying this drug in clinical practice.

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Study of the anti-inflammatory and immunotropic activity of the secretome from multipotent mesenchymal stromal cells induced by erythropoietin, valproic acid or dexamethasone *in vitro*

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ABSTRACT

The aim of this study was to evaluate the effect of treatment with valproic acid, erythropoietin, and dexamethasone on the anti-inflammatory and immunosuppressive activity of the secretome of adipose-derived multipotent mesenchymal stromal cells (MMSCs) in an *in vitro* experiment.

Materials and methods. MMSCs were isolated from the fat of 6 healthy donors. The cells were grown in the culture up to passage 4. Then they were treated with valproic acid, erythropoietin or dexamethasone for 3 hours, washed from preparations, and incubated in a serum-free medium for 48 hours. Some of the cells were not treated with preparations. Supernatants from the cell cultures were concentrated by ultrafiltration, and protein standardization was performed using a nanophotometer. Then the supernatants were sterilized and added to mononuclear cells from peripheral blood of 8 healthy donors. The mononuclear cells were isolated by Ficoll density gradient centrifugation according to the standard protocol. Concentrations of TNFα, IL-2, IL-4, IL-6, IL-10, and IFNγ cytokines in 24-hour cultures and IL-9, IL-10, IL-17A, and IL-21 cytokines in 48-hour cultures were determined using multiplex analysis.

Results. The production of IL-2, IL-6, TNFα, and IL-10 was reduced by the secretome of MMSCs treated with valproic acid. The production of IL-2, IL-6, and TNFα decreased during incubation of the mononuclear cells with the secretome of MMSCs treated with erythropoietin. The secretome of dexamethasone-treated MMSCs suppressed the production of IFNγ, IL-1β, IL-1ra, IL-2, IL-6, IL-9, IL-10, and IL-17A. No statistically significant differences were revealed in the production of IL-4, IL-5, IL-9, and IL-21.

Conclusion. Among the studied inducers, dexamethasone enhanced the anti-inflammatory and immunosuppressive activity of MMSCs the most, which was manifested through the effect of their supernatants on peripheral blood mononuclear cells.

Keywords: multipotent mesenchymal stromal cells, valproic acid, erythropoietin, dexamethasone, cytokines, inflammation

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

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Conformity with the principles of ethics. All study participants signed an informed consent. The study was approved by the Ethics Committee at the Belgorod State National Research University (Protocol No. 1 of 24.01.2020).

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Изучение противовоспалительной и иммунотропной активности секретома мультипотентных мезенхимальных стромальных клеток, индуцированных эритропоэтином, вальпроевой кислотой или дексаметазоном *in vitro*

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РЕЗЮМЕ

Цель. Исследовать влияние обработки вальпроевой кислотой, эритропоэтином и дексаметазоном на противовоспалительную и иммуносупрессивную активность секретома мультипотентных мезенхимальных стромальных клеток (ММСК) жировой ткани в эксперименте *in vitro*.

Материалы и методы. ММСК выделяли из жира шести здоровых доноров. Клетки растили в культуре до четвертого пассажа, затем обрабатывали вальпроевой кислотой, эритропоэтином или дексаметазоном в течение 3 ч, отмывали от препаратов и инкубировали в бессывороточной среде в течение 48 ч. Часть клеток не обрабатывали препаратами. Супернатанты от культур клеток сконцентрировали ультрафильтрацией, стандартизировали по содержанию белка с помощью нанофотометра, стерилизовали и добавляли к мононуклеарам из периферической крови восьми здоровых доноров. Мононуклеары выделяли в градиенте плотности фиколла по стандартному протоколу. Концентрации цитокинов фактора некроза опухоли альфа (TNFα), интерлейкина (IL) -2, IL-4, IL-6, IL-10, интерферона гамма (IFNγ) в суточных культурах и IL-9, IL-10, IL-17A, IL-21 в 48-часовых культурах определяли с помощью мультиплексного анализа.

Результаты. Продукция IL-2, IL-6, TNFα, IL-10 снижается под действием секретома от обработанных вальпроевой кислотой ММСК. Продукция IL-2, IL-6, TNFα уменьшается при инкубации мононуклеаров с секретомом клеток, обработанных эритпропоэтином. Секретом обработанных дексаметазоном ММСК подавляет продукцию IFNγ, IL-1β, IL-1ra, IL-2, IL-6, IL-9, IL-10, IL-17A. Статистически значимых различий по изменению продукции IL-4, IL-5, IL-9, IL-21 не выявлено.

Заключение. Среди изученных индукторов дексаметазон показал себя более активным в усилении противовоспалительной и иммуносупрессивной активности ММСК, выраженной через влияние их супернатантов на мононуклеары периферической крови.

Ключевые слова: мультипотентные мезенхимальные стромальные клетки, вальпроевая кислота, эритропоэтин, дексаметазон, цитокины, воспаление

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют о финансировании со стороны ООО «Инновационный центр «Бирюч – новые технологии», ООО «Центр клеточных технологий Бирюч» и программы «У.М.Н.И.К.».

Соответствие принципам этики. Все участники исследования подписали информированное согласие. Исследование одобрено этическим комитетом Белгородского государственного национального исследовательского университета (протокол № 1 от 24.01.2020).

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типотентных мезенхимальных стромальных клеток, индуцированных эритропоэтином, вальпроевой кислотой или дексаметазоном in vitro. *Бюллетень сибирской медицины.* 2022;21(1):29–35. https://doi.org/10.20538/1682-0363-2022-1-29-35.

INTRODUCTION

The anti-inflammatory activity of multipotent mesenchymal stromal cells (MMSCs) has been confirmed by numerous studies. Currently, much attention is paid to the regulatory effects of MMSCs, which are determined by the action of biologically active substances in their secretome. Thus, the secretome can be an effective alternative to the use of stem cells and is a good basis for development of innovative drugs. However, the possibility of changing the functional activity of MMSCs and, hence, the composition of their secretome after pharmacological stimulation with erythropoietin (EPO), valproic acid (VA), and dexamethasone (DEX), has not yet been realized in the world [1]. There is evidence that EPO increases the survival rate of MMSCs when they are injected together. EPO also reduces the inflammatory microenvironment of diabetic foot ulcers. The mechanism consists in inhibition of the release of the proinflammatory cytokine tumor necrosis factor-α (TNF-α) by the cells [2].

Therefore, EPO is a potential regulator of the functional activity of cells with receptors for it, including stromal cells [2], however, the exact effects remain poorly studied. It has been shown that the use of VA in therapy inhibits proliferation and differentiation of MSCs, as well as release of proinflammatory cytokines [3]. With the administration of lipopolysaccharide (LPS) and VA to dogs, VA has been shown to reduce the production of proinflammatory cytokines. It is known to increase the anti-inflammatory activity of embryonic fibroblasts under the influence of DEX [4].

The aim of the research was to study the possibility of changing the anti-inflammatory and immunosuppressive activity of the secretome of adipose-derived MMSCs after treatment with VA, EPO, and DEX *in vitro*.

MATERIALS AND METHODS

All individuals included in the study signed an informed consent. The study was approved by the Ethics Committee at Belgorod State National Research University (protocol No. 1 of 24.01.2020). Mononuclear cells (MNCs) from peripheral blood of 8 healthy volunteers were isolated by Ficoll density gradient centrifugation. MMSCs were isolated from the adipose tissue of 6 donors using collagenase type II. MMSCs were grown up to passage 4 in the Minimum Essential Medium (MEM)-**α** with 10% fetal bovine serum under standard conditions of a gas incubator (humid atmosphere, 5% CO_2, 37 °C). Then the cells were seeded in culture plates and treated with 1 IU / ml of EPO (Sandoz, Slovenia), 20 **μ**g / ml of VA (Merck, USA) or 10 **μ**mol / ml of DEX (CSPC Ouyi Pharmaceutical, China) for 3 h. The cells were washed from the preparations and incubated in a serum-free medium for 48 h under standard conditions.

Some of the MMSCs were not treated with any of the pharmacological agents. Before adding to the culture plate, supernatants from MMSC cultures were concentrated using Vivaspin 15 R centrifugal concentrators (Sartorius, Germany) with molecular weight cut-off (MWCO) of 3 kDa, standardized for the total protein content (1 mg / ml) using a nanophotometer (Implen, Germany), sterilized by filtration, and then added to the plates with MNCs. To stimulate MNCs, 10 μg / ml of phytohemagglutinin (PanEco, Russia) and 100 ng / ml of LPS (Merk, USA) were added. After 24 h and 48 h, the plate was centrifuged, and the supernatant was taken for the study of cytokine concentrations. Concentrations of cytokines TNF-α, interleukin (IL)-2, IL-4, IL-6, IL-10, and interferon gamma (IFN-**γ**) in daily cultures were determined using Bio-Plex Pro Human Cytokine 8-Plex and Human Ultrasensitive Cytokine Magnetic 10-Plex Panels (Bio-Rad, Invitrogen, USA). Concentrations of IL-9, IL-10, IL-17A, and IL-21 in 48-hour cultures were determined using the Th9/Th17/Th22 Cytokine 7-Plex Human ProcartaPlex Panel (Invitrogen, USA) according to the instructions on the MAGPIX multiplex reader (Luminex, USA). The concentration of cytokines produced by stimulated MNCs was taken as a control. To confirm the MMSC phenotype, the cells were stained with antibodies to CD105, CD90, CD73, CD31, CD45, and CD34 (BD, Beckman Coulter, USA). Mouse IgG1 conjugated with BV 421 (BD, USA) was used as an isotype control. The expression of markers was determined on

the FACSCanto II flow cytometer with BD FACSDiva software (BD, USA).

Statistical processing of the results was carried out using the SPSS Statistics 17.0 software (IBM, USA). The data obtained were checked for normal distribution using the Shapiro – Wilk test. Descriptive statistics were represented by the median and the interquartile range *Me* $[Q_1–Q_3]$. Statistically significant differences were calculated using the Mann – Whitney U test and the Kruskal – Wallis test; the differences were considered statistically significant at $p < 0.05$.

RESULTS

The secretome from DEX-stimulated MMSCs significantly ($p < 0.05$) reduced the production of the regulatory cytokine IFN-γ (Table). Incubation of MNCs with the secretome from MMSCs treated with EPO and VA did not reveal a statistically significant decrease in the level of this cytokine ($p > 0.05$). A decrease in the concentration of IL-2 under the influence of VA, EPO, DEX, and joint incubation with the MMSC secretome was significant in all cases, regardless of the presence or absence of preliminary MMSC treatment (Table). However, the greatest effect in reducing the production of this cytokine was shown by the secretome from DEX-treated MMSCs ($p < 0.05$). The secretome from DEX-treated MMSCs contributed to a decrease in the production of IL-4 and IL-5 by 1.3 and 1.6 times, respectively ($p > 0.05$). A tendency toward suppression of IL-21 production was also noted ($p > 0.05$). The production of proinflammatory cytokines decreased after incubation with the secretome from DEX-treated MMSCs: TNF-α – by 6.0 times, IL-1β – by 2.5 times, IL-6 – by 1.5 times, IL-9 – by 5.6 times, and IL-17A – by 3.2 times (Table, $p < 0.05$).

A pairwise analysis revealed differences between the decrease in TNF-α, IL-1β, and IL-17A after the administration of the secretome from DEX-treated MMSCs and secretomes from native MMSCs and those treated with EPO and VA ($p < 0.05$). A decrease in the IL-6 concentration under the influence of all secretomes was statistically significantly smaller ($p < 0.05$) than in the control. However, the pairwise analysis did not reveal any differences in the co-incubation of secretomes from stimulated and native MMSCs. A decrease in the production of anti-inflammatory cytokines IL-1ra and IL-10 (Table) after treatment of the cells with EPO and DEX was noted. This effect was especially pronounced after exposure of MNCs to the secretome of DEX-treated MMSCs ($p < 0.05$).

DISCUSSION

A statistically significant decrease in IFNγ was observed upon incubation of MNCs together with the secretome from DEX-treated MMSCs ($p < 0.05$), which is consistent with the data on the decrease in IFNγ by glucocorticoids and the effect of MMSCs in treatment of imiquimod-induced psoriasis [5]. IL-2 produced by Th1 lymphocytes decreased upon incubation with the secretome from MMSCs treated with DEX ($p < 0.05$). There are conflicting literature data that glucocorticoids have a positive effect on the production of IL-4, IL-10, and IL-13 by Th2 cells, causing a shift toward humoral immunity without immunosuppression. However, most scientific works have shown that synthesis of IgE *in vivo* is declining [6]. The biological functions of IL-21 include induction of an inflammatory T-cell response and suppression of IgE production. It has been shown that treatment of thrombocytopenia with high doses of DEX leads to a decrease in IL-21 [7]. We revealed only a tendency toward a decrease in IL-21 production under the influence of MNC incubation with the secretome from MMSCs. Confirmation of this phenomenon, along with the revealed trend toward a decrease in IL-4 and IL-5, can be used in correction of IgE-related diseases.

IL-1β was significantly reduced after exposure to the secretome from MMSCs treated with DEX. It is known that there is no significant effect of VA on the production of this cytokine [8], while it moderately increases with the addition of EPO [9]. IL-6 decreased after pharmacological treatment of cells, which is consistent with the article [8].

Recent data confirm that Th1, Th2, and Th17 cells have different sensitivity to glucocorticoids [10, 11]. It has been shown that Th1- and Th17-related cytokines are involved in the development of systemic sclerosis [4], and increased expression of IL-17A is observed during the development of inflammation in psoriasis [5]. The authors demonstrated a significant decrease in the production of IL-1β, IL-6, IL-10, IFNγ, TNF, and IL-17A by *in vitro* exposure of MNCs from patients with systemic sclerosis to DEX [4]. The revealed decrease in IL-17A after exposure to the secretome of DEX-treated MMSCs may be used in treatment of systemic sclerosis and psoriasis.

In addition, a tendency toward a decrease in IL-17A under the influence of the secretome from EPO-treated MMSCs was revealed, which can be potentially used in chronic inflammatory diseases, such as colitis [12]. It was shown that the DEX-induced decrease in

Table

Concentration of cytokines produced by different groups of cells (pg / ml), *Me* [Q_1–Q_3]

Cells	Cytokine concentration, pg / mL										
	IFNγ	IL-2	IL-4	IL-5	IL-21	TNFα	IL-1β	IL-6	IL-9	IL-17A	IL-1ra	IL-10
MNC	3.4 [2.3–4.5]*	159 [150.6–171.6]*	187 [155.7–229.2]*	70.7 [33.2–120.7]	1.8 [1.8–1.8]*	45.0 [25.2–65.8]	17.4 [12.2–23.6]*	18186.7 [18111.7–18286.7]	0.1 [0.1–0.3]*	1.6 [0.4–1.6]*	1314.2 [1309–1319.3]	11.7 [7.4–14.4]
MNC stim.	2067.5 [837.6–2737.7]	1330.4 [1255.4–1430.4]	445.8 [370.8–545.8]	141.3 [66.3–241.3]	53.3 [52.1–80.6]	3961.2 [3886.2–4061.2]	9880.1 [8479.8–13256.2]	22955.0 [22820.7–23041.2]	45.3 [23.5–153.7]	308.3 [219.3–698.4]	3996.5 [2821.7–4730.5]	6017 [4495.2–7995.4]
MMSC stim.	103.7 [76.1–122.4]*	75.8 [29.4–84.4]*	98.8 [52.8–127.9]*	34.8 [14.4–47]	23.6 [18.2–24.3]*	40.8 [24.4–62.3]*	185.1 [64.8–329.3]*	6271.9 [1147–7513.4]*	6.4 [5–6.9]*	32.8 [28.1–39.1]*	13.7 [9.9–44.5]*	22.6 [5.7–73.9]*
MMSC + MNC stim.	1567.9 [865–2120.4]	624.3 [616.8–809.8]*	319.6 [312.1–397.9]	137.9 [129.2–167.4]	37.9 [12.6–75.7]	2254.7 [1638–2272.5]*	7848.6 [6251–12121.9]	14703.6 [14380.4–15615.5]*	33.0 [20–99.7]	335.3 [328.7–702.8]	4191.7 [3170.8–4918.3]	5522.9 [4074.3–8209]
MMSC + EPO + MNC stim.	1421.2 [1379–1634.7]	731.8 [720.6–838.4]*	386.1 [361.8–390.7]	144.7 [143.3–154.7]	36.4 [35.1–49.5]	1985.6 [1895.4–2297.6]*	7015.0 [6092.2–7050.6]	14304.2 [14072.9–15377.4]*	15.3 [8.2–24.3]	243.9 [51.8–1089.8]	3659 [2561.3–4317.6]	4618.7 [4125.6–5603.2]
MMSC + DEX + MNC stim.	654.9 [203.5–2036.6]*	252.5 [248.7–264.5]*	331 [245.7–350.2]	80.5 [74.3–86.6]	37.4 [10.4–134.2]	657.1 [652.8–685.8]*⊗	3970.8 [1034.7–4806.9]*⊗	11886.7 [11792.4–15446.3]*	8.1 [5.5–9.8]*	96.6 [65.5–261.3 7]*⊗	1924.4 [1312.1–2169.3]*	2457.4 [2085.2–2984]*
MMSC + VA + MNC stim.	1371.8 [1157.3–1577.2]	756.8 [680.1–798.4]*	353.8 [352.3–374.7]	158.9 [132.5–174.3]	37 [34.6–74.7]	2389.5 [1897.1–2449.1]*	6281.3 [6178.3–6387.6]	14755.9 [12630.9–14819.1]*	17.0 [11.7–25.7]	400.7 [146.7–552.3]	4303.5 [3012.5–078.1]	4235.7 [3710.2–4764.3]*

Note: * – statistical significance of the difference in comparison with MNC stim. ($p < 0.05$), ⊗ – statistical significance of the difference in comparison with parameters of other secretomes ($p < 0.05$).

TNF-α was less pronounced after high doses of LPS than after 0.1 ng of LPS. On the contrary, the effect of DEX on IL-10 secretion is biphasic: stimulation at lower doses of LPS and inhibition at higher doses of LPS [13]. We used 10 μmol / ml of DEX and 100 ng / ml of LPS, which is 10 times more than in the cited source, but the effects turned out to be natural: a decrease in the production of TNFα and IL-10. This suggests that the secretome from DEX-treated MMSCs has a more pronounced anti-inflammatory effect than that of the native MMSCs.

Glucocorticoids are known to reduce the production of IL-1β. In this study, the greatest decrease in the production of IL-1β and TNFα was observed under the effect of the secretome from DEX-treated MMSCs ($p < 0.05$), which shows the ability of glucocorticoids to exert an indirect anti-inflammatory effect through the influence on stromal cells. It is known that under the influence of DEX, in the blood serum of patients with thrombocytopenia, the production of TNFα significantly decreases [7], and in patients with Crohn's disease, TNFα and IL-6 decrease and genes associated with phagocytosis are inhibited, which can lead to persistent infection [14]. It may be possible to use glucocorticoids to suppress synthesis of proinflammatory cytokines indirectly, using DEX-treated MMSCs and their secretome, avoiding the increased risk of developing infectious diseases. A decrease in production of IL-9, one of the factors in mast cell differentiation, under the effect of the secretome from DEX-treated MMSCs, can be used in allergic and autoimmune inflammation.

Scientists have shown that bone marrow MMSCs reduce the production of IL-9 by MNCs in patients with rheumatoid arthritis *in vitro* [14]. There is evidence that EPO has no effect on IL-10 production [9] and exerts a suppressive effect on TNFα synthesis [6]. This is consistent with our data: a significant decrease in TNFα under the effect of EPO and a tendency toward IL-10 suppression. It has been shown that DEX reduces IL-10 production by LPS-stimulated MNCs [6]. In this study, the level of anti-inflammatory cytokines produced by MNCs under the effect of EPO and the secretome from EPO-treated MMSCs decreased ($p > 0.05$). This effect can be studied in more detail and, if confirmed, used in therapy. As shown in the article [13], IL-1ra production is reduced by DEX, which is consistent with our data. However, this effect is stronger when using the secretome of DEX-treated MMSCs, which suggests possible limitations of its anti-inflammatory effect with long-term use.

CONCLUSION

Significant suppression of IL-2, IL-6, and TNFα production under the effect of secretomes from MMSCs previously stimulated by EPO and VA was shown. However, this effect was more pronounced when cells were treated with DEX. An increase in the anti-inflammatory and immunosuppressive activity of the secretome from MMSCs after preliminary stimulation with DEX at a concentration of 10^{-5} mol / l was found. This effect was observed in the form of a decrease in the production of regulatory cytokines IFNγ and IL-2 and proinflammatory cytokines TNF-α, IL-1β, IL-6, IL-9, and IL-17A by MNCs under the effect of this MMSC secretome. A significant decrease in the IL-10 and IL-1ra production suggests possible limitations in long-term therapy for inflammatory diseases using the studied secretome.

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Authors contribution

Golubinskaya P.A. – carrying out of experiments, analysis and interpretation of data, drafting of the article. Puzanov M.V. – critical revision of the manuscript for important intellectual content. Sarycheva M.V. – analysis and interpretation of data. Burda S.Yu. – analysis and interpretation of data, drafting of the article. Nadezhdin S.V. – provision of methodological consultation, critical revision of the manuscript for important intellectual content. Korokin M.V. – research leadership, final approval of the manuscript for publication. Burda Yu.E. – conception and design, control over the quality of research results.

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Effect of β-blocker therapy on the level of soluble ST2 protein in the blood serum in patients with heart failure with preserved and mildly reduced ejection fraction

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ABSTRACT

Aim. To study the prognostic value of high serum concentration of soluble ST2 protein (sST2) in the development of cardiovascular events after endovascular myocardial revascularization and the possibility of using this biomarker as a target for β-blocker therapy in patients with chronic heart failure (CHF) with preserved (HFpEF) and mildly reduced (HFmrEF) left ventricular ejection fraction.

Materials and methods. The study included 72 patients (aged 57–69 years, 81.94% were men) with class I–III CHF of ischemic etiology with HFpEF and HFmrEF. The patients were admitted to the cardiology department for endovascular myocardial revascularization. Before myocardial revascularization, serum concentrations of sST2 and N-terminal pro-brain natriuretic peptide (NT-proBNP) in all patients were analyzed by enzyme-linked immunosorbent assay (ELISA). Doses of β-blockers used in all patients were recalculated into a total daily dose equivalent to metoprolol succinate. Patients were divided into 2 groups depending on the median equivalent dose of metoprolol succinate ("high" ≥ 100 mg / day and "low" < 100 mg / day).

Results. In patients of group 1, the serum concentration of sST2 was 30.7% higher ($p < 0.001$) than in patients of group 2 (40.26 [34.39; 48.92] ng /ml and 27.9 [23.05; 35.27] ng / ml, respectively), the serum NT-proBNP level in group 1 was 22.8% higher ($p = 0.049$) than in group 2 (167 [129; 330] ng / ml vs. 129 [125; 147] ng / ml, respectively). In patients receiving an equivalent dose of metoprolol succinate < 100 mg / day, the incidence of cardiovascular events was 34% higher ($p = 0.002$) than in patients receiving an equivalent dose of metoprolol succinate ≥ 100 mg/day. The ROC analysis showed that serum sST2 level ≥ 34.18 ng / ml (sensitivity 78.0%, specificity 90.0%, area under the curve (AUC) 0.906; $p < 0.0001$) predicts a high risk of cardiovascular events within one year. However, the serum NT-proBNP level was not an informative predictor of cardiovascular events.

Conclusion. It was confirmed that increased sST2 serum concentration has high prognostic value in the development of cardiovascular events within a year after endovascular myocardial revascularization. The possibility of using this biomarker as a target for β-blocker therapy in patients with HFpHF and HFmrEF was substantiated. Aggressive use of β-blockers in the group of patients with HFpEF and HFmrEF and sST2 overexpression is preferable in order to reduce the incidence of cardiovascular events.

Keywords: chronic heart failure, left ventricle, preserved and mildly reduced ejection fraction, β-blockers, biomarkers, soluble ST2, N-terminal pro-brain natriuretic peptide, prognosis, endovascular revascularization

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

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Влияние терапии β-адреноблокаторами на уровень растворимой формы белка st2 в сыворотке крови пациентов с сердечной недостаточностью с сохраненной и умеренно сниженной фракцией выброса

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РЕЗЮМЕ

Цель – изучение прогностического значения высокой концентрации в сыворотке крови растворимой формы белка ST2 (sST2) в развитии сердечно-сосудистых событий после эндоваскулярной реваскуляризации миокарда и возможности использования этого биомаркера в качестве мишени для терапии β-блокаторами у пациентов с хронической сердечной недостаточностью (ХСН) с сохраненной (СНсФВ) и умеренно сниженной (СНусФВ) фракцией выброса левого желудочка.

Материалы и методы. В исследование включены 72 пациента (в возрасте 57–69 лет, 81,94% мужчин) с ХСН I–III функционального класса ишемической этиологии с СНсФВ и СНусФВ, госпитализированных в кардиологическую клинику для выполнения эндоваскулярной реваскуляризации ишемизированного миокарда. У всех пациентов перед реваскуляризацией миокарда анализировали концентрацию в сыворотке крови sST2 и N-терминального промозгового натрийуретического пептида (NT-proBNP) с помощью иммуноферментного анализа (ELISA). Дозы применяемых у всех пациентов β-блокаторов были пересчитаны в общую суточную дозу, эквивалентную метопрололу сукцинату. Больные были разделены на две группы в зависимости от медианы эквивалентной дозы β-блокатора метопролола сукцината («высокая» ≥100 мг/сут и «низкая» <100 мг/сут).

Результаты. У пациентов первой группы сывороточная концентрация sST2 была на 30,7% ($p < 0,001$) больше, чем у больных, вошедших во вторую группу (40,26 [34,39; 48,92] нг/мл и 27,9 [23,05; 35,27] нг/мл соответственно), уровень NT-proBNP в сыворотке крови больных первой группы также был выше (на 22,8%; $p = 0,049$), чем у пациентов второй группы (167 [129; 330] нг/мл против 129 [125; 147] нг/мл соответственно). У пациентов, получавших эквивалентную дозу метопролола сукцината <100 мг/сут, частота сердечно-сосудистых событий была выше на 34% ($p = 0,002$), чем у пациентов, получавших эквивалентную дозу метопролола сукцината ≥ 100 мг/сут. По данным ROC-анализа установлено, что сывороточный уровень sST2 ≥ 34,18 нг/мл (чувствительность 78,0%, специфичность 90,0%, AUC 0,906; $p < 0,0001$) позволяет прогнозировать высокий риск развития сердечно-сосудистых событий в течение ближайшего года. Уровень NT-proBNP в сыворотке крови при этом не являлся информативным предиктором сердечно-сосудистых событий.

Заключение. Подтверждено высокое прогностическое значение повышения концентрации в сыворотке крови sST2 в развитии сердечно-сосудистых событий в течение года после эндоваскулярной

реваскуляризации миокарда и обоснована возможность использования этого биомаркера в качестве мишени для терапии β-блокаторами у пациентов с СНсФВ и СНусФВ. Агрессивное применение β-блокаторов в группе пациентов с СНсФВ и СНусФВ и гиперэкспрессий sST2 предпочтительнее с целью снижения частоты сердечно-сосудистых событий.

Ключевые слова: хроническая сердечная недостаточность, левый желудочек, сохраненная и умеренно сниженная фракция выброса, β-блокаторы, биомаркеры, растворимый ST2, N-терминальный промозговой натрийуретический пептид, прогноз, эндоваскулярная реваскуляризация

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Chronic heart failure (CHF) is a serious public health problem with the prevalence of 5.8 to 6.5 mln individuals in the United States, about 8.1 mln individuals in the Russian Federation, and 26 mln people worldwide [1–3]. Despite the advances made in the study of the pathogenesis, course features, clinical manifestations, and treatment methods, the prognosis in CHF patients remains unfavorable [4, 5]. One of the key indicators of the myocardial dysfunction severity in CHF patients is the value of the left ventricular ejection fraction (LVEF), which characterizes its contractility [6].

Based on the data of large epidemiological studies of the last few decades, scientists have come to the conclusion that CHF can also develop in preserved LVEF. CHF with preserved ejection fraction (HFpEF) is detected in about half of all patients with heart failure – they account for 51–63% of the general population [7, 8]. Since life expectancy in economically developed countries tends to increase, the prevalence of HFpEF will continue to grow. Epidemiological data from population-based studies in the United States show that if current trends continue, 8.5 mln Americans will be diagnosed with HFpEF by 2030, with about 70% of them being over 65 years old (6 mln) [9]. Over the past decade, the rate of increase in HFpEF incidence has grown on average by 10–20% relative to the same indicator for heart failure with reduced ejection fraction (HFrEF) [10–14].

Beta-blockers (β-blockers) are a class of drugs used to control CHF symptoms and improve survival, especially in patients with left ventricular (LV) systolic dysfunction [15]. Co-administration of this group of drugs with other drugs that are commonly used to treat CHF, such as angiotensin-converting enzyme (ACE) inhibitors, diuretics, angiotensin II receptor blockers (ARBs), and mineralocorticoid receptor antagonists, regresses LV remodeling and decelerates the progression of systolic CHF [15, 16]. In theory, β-blockers can also be used to treat patients with diastolic CHF, which is characterized by an increase in LV myocardial stiffness. In this case delayed or incomplete ventricular relaxation leads to slowdown in diastolic filling and an increase in ventricular filling pressure. The LV filling is never complete during diastole, although the pumping function remains preserved [17]. Considering the fact that diastolic dysfunction and LV remodeling also play a key role in the mechanisms of CHF progression up to the terminal stage of heart disease and death [18, 19], there is every reason to expect that the use of β-blockers in patients with preserved LVEF will lead to a decrease in CHF manifestations and symptoms and better survival. It is possible due to a decrease in heart rate and an increase in the time for a more complete LV filling and, as a consequence, an increase in blood volume during diastole [20]. However, now there is no con-

vincing evidence that the use of β-blockers in CHF patients with HFpEF and HFmrEF is effective.

Currently, soluble ST2 protein (sST2) is a new biomarker. Along with natriuretic peptides, it plays an important role in the mechanisms (cardiomyocyte and interstitial remodeling of the heart, myocardial dysfunction, and cardiomyocyte apoptosis, etc.) of cardiovascular diseases [21–23]. In contrast to N-terminal pro-brain natriuretic peptide (NT-proBNP), synthesis and secretion of which is determined by increased stretching of cardiomyocytes, the sST2 level also reflects the activity of inflammatory and fibrotic processes in the cardiac muscle tissue [23, 24], which may be more useful for risk stratification and monitoring of treatment efficacy in patients with HFpEF. There is evidence that the dynamic changes in ST2 concentrations during CHF treatment correlate with the frequency of long-term outcomes [25]. Therefore, it can be assumed that comprehensive treatment that slows down LV remodeling or provides its "reverse" remodeling, which ultimately leads to a decrease in the incidence of cardiovascular complications, will significantly improve the prognosis for HFpEF patients with an initially elevated sST2 level [18].

The aim of the study was to evaluate the prognostic value of high serum concentration of sST2 in the development of cardiovascular events after myocardial revascularization and the possibility of using this biomarker as a target for β-blocker therapy in patients with HFpEF and HFmrEF.

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local Ethics Committee at Cardiology Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences. All patients signed an informed consent to participate in the study.

This study was a prospective, observational, single-center study. The study included 72 patients (aged 57–69 years, 81.9% were men) with CHF of ischemic etiology corresponding to functional class (FC) I–III according to the classification of the New York Heart Association (NYHA), with preserved and mildly reduced LVEF. Patients were admitted to hospital for endovascular revascularization (Table 1).

Table 1

Clinical and demographic characteristics of patients at the time of inclusion in the study

Parameter	Group 1, $n = 40$ BB ≥100 mg / day	Group 2, $n = 32$ BB <100 mg / day	p
Age, years, *Me* (Q_{25}; Q_{75}) Men, *n* (%)	62 (57; 69) 32 (80.0)	61.5 (53.5; 68.5) 27 (84.4)	0.426 0.631
CHF duration, months, *Me* (Q_{25}; Q_{75})	12 (6; 17)	11 (7; 18)	0.374
IHD duration, years, *Me* (Q_{25}; Q_{75})	5 (2; 11)	5 (2; 10)	0.861
6-minute walk test, m, *Me* (Q_{25}; Q_{75})	335 (275; 385)	300 (225; 385)	0.439
FC of CHF by NYHA, *n* (%) I II III	 2 (5.0) 29 (72.5) 9 (22.5)	 3 (9.4) 17 (53.1) 12 (37.5)	 0.835 0.089 0.056
GFR, ml / min/1.73 m^2, *Me* (Q_{25}; Q_{75})	42.4 (29.3; 59)	77 (72; 87)	0.492
Body mass index, *Me* (Q_{25}; Q_{75})	27.1 (24.9; 31.0)	28.8 (25.9; 30.9)	0.439
Hypertension, *n* (%)	34 (85.0)	26 (81.2)	0.778
Type 2 diabetes mellitus, *n* (%)	8 (20.0)	5 (15.6)	0.631
COPD, *n* (%)	3 (7.5)	2 (6.2)	0.872
Atrial fibrillation, *n* (%)	6 (15.0)	2 (6.2)	0.665
History of myocardial revascularization, *n* (%)	23 (57.5)	20 (62.5)	0.667
Systolic blood pressure, mm Hg, *Me* (Q_{25}; Q_{75}) Diastolic blood pressure, mm Hg, *Me* (Q_{25}; Q_{75}) Heart rate, bpm, *Me* (Q_{25}; Q_{75})	120 (120; 130) 80 (70; 80) 61 (55; 67)	120 (110; 130) 80 (80; 80) 66 (61; 82)	0.779 0.624 0.061
Smoking, *n* (%)	12 (30.0)	4 (12.5)	0.327
History of acute CVA, *n* (%)	6 (15.0)	3 (9.4)	0.331

Table 1 (continued)

Parameter	Group 1, n = 40 BB ≥100 mg / day	Group 2, n = 32 BB <100 mg / day	p
Family history of CVD, n (%)	7 (17.5)	5 (15.6)	0.823
Therapy, n (%)			
ACE inhibitors or ARBs	36 (90.0)	27 (84.4)	0.435
Spironolactone / eplerenone	11 (27.5)	9 (28.1)	0.471
Loop diuretics	13 (32.5)	8 (25.0)	0.660
Amiodarone	4 (10.0)	2 (6.2)	0.675
Statins	38 (95.0)	29 (90.6)	0.912
Total cholesterol, mmol / l	4.65 (3.67; 5.25)	4.65 (3.67; 5.11)	0.932
LDL, mmol / l	3.03 (1.95; 3.41)	2.49 (2.25; 3.43)	0.856
HDL, mmol / l	1.07 (0.85; 1.31)	1.06 (0.96; 1.26)	0.889
Triacylglycerols, mmol / l	1.44 (1.13; 1.93)	1.67 (1.22; 1.92)	0.870
Hemoglobin, g / l	142 (131; 153)	147 (138; 152)	0.464
hsCRP, mg / l	6 (5; 7)	6 (4; 7)	0.596
HbA1c, %	4.8 (4.5; 6.6)	5.1 (4.7; 6.7)	0.445
sST2, ng / ml	27.9 (23.05; 35.27)	40.26 (34.39; 48.92)	<0.001
NT-proBNP, pg /l	129 (125; 147)	167 (129; 330)	0.049

Note: HbA1c – glycated hemoglobin; NT-proBNP – N-terminal pro-brain natriuretic peptide; NYHA – New York Heart Association; sST2 – soluble ST2; BP – blood pressure; BB – β-blockers, ARBs – angiotensin-II receptor blockers; hsCRP – highly sensitive C-reactive protein; CHF – chronic heart failure; ACE inhibitors – angiotensin-converting enzyme inhibitors; IHD – ischemic heart disease; HDL – high-density lipoproteins; LDL – low-density lipoproteins; GFR – glomerular filtration rate; HR – heart rate; COPD – chronic obstructive pulmonary disease; FC – functional class; CVD – cardiovascular disease.

According to modern criteria, CHF with preserved and mildly reduced LVEF was diagnosed in the presence of signs and / or symptoms of heart failure, preserved LV systolic function (LVEF ≥ 40%), NT-proBNP level ≥125 pg / ml, as well as signs of LV diastolic dysfunction [26].

Exclusion criteria were age older than 75 years, GFR lower than 50 ml / min / 1.73 m^2 (CKD-EPI equation), bronchial asthma and severe chronic obstructive pulmonary disease, autoimmune diseases, pregnancy, malignant neoplasms, less than six months after acute coronary or cerebrovascular events or failure to sign an informed consent form.

All patients received standard treatment and were followed up for 12 months after myocardial revascularization. Patients were divided into 2 groups depending on the median equivalent dose of the β-blocker metoprolol succinate ("high" ≥ 100 mg / day and "low" < 100 mg / day). Doses of β-blockers used in all patients were converted into a total daily dose equivalent to controlled-release metoprolol succinate (in accordance with the data of the PROTECT study) in the following ratios: immediate-release metoprolol tartrate, carvedilol × 4, bisoprolol × 20, propranolol × 0.833, and sotalol × 1.2. Group 1 included 40 patients who received an equivalent dose of metoprolol succinate ≥ 100 mg / day, group 2 included 32 patients who received < 100 mg / day (Table 1).

The primary composite endpoint was considered a set of events: cardiovascular death, fatal or non-fatal stroke, any coronary event (sudden cardiac death, fatal or non-fatal myocardial infarction, myocardial revascularization or hospitalization for unstable angina), aggravation of CHF (appearance of new symptoms / signs or progression of symptoms / signs requiring unplanned intensification of diuretic therapy or hospitalization). In total, two deaths were recorded: in the first case – due to acute myocardial infarction 11 months after revascularization, in the second case – in the postoperative period, one month after coronary artery bypass grafting performed due to the CHF progression.

Blood samples were obtained by venipuncture from 8 AM to 9 AM and the corresponding blood serum samples after centrifugation were stored at –24 ° C with one freeze – thaw cycle. Serum sST2 and NT-proBNP levels were analyzed from the same blood sample by enzyme-linked immunosorbent assay (ELISA) prior to myocardial revascularization. Soluble ST2 was measured using a high-

ly sensitive monoclonal sandwich immunoassay (Presage® ST2 assay, Critical Diagnostics, USA). NT-proBNP levels were determined using a sandwich immunoassay (Biomedica, Austria).

Statistical processing of the study results was carried out using the STATISTICA 10.0 (StatSoft, Inc., USA) and MedCalc 11.5.0.0 (MedCalc Software Ltd, USA) programs. To test statistical hypotheses when comparing two independent groups, the Mann – Whitney U test was used. The Wilcoxon W test and the sign test were used to compare two dependent variables. When analyzing qualitative features, contingency tables were analyzed using the Pearson's χ^2 test or the Fisher's exact test, when the expected value in any of the table cells with specified boundaries was below 10. A ROC analysis was performed to identify predictors of unfavorable cardiovascular events. The characteristic curves were constructed and the area under the curve (AUC) was calculated. An AUC value exceeding 0.70 was considered significant. To identify factors that have a significant impact on the disease course and prognosis, the odds ratio (OR) and a 95% confidence interval (CI) were calculated. The data were presented as the median and interquartile range *Me* (Q_{25}; Q_{75}). The critical level of statistical significance (p) in all analyses was equal to 0.05.

RESULTS

Therapy with low doses of β-blockers was associated with high serum levels of sST2 and NT-proBNP (Table 1). In group 1, sST2 serum concentration was 30.7% higher ($p < 0.001$) than in group 2 (40.26 (34.39; 48.92) and 27.9 (23.05; 35.27) ng / ml, respectively). The NT-proBNP level in group 1 was also 22.8% higher ($p = 0.049$) than in group 2 (167 (129; 330) ng / ml vs. 129 (125; 147) ng / ml, respectively). Echocardiographic parameters in the groups did not differ significantly at the time of inclusion in the study (Table 2).

Table 2

Echocardiographic characteristics of patients at the time of inclusion in the study, *Me* (Q_{25}; Q_{75})

Parameter	Group 1, $n = 40$ beta-blocker ≥ 100 mg / day	Group 2, $n = 32$ beta-blocker < 100 mg / day	p
Left ventricular ejection fraction, %	64 (50.5; 65.0)	61 (48.5; 65.0)	0.083
End-systolic dimension, mm	33.0 (31.5; 35.0)	33.0 (32.5; 40.5)	0.524
End-diastolic dimension, mm	50.25 (48.0; 52.5)	51.0 (48.7; 53.0)	0.307
End-systolic volume, ml	43.0 (36.5; 48.0)	44.5 (39.5; 64.0)	0.065
End-diastolic volume, mm	116 (100.5; 125.5)	116.5 (108.5; 129.0)	0.224
LVMI, g / m^2	94.5 (88.0; 105.0)	98.0 (88.5; 114.5)	0.276
EDVI, ml / m^2	57.3 (53.3; 64.45)	60.4 (56.5; 72.9)	0.056
ESVI, ml / m^2	20.9 (19.2; 24.1)	23.1 (20.4; 27.6)	0.276

Note. LVMI – left ventricular mass index; EDVI – end-diastolic volume index, ESVI – end-systolic volume index.

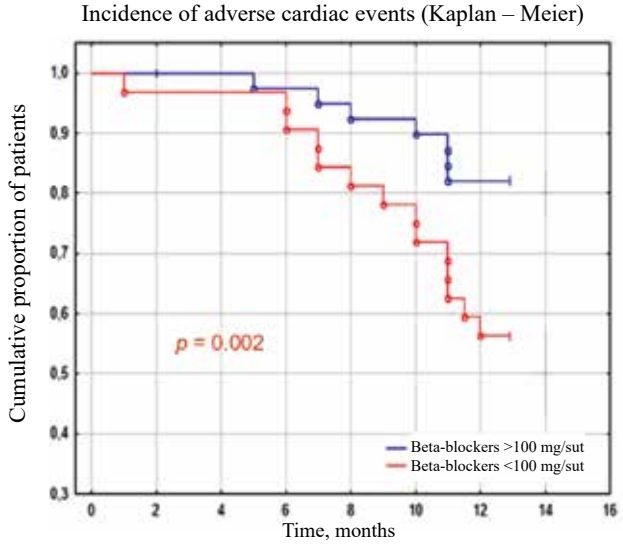

Fig. 1. Development of cardiovascular events within a year in groups of CHF patients with preserved and mildly reduced ejection fractions, formed depending on the β-blocker dosage (the Kaplan – Meier method)

Intergroup differences were found in the incidence of cardiovascular events within a year after endovascular revascularization (Fig. 1). Patients who received an equivalent dose of metoprolol succinate < 100 mg / day had a 34% higher rate of cardiac events ($p = 0.002$) than patients who received an equivalent dose of metoprolol succinate ≥ 100 mg / day.

According to the ROC analysis, it was found that an increase in the level of sST2 ≥ 34.18 ng / ml (sensitivity 78.0%, specificity 90.0%, AUC 0.906; $p < 0.0001$) predicted a high risk of cardiovascular events within the following 12 months. Serum NT-proBNP levels did not have any predictive value for risk stratification (Fig. 2).

Data analysis showed that in patients with sST2 overexpression ≥ 34.18 ng / ml who received a low dose of β-blockers, cardiovascular events developed more frequently (OR 4.18; $p < 0.0001$), while in patients with the level of the studied biomarker in the blood lower than 34.18 ng / ml and a high dose of β-blockers, no adverse events were recorded in any of the cases during the 12-month follow-up. Patients with sST2 < 34.18 ng / ml who received a low dose of β-blockers and patients with overexpression of sST2 ≥ 34.18 ng / ml who received β-blockers at a high dose had intermediate incidence of cardiovascular events (OR 1.79; $p = 0.003$ and 2.09; $p = 0.023$, respectively). The addition of NT-proBNP to sST2 analysis models did not increase the accuracy of risk stratification.

After 12 months, in patients receiving low doses of β-blockers, LVEF decreased by 6.3% ($p = 0.043$), and end-systolic dimension increased by 10.8% ($p = 0.049$) (Table 3), which indicated the progression of LV remodeling and, as a consequence, manifestations of heart failure.

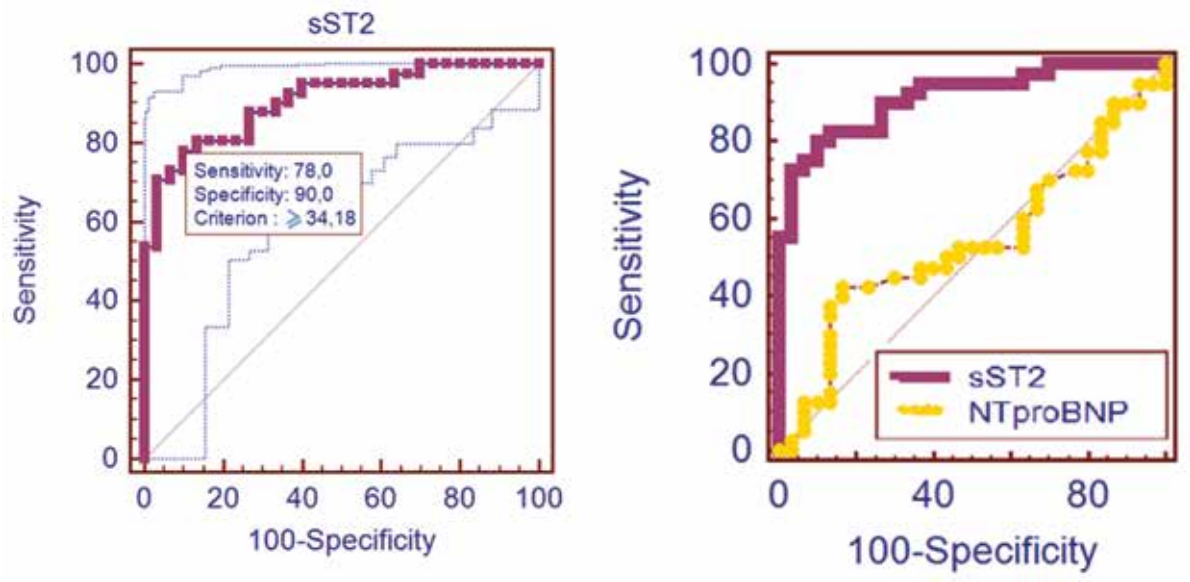

Fig. 2. Sensitivity and specificity of sST2 values in cardiovascular event risk stratification in CHF patients with preserved and mildly reduced ejection fraction (ROC analysis)

Table 3

Echocardiographic and laboratory data in groups of CHF patients during the 12-month follow-up depending on the dose of β-blockers, *Me* (Q_{25}; Q_{75})

Parameter	Baseline		After 12 months		
	Group 1 (BB ≥ 100 mg/day) $n = 40$	Group 2 (BB < 100 mg/day) $n = 32$	Group 1 (BB ≥ 100 mg/day) $n = 40$	Group 2 (BB < 100 mg/day) $n = 32$	p
LVEF, %	64 (60.5; 65.0)	63 (58.5; 65.0)	64 (61; 65)	59 (52.0; 62.0)#	0.043
ESD, mm	33.0 (31.5; 35.0)	33.0 (32.5; 40.5)	33.0 (32.0; 34.0)	37.0 (32.0; 40.0)#	0.052
EDD, mm	50.25 (48.0; 52.5)	51.0 (48.7; 53.0)	50.5 (49; 52)	53.0 (50.0; 54.0)	0.057

Table 3 (continued)

Parameter	Baseline		After 12 months		
	Group 1 (BB ≥ 100 mg/day) *n* = 40	Group 2 (BB < 100 mg/day) *n* = 32	Group 1 (BB ≥ 100 mg/day) *n* = 40	Group 2 (BB < 100 mg/day) *n* = 32	*p*
ESV, ml	43.0 (36.5; 48.0)	44.5 (39.5; 64.0)	41.5 (37.0; 44.5)	45.5 (40.0; 60.0)	0.085
EDV, ml	116 (100.5; 125.5)	116.5 (108.5; 129.0)	115.5 (102; 119.5)	118.0 (111.0; 126.0)	0.144
LVMI, g / m^2	94.5 (88.0; 105.0)	98.0 (88.5; 114.5)	93.5 (86.5; 102.0)	98.0 (89.0; 105.0)	0.237
EDVI, ml / m^2	57.3 (53.3; 64.45)	60.4 (56.5; 72.9)	58.4 (53.2; 64.15)	60.6 (57.1; 65.5)	0.066
ESVI, ml / m^2	20.9 (19.2; 24.1)	23.1 (20.4; 27.6)	21.35 (19.2; 23.85)	24.4 (21.4; 31.4)	0.076
sST2 (ng / ml)	27.9 (23.05; 35.27)	40.26 (34.39; 48.92)	27.98 (23.4; 30.17)	39.6 (32.61; 49.66)	0.001
NT-proBNP (ng / l)	129 (125; 147)	167 (129; 330)	–	–	–

Note. LVEF – left ventricular ejection fraction; EDD – end-diastolic dimension; ESD – end-systolic dimension; ESV – end-systolic volume; EDV – end-diastolic volume.

DISCUSSION

Currently, β-blockers are a class of drugs widely used for the treatment of heart failure which undoubtedly improve survival in patients with LV systolic dysfunction and reduce the incidence of adverse cardiovascular outcomes and hospitalizations for decompensated heart failure [15]. However, there are contradictory data on their effectiveness in CHF patients with preserved LVEF.

The OPTIMIZE-HF registry evaluated the endpoints of over 7,000 elderly patients hospitalized with heart failure. It was shown that β-blockers did not have a significant effect on mortality or the risk of rehospitalization for CHF decompensation in patients with preserved LV systolic function [21]. At the same time, it should be noted that the number of patients included in the registry was small in order to evaluate this fact properly. In 2015, S. Prijic et al. performed a systematic review and meta-analysis of 17 studies with 27,099 heart failure patients. Based on the data obtained, it was shown that the use of β-blockers reduced mortality from all causes by 19%, however, the analysis in the subgroups did not reveal this effect in elderly patients over 75 years of age [27].

Another study, which included 538 HFpEF patients, evaluated the effect of β-blocker therapy on the course of this pathology. At the same time, this group of drugs had no obvious positive effect on CHF severity in patients with preserved LVEF. The exception was patients with IHD and atrial fibrillation, where the use of β-blockers led to a decrease in the CHF FC (according to the NYHA) and the level of brain natriuretic peptide as well as an increase in exercise tolerance [28].

Maladaptive myocardial remodeling is a central feature of heart failure progression. This process can be modulated by various factors and includes hypertrophy and cardiomyocyte apoptosis, which leads to a significant change in the structure and function of the myocardium [29, 30]. One of the most important factors in myocardial remodeling is activation of the sympathetic nervous system. Increased concentrations of norepinephrine cause death of cardiac cardiomyocytes and stimulate gene expression and protein synthesis in fibroblasts, which contribute to CHF progression [15, 31, 32]. Acting directly through β-adrenergic receptors, β-blockers deactivate the sympathetic nervous system and prevent CHF progression, slowing down the processes of unfavorable remodeling. Over the past decade, several randomized clinical trials have shown that the use of β-blockers improves LV function and reduces morbidity and mortality in patients with both acute and chronic heart failure [29, 32, 33].

Soluble ST2 is currently considered a new biomarker involved in the pathophysiology of processes occurring in the myocardium, such as maladaptive cardiac remodeling, ischemic and non-ischemic dysfunction and myocardial apoptosis, and arrhythmogenesis, leading to the development of heart failure and sudden cardiac death [18, 27, 34–38]. Unlike the concentration of NT-proBNP and brain natriuretic peptide (markers of myocardial stress or myocardial dysfunction), the level of sST2 expression does not depend on factors, such as body mass index, gender, age, smoking status, and presence of comorbidities (mainly renal dysfunction), and has the lowest intra- and interindividual variabili-

ty among the main cardiac biomarkers. Taking into consideration the above points, ST2 may be more useful than NT-proBNP for risk stratification and monitoring of treatment effectiveness in HFpEF patients [22].

It was found that sST2 overexpression in the blood serum of patients is closely associated with autoimmune and inflammatory processes, in particular, with type 2 CD4 + cells [39]. It is also known that elevated sST2 levels can be used as a marker for predicting cardiovascular mortality and rehospitalization rates in patients with heart failure [35, 40]. It has been shown that increased baseline sST2 levels and achieved doses of β-blockers are associated with the incidence of cardiovascular events in patients with heart failure with preserved and mildly reduced LVEF, regardless of NT-proBNP concentrations. At the same time, the mechanisms of action of β-blockers on the ST2 interleukin receptors remain unclear.

The study by J. Xia et al. (2017) suggested that β-blocker therapy modulates IL-33 / sST2 signaling, thereby slowing down the processes of ventricular remodeling, but these data were obtained in the animal model of acute myocardial infarction [41]. B-blocker therapy was found to significantly improve LV function, decrease infarction size and enhance IL-33 / ST2 signaling, leading to a decrease in sST2 expression. It is interesting that in this study β-blocker treatment reduced sST2 levels but did not affect elevated IL-33 levels. Thus, it was concluded that β-blocker therapy may play an important role in modulating IL-33 / ST2 signaling and in ventricular remodeling [41].

Analysis of the prospective, randomized PROTECT trial revealed the relationship between changes in β-blocker doses and sST2 levels and the risk of cardiovascular events [42]. The authors of this study analyzed the use of the β-blocker at a wide range of doses and identified the role of sST2 in risk stratification. In this study, patients were randomized into four groups according to the baseline sST2 values (≤ 35 ng / ml versus > 35 ng / ml) and a final β-blocker dose (≥ 50 mg versus < 50 mg daily) in patients with LVEF ≤ 40%. As a result, it was found that β-blocker therapy had a dose-dependent effect, and the measurement of sST2 helped to identify patients with CHF. Higher doses of these drugs may be especially useful for such patients, as they can reduce the incidence of adverse events.

At the same time, the results of the EPHESUS study evaluating the effectiveness of eplerenone on the survival of patients with heart failure after acute myocardial infarction showed that adverse LV remodeling in patients with normal ST2 expression is less common regardless of therapy [20]. The study by W.P. Huang et al. found that patients after ST-segment elevation myocardial infarction and higher baseline sST2 concentrations, which were not titrated with high doses of β-blockers ($p < 0.0001$) before therapy, had higher incidence of cardiovascular events [42].

The data obtained help to understand the mechanisms of involvement of ST2 receptors in the pathogenesis of cardiac remodeling, fibrosis, and apoptosis leading to the onset and progression of CHF with preserved and mildly reduced LVEF. Long-term therapy with β-blockers has anti-ischemic and hemodynamic effects in patients with CHF with preserved and mildly reduced LVEF. It is probably due to an increase in time allowing for more complete LV filling during diastole, which causes an increase in stroke volume. Another effect of β-blockers is their influence on the increased tone of the sympathetic nervous system and the level of catecholamines. As a result, CHF progression is prevented by inhibiting adverse remodeling.

It should be noted that our data in no way suggest that β-blocker therapy should be abandoned if sST2 expression is normal. Moreover, we have shown that in patients with physiological sST2 values, the incidence of cardiovascular events was further reduced due to the use of high doses of β-blockers.

CONCLUSION

Therefore, the study results confirm high prognostic value of the increase in the sST2 serum concentration in the development of cardiovascular events within a year after endovascular myocardial revascularization and substantiate the possibility of using this biomarker as a target for β-blocker therapy in HFpEF and HFmrEF patients. Aggressive use of β-blockers is preferable in the group of patients with sST2 overexpression and HFpEF and HFmrEF in order to reduce the incidence of cardiovascular events.

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Effect of local ozone therapy on inflammatory markers in experimental ulcerative colitis

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ABSTRACT

Aim. To evaluate the effect of rectal insufflations of medical ozone on markers of inflammation in experimental ulcerative colitis.

Materials and methods. The experimental study was performed *in vivo* on 49 white, sexually mature male Wistar rats weighing 250 ± 15 g. The model of ulcerative colitis was reproduced using two-stage oxazolone administration (Sigma-Aldrich, USA). A group of animals received rectal insufflations of medical ozone at a dose of 1.0 mg / l once a day in the volume of 10 ml of ozone / oxygen mixture. The cycle of insufflations lasted 10 days. The ozone / oxygen mixture was obtained using an automated ozone therapy device with an ozone destructor UOTA-60-01"Medozon" (Medozon LLC, Moscow, Russian Federation). According to the disease activity index (DAI) score, the disease activity index was evaluated. The intensity of neutrophil phagocytosis in the blood was detected using polystyrene latex particles. The ability of neutrophils to reduce nitroblue tetrazolium (NBT) was determined using spontaneous and induced NBT tests. The interleukin-17 (IL-17) concentration in the serum was determined by enzyme-linked immunosorbent assay (ELISA) using a test system for rats manufactured by Bender MedSystems (Austria).

Results. Under the conditions of ozone therapy by rectal insufflations in experimental ulcerative colitis, we demonstrated improvement in the clinical presentation of the disease, intensity of phagocytosis, phagocytic index, and spontaneous and induced ability of neutrophils to reduce NBT with normalization of the functional reserve of cells and the level of proinflammatory IL-17 on day 6 of the experiment.

Conclusion. The results obtained allow to verify pronounced anti-inflammatory and immunomodulatory effects of ozone and consider it as one of the most relevant treatment strategies for inflammatory bowel diseases.

Keywords: experiment, ulcerative colitis, inflammation, medical ozone

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Влияние локальной озонотерапии на маркеры активности воспалительного процесса при экспериментальном язвенном колите

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РЕЗЮМЕ

Цель. Оценить влияние ректальных инсуффляций медицинского озона на маркеры активности воспалительного процесса при экспериментальном язвенном колите.

Материалы и методы. Экспериментальное исследование проводили в условиях *in vivo* на 49 белых половозрелых самцах крыс линии Вистар массой 250 ± 15 г. Модель язвенного колита воспроизводили с помощью двухэтапного введения оксазолона (Sigma-Aldrich, США). Группе животных проводили инсуффляции медицинского озона ректально в дозе 1,0 мг/л, 1 раз/сут, в объеме 10 мл озоно-кислородной смеси (ОКС). Курс 10 сут. ОКС получена на озонотерапевтической автоматической установке с деструктором озона УОТА-60-01 «Медозон» (ООО «Медозон», Москва, Россия). В соответствии со шкалой DAI (disease activity index) оценивали индекс активности болезни. Детекцию активности фагоцитоза нейтрофилов крови проводили с использованием частиц полистирольного латекса. НСТ-редуцирующую способность нейтрофилов определяли с применением спонтанного и индуцированного вариантов НСТ-теста. Определение концентрации интерлейкина (IL) 17 в сыворотке определяли методом иммуноферментного анализа с помощью тест-системы для крыс фирмы Bender Medsystems (Австрия).

Результаты. В условиях ректального применения озонотерапии при экспериментальном язвенном колите показано улучшение клинической картины заболевания, нормализация показателей интенсивности фагоцитоза, фагоцитарного числа, спонтанной и индуцированной НСТ-редуцирующей способности с нормализацией показателя функционального резерва клеток и уровня провоспалительного IL-17 на 6-е сут эксперимента.

Заключение. Полученные результаты позволяют констатировать наличие выраженного противовоспалительного и иммуномодулирующего эффектов озона и рассматривать последний в качестве одного из актуальных направлений терапии воспалительных заболеваний кишечника.

Ключевые слова: эксперимент, язвенный колит, воспаление, медицинский озон

Конфликт интересов. Авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic disease of the colon which is characterized by the presence of pronounced inflammation in the mucosa. The incidence of UC in the European population ranges from 0.6 to 24.3 per 100,000 people, and the prevalence reaches 505 cases per 100,000 people [1]. The etiology of UC is multifactorial and understudied. A special role in the pathogenesis of UC is assigned to gut microbiome imbalance [2]. A key defect of innate immunity in UC is hyperactivation of proinflammatory signaling pathways, which leads to impaired recognition of bacterial molecular patterns

by dendritic cells of the colonic mucosa. Infiltration of the colonic mucosa tissue by lymphocytes with signs of plasmocytic differentiation mainly to the Th2-phenotype is accompanied by overexpression of proinflammatory cytokines (tumor necrosis factor- α (TNF-α), interleukin (IL)-6, IL-17), intercellular adhesion molecules (ICAM), and signaling molecules with formation of characteristic morphological changes.

One third of patients with UC are known to be refractory to background therapy, and many patients develop a wide range of adverse effects that limit its use [3]. This dictates the need for search for new, pathogenetically grounded approaches to UC treatment, including the use of genetically engineered immunobiological drugs with anti-cytokine effect, cell adhesion molecule inhibitors, stem cells, and relevant efferent therapy techniques. Among the latter, the use of therapeutic concentrations of medical ozone is of special interest due to its pronounced anti-inflammatory, immunomodulatory, bactericidal, and antioxidant effects. The successful use of ozone therapy in surgery, dermatocosmetology, and ENT disorders is known, and there are some publications on the use of ozone therapy in gastroenterology.

The aim of the study was to evaluate the effect of rectal insufflations of medical ozone on inflammatory markers in experimental UC.

MATERIALS AND METHODS

The study was conducted at the site of the experimental biological clinic (vivarium) of the South Ural State Medical University of the Ministry of Health of the Russian Federation. All procedures were carried out in strict accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETSIN 123, March 18, 1986) and in compliance with the Directive 2010/63/EU of the European Parliament and of the Council of 22.09.2010 and were approved by the local Ethics Committee at South Ural State Medical University (Protocol No. 4 of 22.05.2020) [4]. Experimental studies were carried out *in vivo* on 49 white, sexually mature male Wistar rats weighing 250 ± 15 g. By simple randomization, the male rats were divided into 2 groups: group 1 (n = 9) included intact animals, group 2 (n = 54) – animals with UC.

The model of UC was reproduced by two-stage oxazolone administration (Sigma-Aldrich, USA) with clinical and morphological verification [5]. The first stage was characterized by development of skin sensitization following application of 150 μl of 3% oxazolone solution to the pre-treated interscapular area. At the second stage, the 3% oxazolone solution was rectally injected to a depth of 7 cm. The study was performed on days 2, 4, and 6. After receiving the experimental model of UC, 21 animals were randomly selected for ozone therapy (group 3). Daily, the animals of group 3 received rectal insufflations of medical ozone at a dose of 1.0–1.2 mg / l once a day in the volume of 10 ml of ozone / oxygen mixture. The cycle lasted 10 days. The ozone / oxygen mixture was obtained using an automated ozone therapy device with an ozone destructor UOTA-60-01"Medozon" (Medozon LLC, Moscow, Russian Federation). Blood sampling for the study was carried out under general anesthesia (the drug Zoletil-100 (tiletamine hydrochloride, Virbac Sante Animale, France) at a dose of 20 mg / kg). Blood was collected by a left ventricular puncture in vacuum blood collection tubes "Vacuette" (Greiner Bio-One, Austria) with an anticoagulant (K3-EDTA or heparin) for immunological studies.

The clinical examination was performed by calculating the disease activity index (DAI), according to the modified score for evaluating the studied pathology in rats [6]. The score includes 3 parameters, such as body weight, stool consistency, and the presence of blood in the stool. For the evaluation, a 4-point scale from was used (from 0 to 4). The scores were summed, the minimum index value being 0, the maximum – 12.

The granulocyte population was isolated by ficoll (Pharmacia, Sweden) – verografin (SPOFA, Czech Republic) density gradient centrifugation. Functional assessment of the phagocytic activity of blood neutrophils was performed using polystyrene latex particles (diameter 1.5 μm). The results were considered using immersion microscopy with the calculation of the percentage of cells that engulfed at least one polystyrene latex particle (activity of phagocytosis (AP)), the number of engulfed latex particles (units) in 100 cells (intensity of phagocytosis (IP)), and the number of engulfed latex particles (units) per one phagocyte (phagocytic index (PI)).

The ability of neutrophils to reduce NBT was determined using spontaneous and induced NBT tests, with the calculation of the functional reserve of cells according to the method proposed by A.N. Mayanskiy and M. E. Vixman [7]. The result was expressed in units. The interleukin-17 (IL-17) concentration in the serum was determined on the automated immunoassay analyzer "Personal LAB" using a test system for rats (Bender MedSystems, Austria). The results were expressed in pg / ml.

Statistical processing of the results was carried out using the Statistica 8.0 software package. The data in the tables are presented as the median and interquartile range *Me* (Q_{25}–Q_{75}). The groups were compared using the nonparametric Kruskal – Wallis and Mann – Whitney tests with the Bonferroni correction. The differences were considered statistically significant at $p \leq 0.016$.

RESULTS

The clinical status in experimental UC in the animals of group 2 was evaluated according to the modified Disease Activity Index (DAI) score and showed elevation of DAI on day 2, with an increase on day 4 and 6 (Table 1). Changes in the ethological status were manifested through reduced motor activity, grooming, and feed consumption.

The evaluation of the ability of neutrophils to engulf particles (Table 2) in experimental UC showed that on day 2 of the observation in group 2, the activity of phagocytosis (AP), the intensity of phagocytosis (IP), and the phagocytic index (PI) increased. The maximum values for these parameters were observed on day 4, with a tendency to a decrease in AP and IP on day 6 compared with the intact group.

When assessing the ability of blood neutrophils to reduce NBT in animals with experimental UC, it was found that on day 2, the activity and intensity of the spontaneous and induced NBT tests increased, along with the functional reserve of neutrophils, estimated by the activity and intensity of the NBT test. Day 4 of the experiment was characterized by high values for spontaneous NBT test and functional reserve, however, on day 6, the parameters of spontaneous and induced NBT tests and functional reserve did not differ from those in the intact group. The results of the study on the concentration of proinflammatory IL-17 in the serum of animals with experimental ulcerative colitis (EUC) at runtime are presented in Table 3. The level of IL-17 in group 2 increased by day 4 and reached the maximum value by day 6 of the experiment compared with the group of intact animals.

Under the conditions of rectal ozone therapy in animals with EUC, positive changes in the ethological status were recorded in the form of increased motor and stress-protective activity and higher degree of feed consumption. Against the background of ozone therapy, starting from day 2 of the observation, no visible signs of intestinal bleeding with a lack of blood in the stool, as well as more formed and firmer stool were noted. These signs were manifested through the integrated parameter of DAI assessment, the values of which are presented in Table 1. Therefore, in group 3, the DAI values on day 2 did not differ significantly from those in group 2, with a significant decrease on days 4 and 6. However, despite a significant decrease, the DAI values in these groups on days 4 and 6 did not reach the values in the group of intact animals, which indicates incomplete restoration of the colonic mucosa against the background of local medical ozone administration.

The effect of rectal insufflations of medical ozone in EUC on the functional activity of blood neutrophils was studied (Table 2). Starting from day 2, as well as on days 4 and 6, an increase in AP and IP was revealed compared with the intact group. Compared with group 2, starting from day 2 and on day 4, significantly lower values for IP and PI were recorded, which decreased to the level in the intact group on day 6 of the experiment.

Under the conditions of rectal ozone therapy, on day 2 of the experiment, the parameters of spontaneous and induced NBT test (activity) significantly increased, and the parameters of the functional reserve decreased compared with the intact group. Compared with group 2, induced NBT test and functional reserve values in group 3 decreased. On days 4 and 6, the parameters of spontaneous and induced NBT test (activity) decreased compared with group 2 and reached the level in the intact group. On days 4 and 6, the functional reserve values did not differ from those in the intact group.

Under the conditions of rectal ozone therapy, an increase in IL-17 in the blood (Table 3) was noted on day 4 of the experiment compared with the intact group and group 2. Complete normalization of the parameter was observed on day 6 compared with the intact group.

Table 1

Effect of ozone therapy on the changes in the disease activity index in experimental ulcerative colitis, *Me* (Q_{25}–Q_{75})

Parameter	Group 1 Healthy animals (n = 9)	Group 2 Rats with EUC, day 2 (n = 9)	Group 2 Rats with EUC, day 4 (n = 9)	Group 2 Rats with EUC, day 6 (n = 9)	Group 3 Rats with EUC + ozone rectally, day 2 (n = 9)	Group 3 Rats with EUC + ozone rectally, day 4 (n = 9)	Group 3 Rats with EUC + ozone rectally, day 6 (n = 9)
DAI, units	0	5.0 (2.0–8.0)*	8.0 (7.0–12.0)*	12.0 (10.0–13.0) *	6.0 (4.0–7.0)*	3.0 (2.0–3.0) * #	2.0 (1.0–2.0) * #

Note: the differences between the groups are obtained using the Kruskal – Wallis test and the Mann – Whitney test with the Bonferroni correction (here and in Table 2, 3). Statistically significant differences with group 1 – * , with the corresponding observation day in group 2 – #.

Table 2

Effect of rectal ozone therapy on the functional activity parameters of blood neutrophils in experimental ulcerative colitis, *Me* (Q_{25}–Q_{75})

Parameter	Group 1 Healthy animals (n = 9)	Group 2 Rats with EUC, day 2 (n = 9)	Group 2 Rats with EUC, day 4 (n = 9)	Group 2 Rats with EUC 6 days (n = 9)	Group 3 Rats with EUC + ozone rectally, day 2 (n = 9)	Group 3 Rats with EUC + ozone rectally, day 4 (n = 9)	Group 3 Rats with EUC + ozone rectally, day 6 (n = 9)
Phagocytosis activity, %	34.1 (31.0–45.0)	54.0 (44.0–57.0)*	60.8 (48.0–66.0)*	46.3 (36.0–65.0)*	51.60 (36.0–57.5)*	56.3 (44.0–62.0)*	43.5 (35.0–64.0)*
Phagocytosis intensity, units	0.72 (0.5–0.85)	4.5 (3.83–5.5)*	7.2 (5.7–13.1)*	5.8 (4.5–11.5)*	2.3 (1.32–3.7) *#	1.7 (0.7–1.9)*#	0.9 (0.4–1.2)#
Phagocytic index, units	1.5 (1.2–1.9)	8.2 (7.3–9.3)*	13.8 (12.5–16.0)*	13.6 (9.2–15.3)*	5.6 (4.8–6.2)*#	4.3 (2.3–4.6)*#	1.20 (1.2–4.0)#
Sp. NBT test, activity, %	4.0 (4.0–5.0)	9.0 (8.0–10.0)*	14.0 (13.0–16.0)*	7.5 (5.0–12.0)	6.5 (5.0–10.0)*	4.0 (3.0–5.0)#	3.5 (2.0–4.5)#
Sp. NBT test, intensity, units	0.05 (0.04–0.07)	0.18 (0.16–0.19)*	0.20 (0.19–0.21)*	0.08 (0.03–0.12)	0.14 (0.13–0.15)*	0.12 (0.02–0.15)*#	0.06 (0.05–0.08)
Ind. NBT test, activity, %	5.0 (4.0–6.0)	23.5 (9.0–24.0)*	9.5 (8.0–13.0)	6.0 (4.0–7.0)*	8.0 (7.0–10.0)*#	5.5 (5.0–6.0)#	5.0 (4.0–6.0)#
Ind. NBT test, intensity, units	0.04 (0.03–0.05)	0.26 (0.10–0.26)*	0.06 (0.04–0.2)	0.05 (0.03–0.05)*	0.10 (0.09–0.10)*#	0.05 (0.03–0.05)#	0.04 (0.03–0.05)#
Functional reserve (activity of the NBT test)	1.53 (0.70–1.6)	1.8 (1.4–2.1)*	1.70 (1.3–2.0)*	1.64 (0.53–4.3)	0.78 (0.66–0.9)*#	1.6 (0.8–1.9)	1.58 (0.98–1.7)
Functional reserve (intensity of the NBT test)	1.4 (0.95–1.5)	1.62 (1.52–1.7)*	1.73 (1.3–2.2)*	1.4 (0.3–1.5)	0.75 (0.5–0.85)*#	1.2 (0.8–1.3)	1.5 (0.58–1.6)

Table 3

Effect of rectal ozone therapy on the changes in the level of IL-17 (pg / ml) in the blood serum in experimental ulcerative colitis, *Me* (Q_{25}–Q_{75})

Parameter	Group 1 Healthy animals (n = 9)	Group 2 Rats with EUC, day 2 (n = 9)	Group 2 Rats with EUC, day 4 (n = 9)	Group 2 Rats with EUC 6 days (n = 9)	Group 3 Rats with EUC + ozone rectally, day 2 (n = 9)	Group 3 Rats with EUC + ozone rectally, day 4 (n = 9)	Group 3 Rats with EUC + ozone rectally, day 6 (n = 9)
IL-17, pg / ml	6.3 (3.3–7.4)	5.7 (4.3–8.2)*	15.3 (9.6–22.3)*	17.3 (9.3–64.7)*	5.4 (4.2–7.3)	10.3 (9.52–14.25)#	6.62 (5.6–6.8)#

DISCUSSION

In EUC at runtime, an increase in DAI indicated the presence of inflammatory changes in the colonic wall. Neutrophils and then monocytes migrated to the area of primary alterations, causing local tissue damage due to the release of enzymes, inflammatory mediators, and reactive oxygen species (ROS), which was accompanied by an increase in the ability of blood neutrophils to engulf particles and reduce NBT on day 2. Chemotaxis of lymphocytes to the lesion site was accompanied by

an increase in clonal expansion of lymphocyte subpopulations and a rise in the secretory activity of the latter, which was accompanied by an increase in proinflammatory cytokines, including IL-17, in the blood.

IL-17 is known to be a secretory product of a special subpopulation of memory T cells identified as CD4 CD45RO, the synthesis of which is controlled by IL-23. In some inflammatory bowel diseases, IL-23 is essential in the final differentiation of Th0 to Th17 after exposure to IL-1β, IL-6, and TNF-α, while IL-12 induces polarization of the Th1-dependent immune response with production of IFN-γ, TNF-α, and other cytokines [8]. At the same time, the hematopoietic and proinflammatory activity of IL-17 is mediated by the ability of the latter to stimulate production of TNF-α, IL-1β, PGE2, GM-CSF, IL-6, IL-10, IL-12, IL-1 receptor antagonist (IL-1RA), and stromolysin.

Improvement in the clinical presentation (decrease in the severity of symptoms, decrease in DAI) and normalization of IP, PI, and spontaneous and induced NBT test activity with the restoration of the functional reserve of cells on day 6 of the experiment under the conditions of rectal ozone therapy indicate a positive effect of the latter on inflammation in the intestinal wall due to anti-inflammatory and immunomodulatory effects of ozone. The local effect of average therapeutic concentrations of medical ozone directly on pathologically altered areas of the colonic tissues consists in direct ozonation of bioorganic compounds, as well as an indirect effect of ozonolysis products that form a reserve of ROS with a possibility of their subsequent permanent use in aerobic metabolism for maintaining the relevant level of energy substrates of colonocytes.

ROS act as “molecular phages”, contributing to purification of an ulcerated lesion and activating the chemotaxis of neutrophils and monocytes in the lesion [9]. Hydrotrioxides formed following ozone oxidation of organic substances associated with unsaturated fatty acids are extremely unstable compounds that decompose in a cell with release of molecular oxygen, thereby exerting a metabolic effect on the cell and a non-specific bactericidal effect. It is also known that administration of low concentrations of ozone / oxygen mixture launches free radical reactions in the cell. Subthreshold levels of ROS, in turn, are able to strengthen the antioxidant system in the cell according to the feedback principle [9].

We believe that changes in the functional activity of blood neutrophils under the conditions of local ozone therapy in EUC are due to a decrease in destructive processes in the pathological focus, a decrease in the secretion of inflammatory mediators, and restriction of phagocyte activation, both in the lesion area and in circulating neutrophils in the blood. This assumption is confirmed by a decrease in the concentration of the proinflammatory cytokine IL-17 to the values of the intact group against the background of rectal ozone therapy.

CONCLUSION

Therefore, the obtained results demonstrate pronounced anti-inflammatory and immunomodulatory effects of rectal insufflations of medical ozone in EUC, which allows to consider it as one of the promising areas in the therapy for inflammatory bowel diseases.

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Osikov M.V. – conception of the study and final approval of the manuscript. Davydova E.V. – analysis and interpretation of data. Kaygorodtseva N.V. – collection of material.

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Risk of developing drug abuse in administration of a new hexaazaisowurtzitane derivative-based analgesic (experimental study)

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ABSTRACT

The aim of the study was to assess the probability of developing withdrawal syndrome caused by discontinuation of 5-day administration of thiowurtzine with naloxone challenge test in the experiment.

Materials and methods. The test sample of the analgesic "Thiowurtzine, capsule 120 mg" served as the study object. The active pharmaceutical ingredient is an organic, low molecular weight compound 4-(3,4-dibromothiophene carbonyl)-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo [5,5,0,$0^{3,11}$,$0^{5,9}$]dodecane that was first synthesized according to computer modeling results at the IPCET SB RAS (Biysk).

The likelihood of developing physical dependence was explored by *per os* administration of thiowurtzine and the reference drug tramadol twice a day for 5 days as follows: 1) at 9 a.m. – thiowurtzine 50 mg / kg and tramadol 10 mg / kg, at 3 p.m. – thiowurtzine 50 mg / kg and tramadol 10 mg / kg; 2) at 9 a.m. – thiowurtzine 50 mg / kg and tramadol 10 mg / kg, at 3 p.m. – thiowurtzine 75 mg / kg and tramadol 15 mg / kg; 3) at 9 a.m. – thiowurtzine 75 mg / kg and tramadol 15 mg / kg, at 3 p.m. – thiowurtzine 75 mg / kg and tramadol 15 mg / kg; 4) at 9 a.m. – thiowurtzine 100 mg / kg and tramadol 20 mg / kg, at 3 p.m. – thiowurtzine 100 mg / kg and tramadol 20 mg / kg; 5) at 9 a.m. – thiowurtzine 100 mg / kg and tramadol 20 mg / kg, at 3 p.m. – naloxone 10 mg / kg subcutaneously.

In all the groups, the intensity of the withdrawal syndrome was studied by specific features in outbred male CD1 mice. During one hour following the naloxone injection, health of mice was assessed according to dominant abstinence components and recessive traits of mild withdrawal syndrome. Two hours after the naloxone injection, the number of mice with negative body weight gain was determined. 24 hours after discontinuation of test compound administration, the open-field test was used to determine the impact on animal behavioral patterns (horizontal and vertical motor and exploratory activity, emotionality and its vegetative manifestations). The hot plate test was carried out to measure the analgesic activity (55˚). The criterion of the withdrawal syndrome severity was a decrease in the number of jumping reactions, changes in the general condition of the animals, stimulation of motor activity, manifestations of hyperalgesia, and a decrease in body weight.

Results. No dominant abstinence components and recessive signs of withdrawal syndrome were detected in animals from the thiowurtzine groups. The data obtained in the study (orientation and exploratory behavior, motor activity, emotionality and its vegetative manifestations, grooming, etc.) allow to conclude that thiowurtzine causes no physical dependence in animals after discontinuation of its 5-day administration with naloxone challenge test, as opposed to the reference drug naloxone. A positive disinhibition effect of this analgesic was revealed due to the activated orientation and exploratory behavior (stress caused by the new environment) in the conditions of the open-field test. The animals showed no manifestations of hyperalgesia in the hot plate test. The animals treated with thiowurtzine did not demonstrate any changes in the body weight.

Conclusion. The obtained results prove that thiowurtzine is a non-narcotic analgesic. It evokes no side effects typical of opioid analgesics (tramadol), including development of physical dependence and withdrawal syndrome

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following naloxone challenge test. Previous *in vivo* and *in silico* studies (docking, molecular modeling, molecular dynamics simulation) on the multi-target mechanism of thiowurtzine explain the absence of its morphine-like effect by the fact that the major targets of the analgesic are TRPA1 receptors and voltage-gated Ca^{2+} channels. With a high degree of probability, the conclusions made herein predict no drug abuse development when thiowurtzine is used in the clinical setting. Absence of ulcerotoxicity found earlier will enable to administer thiowurtzine in long-term cycles for chronic pain syndrome.

Keywords: hexaazaisowurtzitane, thiowurtzine, analgesic activity, withdrawal syndrome, physical dependence, naloxone, tramadol

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

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Риск развития лекарственной зависимости при применении нового анальгетика на основе производного гексаазаизовюрцитана (экспериментальное исследование)

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РЕЗЮМЕ

Целью данного исследования явилось изучение вероятности развития синдрома отмены, вызываемого прекращением 5-суточного введения тиовюрцина по схеме с провокацией налоксоном в эксперименте.

Материалы и методы. Объект исследования экспериментальный образец анальгетика «Тиовюрцин, капсула 120 мг». Активная фармацевтическая субстанция представляет собой органическое низкомолекулярное соединение 4-(3,4-дибромтиофенкарбонил)-2,6,8,12-тетраацетил-2,4,6,8,10,12гексаазатетрацикло [5,5,0,$0^{3,11}$,$0^{5,9}$]додекан, синтезированное впервые по результатам компьютерного моделирования в ИПХЭТ СО РАН **(**г. Бийск**).**

Возможность развития физической зависимости исследовали при введении тиовюрцина и референс-препарата трамадола *per os* 2 раза/сут в течение 5 сут по схеме: 1) в 9 ч – тиовюрцин 50 мг/кг и трамадол 10 мг/кг, в 15 ч – тиовюрцин 50 мг/кг и трамадол 10 мг/кг соответственно; 2) в 9 ч – тиовюрцин 50 мг/кг и трамадол 10 мг/кг, в 15 ч – тиовюрцин 75 мг/кг и трамадол 15 мг/кг; 3) в 9 ч – тиовюрцин 75 мг/кг и трамадол 15 мг/кг, в 15 – тиовюрцин 75 мг/кг и трамадол 15 мг/кг, 4) в 9 ч – тиовюрцин 100 мг/кг и трамадол 20 мг/кг,

в 15 ч – тиовюрцин 100 мг/кг и трамадол 20 мг/кг; 5) в 9 ч – тиовюрцин 100 мг/кг и трамадол 20 мг/кг, в 15 ч – налоксон в дозе 10 мг/кг подкожно.

Во всех группах интенсивность синдрома отмены изучали по специфическим признакам у аутбредных мышей-самцов сток CD1. На протяжении 1-го ч после введения налоксона регистрировали основные компоненты абстиненции и рецессивные признаки легкого течения синдрома отмены. Через 2 ч после инъекции налоксона определяли число мышей с отрицательным привесом массы тела. Через 24 ч после отмены тестируемых веществ исследовали степень воздействия на структуру поведения животных (горизонтально-вертикальную двигательную и исследовательскую активности, эмоциональность и ее вегетативные проявления) в тесте «открытое поле», наличие анальгетического действия в тесте «Горячая пластина» (55˚). Критерием выраженности синдрома отмены считали уменьшение числа прыжковых реакций, изменение общего состояния животных, стимулирование двигательной активности, проявление гипералъгезии, снижение массы тела у мышей.

Результаты. «Доминантных» компонентов абстиненции и рецессивных признаков синдрома отмены у животных из групп введения тиовюрцина не зафиксировано. Совокупность полученных данных (ориентировочно-исследовательское поведение, двигательная активность, эмоциональность по ее вегетативным проявлениям, груминг и т.д.) позволяет заключить, что тиовюрцин после отмены 5-суточного введения по схеме с провокацией налоксоном не вызывает развития физической зависимости в отличие от референс-препарата трамадола. Выявлено позитивное растормаживающее действие анальгетика за счет активации ориентировочно-исследовательского компонента поведения (стресс новизны) в условиях теста «открытое поле». Отсутствовало проявление гипералъгезии у животных в тесте «горячая пластина». Изменения массы тела животных, получавших тиовюрцин, не наблюдалось.

Заключение. Представленные результаты свидетельствуют о том, что тиовюрцин не является наркотическим анальгетиком. Он не проявляет побочных эффектов, типичных для обезболивающих средств с опиоидным компонентом механизма действия (трамадол), прежде всего развития физической зависимости и формирования синдрома отмены. В подтверждение вышесказанного проведенные ранее исследования *in vivo* и *in silico* (докинг, молекулярное моделирование, моделирование молекулярной динамики) мультитаргетного механизма действия тиовюрцина объясняют отсутствие его морфиноподобного действия тем, что основными мишенями анальгетика являются TRPA1-рецепторы и потенциал-зависимые Ca^{2+}ионные каналы. Полученные выводы позволяют с высокой степенью вероятности прогнозировать отсутствие лекарственной зависимости при клиническом применении тиовюрцина, а выявленное ранее отсутствие гастротоксичности предполагает возможность использования анальгетика продолжительными курсами при хроническом болевом синдроме.

Ключевые слова: гексаазаизовюрцитан, тиовюрцин, анальгетическая активность, синдром отмены, физическая зависимость, налоксон, трамадол

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Many acute and chronic diseases, injuriesб and medical interventions are associated with pain, which dramatically impairs the quality of life and requires the use of painkillers. According to experts from the International Association for the Study of Pain, about 20% of humans suffer from chronic pain syndrome due to low efficacy of symptomatic therapy. According to statistics, the number of

cancer patients, patients diagnosed with myocardial infarction and coronary artery disease, as well as patients with various injuries reaches up to 13 million people a year in the Russian Federation. In this respect, analgesics for treatment of severe and excruciating pain of various etiology (including chronic pain) and opioid antagonists are strategically important categories of medicines [1–3]. At the same time, according to the estimates of anesthesiologists and expert analysts from the Russian pharmaceutical market, only about 10% of the demand for next-generation analgesics (enhanced opioids, combined analgesics, etc.) in the clinical setting is addressed. Moreover, the existing problem of side effects (gastro-, nephro-, hepato-, cardio-, and hematotoxicity, teratogenicity, mutagenicity, development of physical and psychological addiction) is still of great medical and social importance due to high availability and widespread use of painkillers of different groups [1–3]. All the above factors determine the need for search, development, and practical implementation of conceptually new analgesics with low toxicity acting on the molecular mechanisms of pain generation.

Preclinical studies of an innovative analgesic (hereinafter referred to as thiowurtzine, TWZ) based on the 2,4,6,8,10,12hexaazatetracyclo[5,5,0,$0^{3,11}$,$0^{5,9}$] dodecane (hexaazaizowurzitane) derivative for treatment of pain syndrome of different etiology are being currently carried out at Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk NIMC. It has been found that the test sample "Thiowurtzine, capsule 120 mg" has low toxicity and LD_{50} 150–5000 mg / kg (hazard class III in line with GOST R 12.1.007-76). Its pronounced analgesic effect was demonstrated in the hot-plate test of nociception, acute visceral and deep somatic pain model (acetic acid-induced writhing test), chemogenic pain model (formalin test), TRPV1 receptor sensitivity model (capsaicin test), and Randall – Selitto paw pressure test [4, 5]. The use of specific pharmacological analyzers allowed us to hypothesize the involvement of the kappa – opioid system and TRP receptors in the antinociceptive effect of thiowurtzine with a probable impact on serotonin and GABAergic structures of the central nervous system and T-type calcium (Ca^{2+}) channels [6, 7]. The discovered multi-target analgesic mechanism of thiowurtzine is manifested at different levels of perception, implementation, and modulation of nociceptive activity, which requires research on the probability of drug abuse development due to drug administration.

The present study is aimed at exploring the likelihood of withdrawal syndrome development due to discontinuation of the 5-day administration of thiowurtzine with naloxone challenge test in the experiment.

MATERIALS AND METHODS

The experiments were conducted on 60 outbred male CD1 mice (weight 20.5 g, age 7–8 weeks). The animals were obtained from the Department of Experimental Biomodeling at Goldberg Research Institute of Tomsk NRMC (Certificate of Animal Health). Animal maintenance and experiment design were approved by the Bioethics Committee at Goldberg Research Institute (JACUC protocol No. 96092015 of 16.09.2015) and complied with the Directive 2010/63/EU of the European Parliament and the Council of the European Union on the Protection of Animals Used for Scientific Purposes and GOST No. 33044-2014 "Principles of Good Laboratory Practice" of 01.08.2015.

The animals were randomly classified into groups using body weight (± 10%) as a criterion for classification. The mice were euthanized by cervical dislocation. The test sample "Thiowurtzine, capsule 120 mg" was the object of the study. The active pharmaceutical ingredient was an organic low molecular weight compound 4-(3,4-dibromothiophene carbonyl)-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo[5,5,0,$0^{3,11}$,$0^{5,9}$]dodecane that was first synthesized using computer modeling results at IPCET SB RAS (Biysk) [5]. The previous study on the analgesic effect of thiowurtzine determined the effective therapeutic dose of the compound – 100 mg / kg intragastrically [5, 7].

To assess the withdrawal syndrome, thiowurtzine and the reference drug tramadol hydrochloride (Joint Stock Company Organica, Russian Federation) were administered *per os* twice a day (in the morning and in the evening) for 5 days as follows: 1) at 9 a.m. – thiowurtzine 50 mg / kg and tramadol 10 mg / kg, at 3 p.m. – thiowurtzine 50 mg / kg and tramadol 10 mg / kg; 2) at 9 a.m. – thiowurtzine 50 mg / kg and tramadol 10 mg / kg, at 3 p.m. – thiowurtzine 75 mg / kg and tramadol 15 mg / kg; 3) at 9 a.m. – thiowurtzine 75 mg / kg and tramadol 15 mg / kg, at 3 p.m. – thiowurtzine 75 mg / kg and tramadol 15 mg / kg; 4) at 9 a.m. – thiowurtzine 100 mg / kg and tramadol

20 mg / kg, at 3 p.m. – thiowurtzine 100 mg / kg and tramadol 20 mg / kg; 5) at 9 a.m. – thiowurtzine 100 mg / kg and tramadol 20 mg / kg, at 3 p.m. – naloxone 10 mg / kg subcutaneously [8–10].

Naloxone (Moscow Pharmaceutical Factory, Russian Federation) is a non-selective opioid antagonist. The animals were divided into 6 groups for the experiments. Group 1: control (purified water) according to the regimen. Group 2: control (purified water) according to the regimen + naloxone. Group 3: thiowurtzine (decapsulated) according to the regimen. Group 4: thiowurtzine (decapsulated) according to the regimen + naloxone. Group 5: tramadol according to the regimen. Group 6: tramadol according to the regimen + naloxone.

During 1 hour following the naloxone injection, the overall health status of mice was assessed according to dominant withdrawal syndrome components and recessive traits of mild withdrawal syndrome (abnormal acoustic and corneal reflexes, the presence of convulsions, writhing movements, tremor as if beating a drum, ptosis, jumping, shaking, chattering teeth, Straub reaction, itching, sneezing, lateral position). The weight of the rodents was measured during the randomization and 2 h after the naloxone injection. 24 hours after withdrawal of the test compounds, the open-field test was used to determine the degree of impact on animal behavioral patterns (emotionality, horizontal and vertical motor and exploratory activity) [8, 11]. The mice were placed at the center of the open-field arena. Then the behavioral patterns of each animal were recorded for 2 min: horizontal activity (the number of crossed squares); vertical activity (the number of rearings with and without support of the cage edge); exploratory activity (the number of holepoking movements); emotional reaction and its vegetative manifestations (defecation); grooming.

Inactive animals with more intense defecation in the open-field maze were considered more emotional than animals who moved a lot but showed less intense defecation. Statistically significant variation in the parameters of horizontal and vertical motor activity was considered a criterion of sedative or stimulating effect. The analgesic activity was studied using a Hot Plate Analgesia Meter (Columbus Instruments, USA) at 55ºC. Latency of the pain response was recorded via animal's licking their hind paws, which is a sign that the pain threshold was reached. The analgesic effect was presented as the average latency in the group, while a significant increase in the response latency after the administration of the compounds was considered a hyperalgesia criterion [8].

The statistical analysis of the obtained data was performed using *Statistica 6.0*. The mean (X) and standard deviation (m) were calculated for all data. Along with the value of n (number of variants), they are presented in the final tables. The intergroup differences were checked using the non-parametric Kruskal – Wallis and Mann – Whitney – Wilcoxon (U) tests. The Fisher's angular transformation criterion (φ) was used to compare frequencies. The differences were considered statistically significant at $p \leq 0.05$ [8].

RESULTS

The control experiments demonstrated no signs of physical dependence while the withdrawal syndrome with naloxone challenge test was examined (table 1).

Table 1

Manifestation of reflexes in outbred male CD1 mice after discontinued administration of thiowurtzine (50–100 mg / kg) and tramadol (10–20 mg / kg) with naloxone challenge test (10 mg / kg s/c)

Reflex	Control, $n = 10$	Control + naloxone, $n = 10$	Thiowurtzine, $n = 10$	Thiowurtzine + naloxone, $n = 10$	Tramadol, $n = 10$	Tramadol + naloxone, $n = 10$
	Number of animals with reflex manifestations, %					
Acoustic	100	100	100	100	100	100
Corneal	100	100	100	100	100	100
Convulsions	0	0	0	0	30*	20*
Tremor	0	0	0	0	20*	20*
Ptosis	0	0	0	0	0	10
Jumping	0	0	0	0	0	10
Shaking	0	0	0	0	20*	0
Chattering teeth	0	0	0	0	0	0

Table 1 (continued)

Reflex	Control, $n = 10$	Control + naloxone, $n = 10$	Thiowurtzine, $n = 10$	Thiowurtzine + naloxone, $n = 10$	Tramadol, $n = 10$	Tramadol + naloxone, $n = 10$
	Number of animals with reflex manifestations, %					
Straub reaction	0	0	0	0	0	20*
Itching	100	100	100	100	100	100
Sneezing	0	0	0	0	0	0
Lateral position	0	0	0	0	0	0

*$p < 0.01$ compared with the control (Fisher's test)

Recessive signs of abuse were recorded in the animals treated with tramadol: tremor (20%), shaking (20%), and convulsions as the withdrawal syndrome component (30%) (Table 1). During tramadol administration according to the regimen, naloxone evoked not only such signs of mild withdrawal syndrome as shaking (20%), tremor as if beating a drum (20%), and ptosis (10%), but also jumping activity (10%), Straub reaction (20%), and convulsions (20%), which are dominant withdrawal syndrome components (Table 1). The mice treated with thiowurtzine according to the regimen in combination with naloxone had none of the physical dependence signs.

The study results for exploratory activity of the animals in the open-field test suggest that thiowurtzine after administration according to the regimen with naloxone challenge test did not change the number of vertical postures and the number of holepoking movements throughout the entire observation period (Table 2).

Table 2

Effect of cycle administration of thiowurtzine according to the ascending dose regimen (50–100 mg / kg) on behavioral patterns of outbred male CD1 mice in the open-field test against the background of administration discontinuation with naloxone challenge test (10 mg / kg s / c), $X \pm m$

Study group (number of animals, $n = 10$)	Overall motor activity	Horizontal activity	Vertical activity	Hole exploratory behavior	Grooming	Defecation	Asymmetry coefficient
1st min of observation							
1.Water control (according to the regimen)	42.3 ± 4.0	25.5 ± 2.9	3.1 ± 1.1	13.5 ± 1.9	0.2 ± 0.1	0 ± 0	60.6 ± 3.9
2. Control (according to the regimen) + naloxone	43.1 ± 3.8	28.3 ± 2.8	3.4 ± 0.8	10.7 ± 1.8	0.1 ± 0.1	0.6 ± 0.3	65.4 ± 3.1
3. Thiowurtzine (according to the regimen)	44.0 ± 4,2	33.4 ± 0.1	2.9 ± 0.7	10.9 ± 1.8	0 ± 0	0.2 ± 0.2	66.8 ± 4.1
4. Thiowurtzine (according to the regimen) + naloxone	54.1 ± 5.1*	35.0 ± 4.0*	4.0 ± 1.3	15.0 ± 1.5	0 ± 0	0.1 ± 0.1	63.5 ± 2.3
5. Tramadol (according to the regimen)	53.6 ± 4.8	37.0 ± 3.6*	4.6 ± 1.1	11.6 ± 1.7	0 ± 0	0.4 ± 0.3	69.3 ± 3.4
6. Tramadol (according to the regimen) + naloxone	38.2 ± 3.3+	25.3 ± 3.0+	1.9 ± 0.8+	10.5 ± 1.7	0.3 ± 0.2	0.2 ± 0.1	65.8 ± 5.3
2nd–3rd min of observation							
1. Water control (according to the regimen)	73.3 ± 7.1	35.3 ± 4.1	6.5 ± 2.0	30.8 ± 4.1	0.6 ± 0.3	0.1 ± 0.1	48.6 ± 3.4
2. Control (according to the regimen) + naloxone	79.9 ± 5.6	40.1 ± 3.0	8.1 ± 1.6	30.3 ± 3.4	0.9 ± 0.3	0.5 ± 0.3	51.1 ± 3.6
3. Thiowurtzine (according to the regimen)	78.9 ± 9.0	45.7 ± 5.7	6.1 ± 1.0	26.0 ± 3.4	0.9 ± 0.2	0.2 ± 0.1	57.9 ± 3.3
4. Thiowurtzine (according to the regimen) + naloxone	96.9 ± 9.2*	52.3 ± 6.12*	6.5 ± 1.2	37.2 ± 3.7	0.5 ± 0.2	0.4 ± 0.4	53.8 ± 3.6
5. Tramadol (according to the regimen)	102.0 ± 12.1	53.9 ± 9.1	5.9 ± 1.2	41.5 ± 6.1	0.6 ± 0.2	0.4 ± 0.3	51.6 ± 4.2
6. Tramadol (according to the regimen) + Naloxone	83.5 ± 9.45	48.7 ± 7.8	3.8 ± 1.0*#	30.2 ± 2.5	0.4 ± 0.2	0.4 ± 0.2	49.5 ± 6.3
Overall outcome, 1st–3rd min of observation							
1. Water control (according to the regimen)	115.6 ± 10.3	60.8 ± 6.5	9.6 ± 2.9	44.3 ± 5.4	0.8 ± 0.3	0.1 ± 0.1	52.6 ± 2.7
2. Control (according to the regimen) + naloxone	123.0 ± 9.2	68.4 ± 5.1	11.5 ± 2.2	41.0 ± 4.7	1.0 ± 0.3	1.1 ± 0.6	56.1 ± 2.6
3. Thiowurtzine (according to the regimen)	122.9 ± 12.2	75.7 ± 8.3	9.0 ± 1.5	36.9 ± 5.0	0.9 ± 0.2	0.4 ± 0.3	61.1 ± 3.1
4. Thiowurtzine (according to the regimen) + naloxone	151.0 ± 12.8	87.3 ± 8.1*	10.5 ± 2.2	52.2 ± 4.2	0.5 ± 0.2	0.5 ± 0.5	58.0 ± 2.1
5. Tramadol (according to the regimen)	155.9 ± 15.6*	90.9 ± 11.7*	10.5 ± 2.1	53.1 ± 6.7	0.6 ± 0.2	0.8 ± 0.4	57.4 ± 2.5
6. Tramadol (according to the regimen) + naloxone	122.0 ± 12.2+	74.0 ± 10.6	5.7 ± 1.4#+	40.7 ± 3.7	0.7 ± 0.3	0.6 ± 0.3	66.9 ± 8.7

*$p < 0.05$ in comparison with water control, #$p < 0.05$ in comparison with the Control + naloxone group, +$p < 0.05$ in comparison with the Tramadol group (Mann – Whitney – Wilcoxon test).

In addition, the absence of statistically significant changes in the grooming and defecation parameters indicates that the drug did not affect the emotional level of the animals. A 1.3-fold increase in the overall motor activity ($p < 0.05$) due to a 1.4-fold increase in the horizontal motor activity (by the number of squares crossed) ($p < 0.05$) during the 1st min of observation and a 1.3-fold ($p < 0.05$) and 1.5-fold increase ($p < 0.05$) in the 2nd–3rd min of the experiment, respectively, relative to similar control data is worth noting.

The revealed moderate stimulating effect of thiowurtzine on the horizontal activity can be explained by the activated orientation and exploratory behavior of mice due to the disinhibition effect of the drug on behavioral reactions of the animals under stress caused by new conditions [8, 11]. The data obtained for all the analyzed parameters (horizontal, vertical and exploratory activity, emotionality by its vegetative manifestations, grooming, etc.) allow us to state that withdrawal syndrome was not formed. In contrast, a statistically significant decrease in overall, horizontal, and vertical motor activity of the mice treated with tramadol with naloxone challenge test, in comparison with similar parameters in the animals of the tramadol group, complied with the sedative activity criterion and indicated withdrawal syndrome development (Table 2).

The data presented in Table 3 allow to conclude that the animals that received naloxone injections after tramadol administration was discontinued developed hyperalgesia, since the response latency exceeded that of the Tramadol group by 1.2 times ($p < 0.05$).

The baseline value for thiowurtzine-suppressed pain sensitivity in the case of a single injection was 50% in the hot plate test, while 24 h after 5-day administration of thiowurtzine was discontinued, the antinociceptive effect of the drug appeared to be statistically significant and reached 23.9% relative to the water control (Table 3). In the same period of observation, the naloxone challenge test resulted in a 21% decrease in the thiowurtzine activity when compared to the same effect in the above-mentioned group, which allows for the conclusion that the animals did not develop hyperalgesia.

The body mass of test animals is one of the important parameters for veterinary monitoring in a long-term experiment.

Table 3

Body mass index of outbred male CD1 mice and antinociceptive response value in the hot plate test in withdrawal syndrome with naloxone challenge test, $X \pm m$

Animal group (number of animals, $n = 10$)	Body mass of mice, g		Response latency development, sec
	before injections	after injections were discontinued	
1. Control, purified water (according to the regimen)	25.5 ± 0.7	25.3 ± 0.9	17.6 ± 2.2
2. Control, purified water (according to the regimen) + naloxone, 10	25.6 ± 0.5	25.7 ± 0.4	17.9 ± 2.0
3. Thiowurtzine (according to the regimen)	25.5 ± 0.6	26.2 ± 0.4	21.8 ± 2.7*
4. Thiowurtzine (according to the regimen) + naloxone, 10	25.6 ± 0.5	26.2 ± 0.5	17.2 ± 1.4
5. Tramadol (according to the regimen)	25.6 ± 0.5	25.5 ± 0.6	17.8 ± 1.3

* $p < 0.05$ in comparison with the Thiowurtzine + naloxone group; $^{\#}p < 0.05$ in comparison with the Control + naloxone group; $^{+}p < 0.05$ in comparison with the Tramadol group (Mann – Whitney – Wilcoxon test).

It can indicate an overall adverse effect of a test compound on metabolism, as well as undesirable and side effects when combined with toxicity testing data. No negative changes in the body mass of the test animals treated with thiowurtzine with naloxone challenge test, which induces withdrawal syndrome, were noted (Table 3).

CONCLUSION

The data obtained on the degree of impact on the animal behavior in the open-field test (horizontal activity, vertical and exploratory activity, emotionality and its vegetative manifestations, grooming, etc.) allow for the conclusion that thiowurtzine causes no sedation or euphoria in animals after discontinuation of its 5-day administration regimen with naloxone challenge test. A positive feature of this analgesic is its disinhibition effect due to the activated orientation and exploratory behavior in the afferentation conditions of the open-field test. It should be noted that dominant withdrawal syndrome components and recessive signs of withdrawal syndrome were not detected in the animals after the thiowurtzine administration, as opposed to the tramadol-dependent mice. The mice that received the analgesic did not show a decrease in body mass, which points to the absence of a toxic effect on the metabolism in the test animals. Discontinuation of the thiowurtzine administration with naxolone challenge test caused no hyperalgesia in the animals in the hot plate test.

The findings presented herein indicate that thiowurtzine evokes no side effects typical of opioid analgesics (tramadol), including physical dependence and withdrawal syndrome development following naloxone challenge test. Previous *in vivo* and *in silico* studies (docking, molecular modeling, molecular dynamics simulation) on the multi-target mechanism of thiowurtzine explain the absence of its morphine-like effect by the fact that the major targets of the analgesic are TRPA1 receptors and voltage-gated Ca^{2+} channels [6, 12]. With a high degree of probability, the conclusions made herein predict no drug abuse development when thiowurtzine is used in the clinical setting. The absence of ulcerotoxicity found earlier will enable to administer thiowurtzine in long-term cycles with no risk of gastrotoxicity for patients with chronic pain syndrome.

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Authors contribution

Krylova S.G. – conception and design of the experiments, participation in the experiments, critical revision of the manuscript for important intellectual content. Lopatina K.A. – carrying out of the experiments, statistical analysis, writing of the article. Zueva E.P. – critical revision of the manuscript for important intellectual content. Povetieva T.N. – conception and design of the experiments, participation in the experiments. Suslov N.I. – critical revision of the manuscript for important intellectual content. Nesterova Y.V. – participation in the experiments, statistical analysis. Afanasieva O.G., Kiseleva E.A., Kulpin P.V. – participation in the experiments. Sysolyatin S.V. – organization of the research object synthesis. Kulagina D.A. – provision of the research object and its synthesis. Zhdanov V.V. – analysis of the results of computer activity prediction, final approval of the manuscript for publication.

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Assessment of the functions of cultured lymphocytes when exposed to drugs used in cosmetology

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ABSTRACT

The aim of the study was to determine the number of lymphocytes, intracellular cytokines produced by lymphocytes, and the cell cycle of lymphocytes isolated from the blood of patients when exposed to various drugs, as well as to assess the functions of cultured lymphocytes when exposed to drugs *in vivo* and *in vitro*.

Materials and methods. The study involved lymphocytes isolated from the blood of healthy women under various conditions. At the first stage of the study, T-lymphocytes were isolated from the blood of patients before exposure to the drug. The absolute and relative lymphocyte count, the number of intracellular cytokines, and the cell cycle were determined.

At the second stage, the drugs were added to the nutrient medium, where lymphocytes isolated from the blood of patients who did not receive systemic drugs were cultured. The placental extract preparation was added to the lymphocytes isolated from the first group of patients, while the hyaluronic acid preparation was added to the lymphocytes isolated from the second group of patients.

At the third stage, the lymphocytes isolated from the blood of patients after systemic exposure to the placental extract preparation or hyaluronic acid preparation were isolated and cultured, after which the same lymphocyte parameters were determined.

Results. The number of T-lymphocytes increased with the systemic use of the placental extract and hyaluronic acid preparations and practically did not change compared with the baseline data, when these drugs were added to the nutrient medium. Placental extract and hyaluronic acid had a positive effect on the mitotic activity of cells; it is worth noting that the effect of placental extract was greater than that of hyaluronic acid. Both drugs did not have a negative effect on apoptosis of T-lymphocytes. Under the effect of placental extract, lymphocytes secreted more interleukins, which contributed to proliferation of keratinocytes.

Conclusion. The placental extract and hyaluronic acid preparations have a stimulating effect on keratinocytes. The placental extract preparation has a stimulating effect on T-lymphocytes after systemic exposure of the body to it.

Keywords: lymphocyte culture, intracellular cytokines, placental extract, hyaluronic acid

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Оценка функций культивированных лимфоцитов при воздействии препаратов, использующихся в косметологической практике

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РЕЗЮМЕ

Цель – определить количество лимфоцитов, внутриклеточных лимфоцитарных цитокинов, клеточный цикл лимфоцитов, выделенных из крови пациентов при воздействии различных препаратов, оценить функции культивированных лимфоцитов при воздействии препаратов *in vivo* и *in vitro*.

Материалы и методы. Исследованию подвергались лимфоциты, выделенные из крови здоровых женщин при различных условиях. На первом этапе исследования выделялись Т-лимфоциты из крови пациентов до воздействия препарата. Определялись: абсолютное и относительное количество лимфоцитов, внутриклеточные цитокины, клеточный цикл.

На втором этапе в питательную среду, где культивировались лимфоциты, выделенные из крови пациентов, которые не получали системно препараты, добавлялись препараты. К лимфоцитам первой группы пациентов в питательную среду добавлялся препарат экстракта плаценты, к лимфоцитам второй группы пациентов – препарат гиалуроновой кислоты.

На третьем этапе выделялись и культивировались лимфоциты, которые были выделены из крови пациентов после системного воздействия на организм препарата экстракта плаценты или препарата гиалуроновой кислоты, после чего определялись те же показатели лимфоцитов.

Результаты. Количество Т-лимфоцитов увеличивалось при системном использовании препаратов экстракта плаценты и гиалуроновой кислоты и практически не менялось по сравнению с исходными данными при добавлении этих препаратов в питательную среду. Экстракт плаценты и гиалуроновая кислота положительно влияют на митотическую активность клеток, экстракт плаценты в большей степени, чем гиалуроновая кислота. Оба препарата не оказывают негативного влияния на процессы апоптоза Т-лимфоцитов. При действии экстракта плаценты лимфоциты выделяют больше интерлейкинов, которые способствуют пролиферации кератиноцитов.

Заключение. Препараты экстракта плаценты и гиалуроновой кислоты оказывают стимулирующее действие на кератиноциты. Препарат экстракта плаценты оказывают стимулирующее действие на Т-лимфоциты при системном воздействии на организм.

Ключевые слова: культивация лимфоцитов, внутриклеточные цитокины, экстракт плаценты, гиалуроновая кислота

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

The use of drugs based on human placental extract is one of the actively studied and developing areas in aesthetic medicine and many other branches of medical science. The human placental extract is an active complex that encompasses amino acids, enzymes, including those for antioxidant defense, vitamins, minerals, growth factors, immunotropic substances, etc. There are published data on the stimulating effect of human placental extract preparations on regeneration of various tissues [1, 2], as well as on the regulating effect of the placental extract on inflammatory processes [3].

In cosmetology, hyaluronic acid preparations are widely used to improve the quality of the skin. Hyaluronic acid promotes water retention in the dermis, has a stimulating effect on fibroblasts, which leads to an increase in the number of collagen fibers in the dermis, and neutralizes the action of proinflammatory interleukins in the skin, which contribute to skin aging [4–7].

The literature presents an increasing amount of data on changes in the skin after exposure to drugs that promote rejuvenation, but there is practically no information about the immune response to administration of such drugs.

The aim of the study was to determine the number of lymphocytes, intracellular cytokines produced by lymphocytes, and the cell cycle of lymphocytes isolated from the blood of patients when exposed to various drugs, as well as to assess the functions of cultured lymphocytes when exposed to drugs *in vivo* and *in vitro*.

MATERIALS AND METHODS

The study involved lymphocytes isolated and cultured from the blood of healthy women under various conditions. At the first stage of the study, T-lymphocytes were isolated from the blood of patients before they were injected with placental extract and hyaluronic acid preparations. These lymphocytes were cultured in a nutrient medium, after which the absolute and relative lymphocyte count, the number of lymphocytes undergoing apoptosis and intracellular cytokines produced by lymphocytes, and the cell cycle were determined.

At the second stage, drugs were added to the nutrient medium where lymphocytes isolated from the blood of patients who did not receive the drugs systemically were cultured. A placental extract preparation was added to the nutrient medium with lymphocytes isolated from the first group of patients, and a hyaluronic acid preparation was added to the lymphocytes isolated from the second group of patients (exposure to the drugs *in vitro*). After a certain incubation period, the same parameters were determined as at the first stage of the study.

The third stage involved isolating and culturing lymphocytes from the blood of patients after systemic exposure of the body to the placental extract or hyaluronic acid preparation (exposure to the drugs *in vivo*), after which the same parameters of lymphocytes were measured.

The data obtained were subject to statistical processing. According to the Kolmogorov – Smirnov test, the sampling distribution was incorrect; therefore, nonparametric methods of statistical processing of the obtained data were used. The *Mo* mode, median, and interquartile range *Me* (*Q*25/ *Q*75) were determined. The calculation was carried out using the IBM SPSS Statistics 2 software packages.

RESULTS

At the first stage, the initial mean absolute cell count of T-lymphocytes isolated from the blood of patients who were not exposed to any drugs was 15,448 cells, and the relative value was 97.2%. After exposure to the placental extract preparation *in vitro*, these parameters practically did not change and amounted to 15,749 cells and 97.0%, respectively. When exposed to the placental extract preparation *in vivo*, the absolute cell count of T-lymphocytes increased significantly and amounted to 19,402.0 cells in the nutrient medium.

When exposed to hyaluronic acid, the absolute and relative cell count of T-lymphocytes decreased both *in vivo* and *in vitro*. *In vitro,* the number of T-lymphocytes was 14,888 cells, *in vivo* – 12,349 cells, which indicated a more pronounced decrease in the lymphocyte count with systemic exposure of the body to the hyaluronic acid preparation. The same tendency was observed in the change in the relative lymphocyte count: 95.3 and 95.1% in *in vitro* and *in vivo* exposure, respectively.

Evaluating the number of proliferating cells that were in the G2 stage and M stage of the cell cycle (the synthetic and mitotic phases, indicating active cell proliferation), we found an increase in this parameter in patients of both groups who received the placental extract and hyaluronic acid preparations. The increase occurred when the individuals were exposed to drugs both *in vitro* and *in vivo*. So, when exposed to the placental extract, the absolute number of proliferating cells increased from 143 to 276 *in vitro* and to 887 *in vivo*, and when exposed to hyaluronic acid, the cell count rose to 396 and 194 cells, respectively. If we compare the degree of increase when exposed to different drugs, we can note a more pronounced degree of cell growth during exposure to the placental extract, which is probably due to the immunomodulating properties of this preparation.

The relative value of proliferating cells also increased, and the tendency was the same as with the increase in the absolute count of proliferating T-lymphocytes. The preparations had practically no effect on the number of apoptotic cells. The absolute and relative lymphocyte count in apoptosis practically did not change when exposed to both preparations either *in vitro*, or *in vivo*, which may indicate that the placental extract and hyaluronic acid preparations did not have a negative effect on the body.

The DNA index, that is the ratio of cells whose cell cycle was in the G1 and G2 phases, practically did not change when exposed to different drugs. The results are presented in Table 1.

Table 1

The number of cultured lymphocytes and lymphocyte cell cycle parameters before and after exposure to drugs *in vitro* and *in vivo*

Parameter	Statistical parameters	Lymphocyte culture				
		without exposure, *n* = 12	with the addition of placental extract *in vitro*, *n* = 12	after systemic exposure to placental extract *in vivo*, *n* = 12	with the addition of hyaluronic acid *in vitro*, *n* = 12	after systemic exposure to hyaluronic acid *in vivo, n* = 12
Absolute count						
Normal cells (G0/G1)	Mo	2,480	15,874	8,708	11,153	14,333
	Me (*Q*25/*Q*75)	15,448 (1,476.0/1,570.0)	15,749 (13,704.0/17,169.0)	19,402 (12,184.0/46,790.0)**	14,888 (9,321/16,291)	12,349 (6,765/14,223) **
Proliferating cells (G2, M)	Mo	28	31	18	6	70
	Me (*Q*25/*Q*75)	143 (26.25/443.7)	276 (83.7/415.5)*	887 (242.0/1,556.0)**	396 (119.7/586.0)**	194 (80.2/432.7)
Apoptotic cells	Mo	10.0	4.0	0.8	42.0	4.0
	Me (*Q*25/*Q*75)	37 (3.75/55.7)	31.5 (10/53)	42 (4.5/81.5)	19.5 (4.5/42.5)	37.5 (14/66)
Relative count, %						
Normal cells (G0/G1)	Mo	95.2	97.8	82.7	62.3	82.1
	Me (*Q*25/*Q*75)	97.2 (95.2/98.7)	97 (93.6/97.8)	96.4 (85.6/99.2)	95.3 (90.7/97.3)	95.1 (89.0/97.2)
Proliferating cells (G2, M)	Mo	0.1	0.2	0.1	0.2	0.51
	Me (*Q*25/*Q*75)	0.15 (0.1/0.91)	0.58 (0.17/1.62) **	1.4 (0.42/4.35) **	0.64 (0.31/1.22) **	0.87 (0.62/1.32) **
Apoptotic cells	Mo	0.1	0.1	0.3	0.3	0.4
	Me (*Q*25/*Q*75)	0.3 (0.1/2.3)	0.4 (0.1/1.67)	0.3 (0.15/1.45)	0.35 (0.3/0.55)	0.55 (0.32/0.9)
DNA index (G1/G2)	Mo	1.93	1.96	1.67	1.96	0.97
	Me (*Q*25/*Q*75)	1.93 (1.84/2.08)	1.97 (1.91/12.11)	2.01 (1.9/6.52)	1.97 (1.96/2)	1.96 (1.85/2)

** $p < 0.01$, *$p < 0.05$ – significance of differences between the parameters before and after exposure to drugs *in vitro* and *in vivo*.

When assessing the level of intracellular cytokines that cultured T-lymphocytes secreted into the nutrient medium, we found a decrease in the content of interleukin (IL)-1b produced by the CD4+ subpopulation. A decrease in the level of this cytokine occurred when both the placental extract and hyaluronic acid were added to the nutrient medium. The lymphocyte subpopulation with the CD8+ phenotype produced this cytokine more with the addition of placental extract than with the addition of hyaluronic acid. Interleukin IL-17A increased in the nutrient medium where lymphocytes with the CD4+ phenotype were cultured with the addition of placental extract and decreased when hyaluronic acid was added. The content of interleukin IL-17A produced by lymphocytes with the CD8+ phenotype increased with the addition of placental extract and hyaluronic acid. The data are presented in Table 2.

Table 2

The number of intracellular cytokines before exposure to drugs and after adding drugs to the nutrient medium

Parameter	Statistical parameter	Spontaneous cytokine production		
		by lymphocytes, *n* = 12	with the addition of placental extract to the medium, *n* = 12	with the addition of hyaluronic acid to the medium, *n* = 12
CD4+(IL1b)	Mo	4.0	25.0	3.0
	Me (*Q*25/*Q*75)	12.0 (4.0/61.25)	8.0 (3.75/26.75)**	8.5 (4.5/56.0)**
CD8+(IL1b)	Mo	7.0	17.0	7.0
	Me (*Q*25/*Q*75)	8.5 (2.0/20.25)	10.5 (6.25/17.0) **	7.0 (6.0/7.5)
CD4+(IL-17A)	Mo	1.0	2.0	4.0
	Me (*Q*25/*Q*75)	6.0 (1.0/19.0)	9.0 (6.5/24.0)**	4.0 (3.0/4.0)**
CD8+(IL-17A)	Mo	4.0	8.0	9.0
	Me (*Q*25/*Q*75)	6.0 (4.0/10.0)	8.5 (7.75/18.0)*	9.0 (9.0/12.0)*

**$p < 0.01$, *$p < 0.05$ – significance of differences between the parameters before and after exposure to drugs *in vitro* and *in vivo*.

DISCUSSION

The number of T-lymphocytes upon exposure to placental extract increased *in vivo* and practically did not change *in vitro*. This may indicate that the described immunomodulating effect of the placental extract will be realized under the condition of a complex effect on the body immune system, where all the links of the immune system are connected with each other and are in close interaction.

On the other hand, the number of lymphocytes upon exposure to hyaluronic acid decreased *in vivo* and did not change *in vitro*. Apparently, this is due to the need to eliminate a foreign substance, which is hyaluronic acid, administered via an intradermal injection, and lymphocytes are consumed in this process.

Both placental extract and hyaluronic acid stimulate mitosis and cell proliferation. With the systemic effect of the placental extract, this process is more active in comparison with the systemic effect of hyaluronic acid, which may be due to a more pronounced positive effect of the placental extract on the immune system and the body as a whole. These drugs have no effect on apoptosis of lymphocytes, which may indicate safety of these drugs for the immune system.

Estimating the content of interleukins that have a stimulating effect on proliferation of keratinocytes, it can be said that the total level of IL-1b and IL-17A increases to a greater extent under the effect of placental extract than hyaluronic acid. On this basis, we can speculate about greater effectiveness of the placental extract in relation to skin cell renewal, although hyaluronic acid has a similar effect.

CONCLUSION

Placental extract has a stimulating effect on lymphocytes upon systemic exposure of the body to it. The results of the study allow us to speculate about the stimulating effect of placental extract and hyaluronic acid not only on the immune system, but also on the quality of the skin.

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The impact of affective disorders on self-assessment of the quality of life in patients with chronic coronary artery disease

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ABSTRACT

Aim. To determine the differences in self-assessment of social functioning by patients with chronic coronary artery disease (CAD), depending on the presence of comorbid affective disorders (ADs).

Materials and methods. Using the Social Adaptation Self-Evaluation Scale (SASS), which makes it possible to assess the degree of social functioning and satisfaction with various aspects of social life, we studied the features of the social functioning of heart hospital patients with chronic CAD with (n = 248) and without AD (n = 291). In 290 patients (average age 56.6 ± 6.7 years) with chronic CAD, chronic ADs (45%) were revealed; depressive episodes (DEs) were diagnosed for the first time in 24% of patients, and 24.5% of patients had recurrent DEs. Bipolar disorder was found in 6.5% of cases. Qualitative and quantitative parameters were investigated using the Mann – Whitney U test and Student's t-test. To assess the frequencies, the Pearson's chi-squared test was used.

Results. The mean total SASS score in the patients with chronic CAD with AD corresponded to difficult social adaptation (33.7 [29.5; 39]), while the patients without AD had good social adaptation score of 40.8 ± 6.3 ($p < 0.05$). In the group without AD, patients with normal social adaptation prevailed (n = 215; 73.8%), while patients with AD more often had difficulties with social adaptation (n = 148; 59.7%). In the CAD patients, depending on the presence of AD, the frequency of disturbances in various spheres of social adaptation differed: employment, interest in and pleasure from activities, disposition of income, pleasure from and interest in seeking information, social support ($p = 0.001$).

Conclusion. Higher frequency of pronounced impairment in social functioning in patients with chronic CAD with AD determines the need for taking this fact into consideration when planning rehabilitation measures in this group of patients.

Keywords: affective disorders, chronic coronary artery disease, self-assessment of quality of life and social functioning, gender differences

Conflict of interests. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

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Conformity with the principles of ethics. All patients signed an informed consent. The study was approved by the local Ethics Committee at the Mental Health Research Institute of Tomsk NRMC of RAS (Protocol No. 115 of 26.11.2018).

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Влияние аффективных расстройств на самооценку качества жизни у больных хронической ишемической болезнью сердца

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РЕЗЮМЕ

Цель – определить различия самооценки социального функционирования пациентов с хронической коронарной болезнью в зависимости от наличия коморбидных аффективных расстройств (АР).

Материалы и методы. С помощью опросника самооценки социальной адаптации (SASS), позволяющего оценить уровень социального функционирования и удовлетворенность различными аспектами социальной жизни, изучены особенности социального функционирования больных кардиологического стационара с хронической ишемической болезнью сердца (ИБС) с АР (n = 248) и без (n = 291). У 290 пациентов (средний возраст – 56,6 ± 6,7 лет) с хронической ИБС выявлялись хронические АР (45%), депрессивные эпизоды (ДЭ) впервые возникшие диагностированы у 24% пациентов, а рекуррентные ДЭ – у 24,5%. В 6,5% случаев обнаружено биполярное аффективное расстройство. Качественные и количественные показатели исследованы с помощью U-критерия Манна – Уитни и T-критерия, для оценки частот применялся метод $\chi 2$ по Пирсону.

Результаты. Общий средний балл по SASS у больных хронической ИБС с АР соответствовал уровню затрудненной социальной адаптации 33,7 [29,5; 39], а группе без АР – хорошей социальной адаптации 40,8 ± 6,3 (p < 0,05). В группе без АР преобладали пациенты с нормальной социальной адаптацией (n = 215; 73,8%), а у больных с АР социальная адаптация была чаще затруднена (n = 148; 59,7%). У пациентов хронической ИБС в зависимости от АР различалась частота нарушений в разных сферах социальной адаптации: занятость, интерес и удовольствие от деятельности, распоряжение своими доходами, удовольствие и интерес от поиска информации, социальная поддержка (p = 0,001).

Заключение. Более высокая частота выраженного нарушения социального функционирования пациентов с хронической ИБС и АР определяет необходимость учета данного факта при планировании реабилитационных мероприятий в данной группе пациентов.

Ключевые слова: аффективные расстройства, хроническая ишемическая болезнь сердца, качество жизни, половые различия

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Socio-demographic and psychosocial factors contribute to the development of depression and cardiovascular diseases (CVDs). Since psychosocial risk factors (RFs) influence the prognosis of CVDs, their screening using standardized questionnaires in persons with high cardiovascular risk (CVR) and patients with CVDs with subsequent correction is of great importance [1]. Depression in patients with coronary artery disease (CAD) is the most significant factor determining their quality of life [2–4].

In the third National Health and Nutrition Examination Survey (NHANES III), major depression or a suicide attempt in the medical history is associated with a practically 15-fold increased risk of developing CAD in females and more than 3-fold increased risk in males [5]. A prospective study of mental health detected that females under 40 years with depression have a 6-fold increased risk of developing CAD than females of the same age without depression, and depression appeared to be not associated with CAD in males and the elderly. On the whole, scientific data confirm a closer association of depression and CAD in females of a younger age.

Recent reviews concerning the association between depression and CAD were limited by unavailability of data about the course of depression. Mainly, they assumed an association between specific biological or socio-demographic variables and depression at the initial level, however, a response to antidepressant therapy in patients with concomitant CAD was studied less [6, 7]. Besides, many RFs for coronary pathology are known to be markers of depression and low response to antidepressant therapy, for example, elevated inflammatory markers, absence of physical activity, and thyroid disorders [8].

F. Vitinius et al. [9] detected an association of an older age, absence of data on experienced myocardial infarction, and higher heart rate variability with a favorable outcome of depression. At the same time, hyperuricemia, high triglyceride level, NYHA functional class III, state after resuscitation, and the use of thyroid hormones, antidiabetic drugs, pain relievers, beta-blockers, and antihyperuricemic and anticholinergic agents had an adverse impact on the depression score according to the Depression Subscale of the Hospital Anxiety and Depression Scale (HADS-D) [10]. When evaluating complete blood count and blood biochemistry tests, no significant differences were found between the groups, and concomitant diseases did not have a significant impact on the results. Studies on the contribution of affective disorders (AD) to self-assessment of social functioning and quality of life in patients with chronic CAD are few.

The aim of the study was to determine the differences in self-assessment of social functioning and quality of life in patients with chronic CAD with concomitant AD and without it.

MATERIALS AND METHODS

At a heart hospital, two groups of patients with chronic CAD were formed: group 1 – with detected AD (n = 248), group 2 – without AD (n = 291). In 290 patients (under 70 years) with chronic CAD, chronic ADs (45%) were detected, newly identified depressive episodes (Des) were diagnosed in 24% of patients, and recurrent DEs – in 24.5% of cases. In 6.5%, of cases bipolar disorder was detected. In 91.7% of cases, depression was accompanied with anxiety (54.8%) [11]. 248 patients from this group filled in the self-evaluation scale of social adaptation, of them 194 individuals were males (78.2%) and 54 – females (21.8%). The average age in the AD group was 56.6 ± 6.7 years. The average age of males was 57.2 ± 6.5 years, and the average age of females was 59.3 ± 7.1 years (p = 0.04).

According to the Beck's Depression Inventory, the depression score in the AD group was 9 [6; 13], which corresponded to a clinically significant level. The severity of anxiety in the AD group reached 38 according to the Sheehan Patient-Rated Anxiety Scale [22,5; 58]. AD lasted 10 [1.5; 20] years. CAD more often developed against the background of a manifested AD.

Group 2 included patients with chronic CAD without AD (n = 291) under 70 years. The average age in the group without AD was 55.8 ± 7.1 years. Males in group 2 prevailed (84.5%, n = 246), females com-

prised 15.5% (n = 45). The average age of males in group 2 was 56.9 ± 6.9 years, and the average age of females was 60.7 ± 6.4 years (p = 0.001).

The mean score according to the Beck's Depression Inventory in this group was less than 6, namely 2 [1; 3], which was clinically insignificant, no anxiety and depressive disorders (F3, F4, F06.3-F06.4) were detected. The severity of anxiety according to the Sheehan Patient-Rated Anxiety Scale in the group without AD scored 17 [11; 27], which corresponds to its absence as a clinical syndrome.

Groups 1 and 2 did not differ in sex, age, and severity of the physical condition (functional class of angina of effort (FC AE) and functional class of chronic heart failure (FC CHF), presence of concomitant diseases (transient ischemic attack (TIA) / acute cerebrovascular accident (ACVA)). However, they differed in the presence of glucose tolerance disorder (GTD) and diabetes mellitus (DM), the method of CAD therapy, recentness of myocardial infarction (postinfarction cardiosclerosis (PICS)), and exercise tolerance (ET) with veloergometry (VEM) (Table 1).

Table 1

Comparative characteristics of some aspects of the physical state of patients with chronic CAD with and without AD

Parameter	Group 1 (CAD + AD)	Group 2 (CAD)	p
Functional class of angina of effort, *Me* [Q_1; Q_3]	2 [2; 3]	2 [2; 3]	–
Functional class of CHF, *Me* [Q_1; Q_3]	2 [1; 3]	2 [1; 3]	–
Duration of CAD, years, *Me* [Q_1; Q_3]	3.5 [1.5; 8]	3.5 [1.5; 8]	–
The incidence of PICS, %	73.6 (183/248)	66.3 (193/291)	–
Recentness of PICS, months, *Me* [Q_1; Q_3]	21 [5; 60]	33 [11; 72]	**
LV EF, %, *Me* [Q_1; Q_3]	64 (52; 86]	62 [53; 65]	–
ET, Watt, *Me* [Q_1; Q_3]	25 [25; 50]	50 [25; 75]	**
Six-minute walk test, m, *Me* [Q_1; Q_3]	350 [250; 432]	450 [350; 500]	–
The incidence of GTD (or DM), %	32.9 (or 166)	24.3 (or 220)	**
The incidence of TIA (or ACVA), %	6.5 (or 232)	3.4 (or 281)	–
Frequency of conservative treatment for CAD, %	31.5 (78/248)	5.8 (17/291)	***

Note: LV EF – left ventricular ejection fraction.
** $p < 0.05$; *** $p < 0.001$.

The groups did not differ in gender (p = 0.07). GTD and DM were statistically significantly more often absent in patients without AD – 75.7% vs. 67.1% in the group with AD ($p < 0.05$). ACVA and TIA were more often revealed in the group with AD, 6.5% vs. 3.4% in the group without AD ($p > 0.05$). Endovascular interventions for CAD treatment were significantly more often used in the group without AD, while patients with AD usually received conservative treatment (31.5% vs. 5.8% in individuals without AD, $p < 0.001$).

To study self-evaluation of social adaptation, a scale with the same name was used, which allowed to assess subjective evaluation of patients' satisfaction with various spheres of their life and their level of social functioning [12]. Statistical processing of data was performed using Statistica 8.0 software (StatSoft Inc., USA).

Parametric parameters were assessed using the Student's t-test for independent samples (with a normal distribution of variables); nonparametric parameters were analyzed using the Mann – Whitney U test. To assess the frequencies, the Pearson's chi-squared test was used.

RESULTS

The mean total SASS score in the patients with chronic CAD with AD corresponded to difficulties in social adaptation (33.7 [29.5; 39]), while the patients without AD had good social adaptation score of 40.8 ± 6.3, which showed statistically significant differences ($p < 0.05$). In the group without AD, patients with normal social adaptation prevailed (73.8%), and patients with AD more often had difficulties in social adaptation (59.7%) (Table 2). 2.8% of patients with AD and CAD were characterized by social maladaptation, 37.5% of patients had normal adaptation in society.

Below, parameters of self-assessment and the presence of parameters of social functioning and quality of life according to the SASS in the study groups are presented (Table 3).

Table 2

Differences in the level of social adaptation in patients with chronic CAD with and without AD

Level of social adaptation	Social maladaptation (score up to 22)	Difficulties in adaptation (score 23–35)	Normal adaptation (score 36–52)	Very good adaptation (score of more than 53)
Group 1	2.8% (7/248)	59.7% (148/248)	37.5% (93/248)	0
Group 2	0	21.4% (62/291)	73.8% (215/291)	4.8% (14/291)

Table 3

Differences in self-assessment of parameters of social functioning and satisfaction with various spheres of life between patients with chronic CAD depending on the presence of AD

Parameter	Presence of the symptom according to self-assessment data in the patients with CAD and AD	Presence of the symptom according to self-assessment data in the patients with CAD without AD	*p*
Employment / unemployment	56.0% (139/248)	41.4% (120/291)	***
Interest in employment	39.5% (98/248)	15.7% (46/291)	***
Satisfaction with employment	47.7% (118/248)	25.7% (75/291)	***
Interest in hobbies	28.4% (70/248)	5.7% (17/291)	***
Quality of spare time	69.3% (172/248)	28.6% (83/291)	***
Seeking communication with family	25.0% (62/248)	8.6% (25/291)	***
Assessment of family relationships	27.3% (68/248)	7.1% (21/291)	***
Number of contacts outside the family	38.6% (96/248)	21.8% (63/291)	***
Active behavior in relationships outside the family	78.4% (194/248)	58.6% (171/291)	***
Assessment of relationships with other people on the whole	29.5% (73/248)	17.1% (50/291)	n/a
Appreciation of relationships with others	25.0% (62/248)	8.5% (25/291)	***
The frequency of other people's seeking communication with the patient	18.6% (46/248)	32.9% (96/291)	***
Patient's compliance with social norms	6.8% (17/248)	0% (0/291)	***
Involvement in community activities	69.6% (173/248)	64.3% (187/291)	n/a
Satisfaction from search for information	39.8% (99/248)	25.7% (75/291)	***
Interest in acquiring information	34.1% (85/248)	21.4% (62/191)	***
Difficulties in expressing an opinion	10.2% (25/248)	5.7% (17/291)	***
Feeling rejected	4.5% (11/248)	0% (0/291)	***
Importance of physical attractiveness	47.7% (118/248)	35.7% (104/291)	**
Difficulties in disposing income	31.9% (79/248)	11.4% (33/291)	***
The ability to control life	52.2% (129/248)	38.6% (112/291)	***

Note: the studied phenomenon is presented as moderately or significantly pronounced (according to the SASS, 1 and 0 points). N/a – not available.

In patients with chronic CAD, depending on the presence of AD, the frequency of essential disturbances in various aspects of social adaptation differed significantly ($\boldsymbol{p} < 0.001$). When analyzing the ability to work in the group with AD, it was revealed that disability was found in 18.7% (46 / 248) of patients, 40.7% (101 / 248) of patients were temporarily disabled, 30.8% (76 / 248) of individuals were retired, and 9.9% (25 / 248) of patients did not work.

In the group without AD, the ability to work differed as follows: disability was found in 12.1% (35 / 291) of patients, 63.8% (186 / 291) of patients were temporarily disabled, 10.3% (30 / 291) of individuals were retired, and 8.6% (25 / 291) did not work. Thus, in the group with AD, retired individuals were revealed statistically significantly more often ($p < 0.05$), and in the group without AD, the patients statistically significantly more often ($p < 0.05$) were temporarily disabled.

Employment, interest in it, and satisfaction from work also statistically significantly differed in the groups. 31.9% of the patients with AD and 11.4% of individuals without AD stated that they "often" or "always" experience difficulties in disposing their income.

The assessment of the quality of spare time appeared to be significantly low (69.3% of persons with AD vs. 28.6% without AD). Up to 39.8% of patients

with AD and 25.7% of persons without AD reported a significant decrease in satisfaction from and interest in searching for information. The sphere of hobbies had essential disturbances (28.4% of persons with AD vs. 5.7% of patients without AD). It may be due to a decrease in hedonism, as well as limited activity and exercise tolerance.

Before the assessment of satisfaction with social relationships, marital status and the presence of children were investigated. In the group without AD, 88.7% (256 / 291) of patients were married and 2% of patients were childless. In the group with AD, 77.2% (191 / 248) of patients had a partner, and 11.1% of patients had no children (females statistically significantly more often than males lost their children or were childless). Females more seldom had a spouse ($p = 0.03$). It may be associated with the fact that females more seldom were married repeatedly than males ($p = 0.02$).

38.6% of patients with CAD and AD were not satisfied with relations in the family, as opposed to 21.8% of patients without AD. A quarter of the patients with AD did not seek communication and support in the family (vs. 8.6% in group 2).

A quarter of patients with CAD and concomitant depression regarded relations with other people insignificant (as opposed to 8.5% of CAD patients without depression). 29.5% of patients with AD negatively assessed relations with other people on the whole, compared with 17.1% of individuals in the group without AD. Probably, due to this fact, 78.4% of persons with AD (vs. 58.6% of people without AD) were not active in relations outside the family.

47.7% of patients with AD and 35.7% of individuals without AD reported unimportance of their physical attractiveness as one of the self-assessment parameters. 10.2% of persons with AD and 5.7% of patients without AD identified difficulties in expressing their opinion. 32.9% of patients with AD, predominantly males (vs. 18.6% of patients without AD), thought that people around were not willing to communicate with them. The level of involvement in community activities was low in both groups without statistically significant differences (69.6% vs. 64.3%; $p > 0.05$). At the same time, a feeling of being rejected was noted by 4.5% of patients with AD and no one from the group without AD. Non-compliance with social norms was reported by 6.8% of patients with AD and no one in the group without AD. These data confirm pronounced passive-aggressive behavior and negativism in patients with AD.

52.2% of persons with AD (vs. 38.6% of patients without AD) had a significantly decreased ability to shape their close circle of friends according to their desire. These disturbances may be associated with AD symptoms based on anergia and low mood and anhedonia associated with it. Patients with CAD and AD are characterized by disturbances of social functioning in many spheres of life, which are more severe and more frequent than in patients with chronic CAD without AD.

DISCUSSION

Mental disorders lead to profound changes in most parameters of the quality of life. Parameter values of self-assessment of social functioning in these patients are worse than in patients with CVDs [13]. Depressive disorders affect the quality of life more than other mental diseases. In mood disorders, the social role and social activity are impaired, and recovery from concomitant physical health disorders decreases. Depressive rumination, pessimism, and passivity prevent emergence of compliance (patients do not adhere to doctor's prescriptions, do not participate in rehabilitation activities, and recover more slowly). In the group of patients with chronic CAD and AD, retired and disabled individuals are revealed significantly more often ($p < 0.05$), and in the group without AD patients are statistically significantly more often ($p < 0.05$) temporarily disabled.

Doctors and patients assess professional and social aspects of the quality of life differently, opposite to identification of the clinical and physical status. The revealed differences in the quality of life influence the diagnostic approach and subsequent treatment strategy. Therefore, the use of self-assessment questionnaires at runtime is important in the assessment of psychosocial functioning when measuring the quality of life. The use of clinical scaling systems allows to register the severity of the disease, the efficiency of the therapy, and adverse effects of drugs.

In patients with chronic CAD and AD, low involvement in community activities, small circle of friends, and distrustful and hostile attitude to surrounding people are revealed, which deprives them of the opportunity to receive social support in stressful situations and during illness. For lonely people, deprived of the support from relatives, relationships outside the family are essential. Weak ties outside the family do not allow them to receive necessary support from their friends or other people. The presence of social sup-

port essentially lowers or reduces to a minimum negative influence of distress and positively affects mental health.

The conducted study shows a relationship between depressive disorders and satisfaction with life and the level of social functioning [14]. Depressive disorders significantly reduce the level of social functioning and the quality of life in patients, especially communication with people around and the ability to shape their inner circle as they wish. The present study allows to suppose that AD may result in the development of CAD in females of a younger age who face adverse events in their lives and have chronic stress and no social support. Social functioning may be considered as an external factor in self-assessment of the quality of life. Relationships, work, and spare time have principal effects on the quality of life.

Patients with chronic CAD, depending on the presence of AD, were characterized by differences ($p < 0.001$) in the frequency of moderate or severe disturbances in such spheres of social adaptation as work, disposition of income, interest in and satisfaction with activities, search for information, interests and hobbies, quality of spare time, and physical unattractiveness. This also concerned social support (absence of a spouse, childlessness, and loss of children). The patients with AD had statistically significant differences in dissatisfaction with family relationships, unwillingness to seek communication and support, devaluation of relationships with people and attributing negative attitudes to others (it was mostly typical of males with AD), and inability to shape the inner circle as they wish.

The ability to interact with people around; choice of situations according to needs, feeling of autonomy and healthy self-esteem; a mission to realize the potential; friendly relationships with others; and the presence of a goal in life are essential for satisfaction with life [15]. A direct correlation of these components with satisfaction with life and assessments of happiness and depression was revealed. Patients with CAD and AD showed low satisfaction with some spheres of life and pronounced disturbances of social functioning. It is necessary to consider these factors in treatment of CAD and planning of rehabilitation measures in this group of patients. Patients' self-assessment of their state not always coincides with the objective severity of clinical symptoms, but it should be used for assessing the efficiency of the conducted measures.

CONCLUSION

Low involvement in social activities was typical of both groups of patients with chronic CAD, irrespective of the presence of AD. Social functioning and self-assessment of the quality of life by patients with AD and chronic CAD were significantly more often impaired, compared with CAD patients without depression. They more often had no support from a spouse and offspring (experienced loss of children or were childless). In patients with chronic CAD, depending on the presence of AD, significant differences were noted in the frequency of disturbances in different spheres of social adaptation ($p < 0.001$), such as work, satisfaction from work and spare time, disposition of income, social support, dissatisfaction with relationships (in family and society) with devaluation of relationships with people and projection, physical attractiveness, and ability to control the life.

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Authors contribution

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Impact of immunotherapy with autologous activated T-lymphocytes on clinical parameters and quality of life in patients with allergic bronchial asthma

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ABSTRACT

Aim. To assess the impact of autologous activated T-lymphocyte immunotherapy on clinical parameters and quality of life in patients with allergic bronchial asthma (BA) in comparison with patients with allergic BA who received standard therapy.

Materials and methods. A non-randomized, pilot study included 19 patients with allergic BA of moderate severity (7 men and 12 women aged 23–61 years, average age – 38.5 ± 4.3 years) who received the T-cell vaccine (n = 12) and standard therapy with inhaled glucocorticoids, short- and long-acting β2-adrenergic agonists (n = 7). After signing an informed consent, the patients were subcutaneously injected with autologous activated T-lymphocytes with a frequency of 4 injections 1 time / week, and then 6 injections 1 time / month. The research methods included asthma control measurement according to the ACQ-5 questionnaire and quality of life assessment according to the AQLQ(S) questionnaire. Clinical data were collected during lung function tests and by measuring the total immunoglobulin E (IgE) level.

Results. In the course of the study, the immunotherapy was well tolerated, no systemic adverse reactions were noted. The treatment approach in the patients who received the T-cell vaccine resulted in significant improvement of asthma control parameters (according to the ACQ-5 questionnaire) and parameters of the patients' quality of life (according to the AQLQ(S) questionnaire) within all 4 categories. Besides, their lung function improved by the end of treatment, and the total IgE level decreased. No significant changes in these parameters were observed during the follow-up in patients who received standard therapy. The study was conducted before immunotherapy, after 2 months (after 5 injections), and after 7 months (after 10 injections).

Conclusion. Evaluation of the impact of immunotherapy with autologous activated T-lymphocytes on the clinical parameters and quality of life in patients with BA indicates effectiveness of treatment in patients with allergic BA.

Keywords: bronchial asthma, T-cell therapy, activated T-cells, patients' quality of life, lung function, immunoglobulin E

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

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Влияние иммунотерапии активированными аутологичными Т-лимфоцитами на клинические параметры и качество жизни пациентов с аллергической формой бронхиальной астмы

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РЕЗЮМЕ

Цель. Оценить влияние иммунотерапии активированными аутологичными Т-лимфоцитами на клинические параметры и качество жизни пациентов с аллергической формой бронхиальной астмы (БА) и сравнить с пациентами с аллергической формой БА, получавшими стандартную терапию.

Материалы и методы. В нерандомизированное пилотное исследование включены 19 пациентов с аллергической формой БА (7 мужчин и 12 женщин в возрасте от 23 лет до 61 года, средний возраст – 38,5 ± 4,3 лет) со средней степенью тяжести, получавших Т-клеточную вакцину (n = 12) и стандартную терапию ингаляционными глюкокортикостероидами, β2-адреномиметиками короткого и длительного действия (n = 7). Пациентам после получения информированного согласия вводились аутологичные активированные Т-лимфоциты подкожно с кратностью 4 инъекции 1 раз/нед, а затем 6 инъекций 1 раз/мес. Методы исследования включали оценку степени контроля над астмой по опроснику ACQ(5), оценку качества жизни больных БА по опроснику AQLQ(S). Определение клинических параметров оценивалось путем измерения функции внешнего дыхания и уровня общего иммуноглобулина Е (IgE).

Результаты. В ходе исследования была отмечена хорошая переносимость иммунотерапии, системные побочные реакции отсутствовали. При использовании данного метода показано достоверное улучшение показателей контроля над астмой (по опроснику ACQ5) и качества жизни пациентов (по опроснику AQLQ(S)) по всем четырем сферам влияния, а также увеличение уровня функции внешнего дыхания к окончанию лечения, снижение уровня общего IgE у пациентов, получавших Т-клеточную вакцинацию. У пациентов со стандартной терапией достоверных изменений данных показателей за период наблюдения не отмечено. Исследование проводилось до иммунотерапии, через 2 мес (после 5 инъекций) и через 7 мес (после 10 инъекций).

Заключение. Проведенная оценка влияния иммунотерапии активированными аутологичными Т-лимфоцитами на клинические параметры и качество жизни пациентов с БА свидетельствует об эффективности проведенного лечения у пациентов с аллергической формой заболевания.

Ключевые слова: бронхиальная астма, Т-клеточная терапия, активированные Т-клетки, качество жизни пациентов, функция внешнего дыхания, иммуноглобулин Е

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INTRODUCTION

Bronchial asthma (BA) is a chronic inflammatory disease of the respiratory tract, the pathogenesis of which involves a number of immunocompetent cells and inflammatory mediators, leading to specific pathophysiological changes [1]. Currently, statistics show that about 235 million people worldwide suffer from BA [2]. According to the latest estimates of the World Health Organization, released in December 2016, there were 383,000 asthma-related deaths around the world [2]. The majority of BA patients respond well to conventional therapy, achieving disease control. However, a significant proportion of patients (20–30%) are refractory to standard therapy [3].

It is necessary to search for new approaches to treatment that provide patients with the optimal quality of BA therapy. Regulatory T cells, which play a suppressive role in the immune response, are of great importance in the pathogenesis of BA. In addition to genetic predisposition to IgE hyperproduction and imbalance of Th2 / Th1 cells, a functional decline of regulatory T cells contributes to the development of allergic reactions. Regulatory cells exert a suppressive effect by inhibiting T-lymphocytes and B-lymphocytes, suppressing the production of proinflammatory cytokines and secreting transforming growth factor (TGF)-β and interleukin (IL)-10 [4]. Direct and indirect targets of regulatory T cells include dendritic cells, T-helpers, B-lymphocytes, IgE-producing cells, mast cells, basophils, and neutrophils. One of the innovative treatment approaches is cellular immunotherapy, based, in particular, on injection of autologous activated T cells [5]. The mechanism of action of immunotherapy is based on recognition and killing of T cells carrying activation marker determinants, which enhances the natural regulatory mechanisms in relation to regulatory T cells [6]. This approach has been shown to be effective in regulating allergic inflammation [5].

The aim of the study was to assess the effect of immunotherapy with autologous activated T-lymphocytes on the clinical parameters and quality of life in patients with allergic BA in comparison with patients in the control group receiving standard therapy.

MATERIALS AND METHODS

The study included 19 patients (7 men and 12 women aged 23–61 years, average age – 38.5 ± 4.3 years) with allergic BA of moderate severity, who were treated at the Immunopathology Clinic of the Research Institute of Fundamental and Clinical Immunology. The patients were receiving standard therapy during 12 weeks prior to enrollment in the study. Inhaled glucocorticoids (IGCs) in combination with long-acting β2-adrenergic agonists and antileukotriene drugs were used as backbone therapy. Inclusion criteria were the age range from 18 to 65 years inclusive; the diagnosis of BA verified at least 12 months prior to inclusion in the study; the presence of backbone IGC therapy for at least 12 weeks; a lack of complete control of BA; forced expiratory volume in one second (FEV1) of 50–90%; the absence of chronic infectious and autoimmune diseases and cancer.

The method for obtaining autologous activated T-lymphocytes from peripheral blood was described earlier [7]. In addition to standard backbone BA therapy, patients were administered autologous activated T-lymphocytes subcutaneously 1 time per week (4 injections), followed by 1 time per month (6 injections).

Evaluation methods to identify the clinical efficacy of immunotherapy included assessment of asthma control parameters (according to the ACQ-5 questionnaire) and quality of life parameters (according to the AQLQ(S) questionnaire), lung function tests and determination of the total IgE level. The study was conducted before the immunotherapy, after 2 months (5 injections), and after 7 months (10 injections). The volume and rate of respiration were measured to analyze the external respiration function. The survey data were recorded on a personal computer using the Spida_5 diagnostic software program. Serum IgE levels were assessed by enzyme-linked immunosorbent assay (ELISA). Statistical processing of quantitative data was carried out using Statistica software, version 6.0. The sample was tested for normal distribution using the Shapiro – Wilk test (for small samples). The data were presented as the median and interquartile range $Me\ (Q_{25};\ Q_{75})$, as well as the mean and its error $M \pm m$. To evaluate the research results, the nonparametric Mann – Whitney and Wilcoxon tests were used. The differences were considered statistically significant at $p < 0.05$.

RESULTS

During treatment with autologous activated T-lymphocytes, 2 out of 12 patients experienced single local adverse reactions, such as hyperemia, swelling, and pain at the injection site, which disappeared without a trace within a few hours.

The changes in the clinical parameters resulted in significant improvement of asthma control parameters according to the ACQ-5 questionnaire (Table 1). The mean score before treatment was 2.02; which, according to the ACQ-5 and ACT scores and the level of asthma control according to the Global Initiative for Asthma (GINA), corresponds to uncontrolled BA [8]. The mean score after treatment was 1.27, which corresponds to partially controlled BA [8]. The difference of 0.5 points is clinically significant during treatment [8].

Table 1

Assessment of asthma control parameters according to the ACQ-5 questionnaire during therapy with autologous activated T-lymphocytes, *M* ± *m*

Parameter	Comparison groups of patients with allergic BA					
	T cell therapy, *n* = 12			Standard treatment, *n* = 7		
	Point 1 (before the treatment)	Point 2 (after 2 months – 5 injections)	Point 3 (after 7 months – 10 injections)	Point 1 (before the treatment)	Point 2 (after 2 months – 5 injections)	Point 3 (after 7 months – 10 injections)
ACQ-5	2.02 ± 0.46	1.53 ± 0.35	1.27 ± 0.39*	2.23 ± 0.48	2.26 ± 0.47	2.00 ± 0.65

* significant differences compared with the parameter before the therapy, $p = 0.02$.

The results of quality of life assessment in patients with allergic BA during immunotherapy according to the AQLQ(S) questionnaire are presented in Table 2. Friedman's test of variance showed that in the group of patients receiving immunotherapy, a significant change in parameters was noted by the end of treatment in all categories of the questionnaire, such as "Symptoms", "Activity limitation", "Emotional sphere", and "Environmental influence". In addition, in the course of therapy, the score in the "Activity limitation" category significantly increased compared with the parameter 2 months after the start of therapy.

The level of serum IgE in patients with allergic BA receiving immunotherapy significantly decreased after 2 months of such treatment and continued to significantly decrease after 7 months (Table 3). In the control group receiving standard therapy, no significant changes by the end of treatment were revealed.

Table 2

Assessment of the quality of life in patients with BA according to the AQLQ(S) questionnaire during therapy with autologous activated T-lymphocytes, *Me* (Q_{25}; Q_{75})

Parameter	Comparison groups of patients with allergic BA					
	T cell therapy, *n* = 12			Standard treatment, *n* = 7		
	Point 1 (before the treatment)	Point 2 (after 2 months – 5 injections)	Point 3 (after 7 months – 10 injections)	Point 1 (before the treatment)	Point 2 (after 2 months – 5 injections)	Point 3 (after 7 months – 10 injections)
Symptoms	5.45 (3.2; 6.1)	5.25 (4.9; 6.2)	5.8 (5.08; 6.7)* $p_{3-1} = 0.045$	4.4 (3.8; 5.5)	4.5 (3.6; 5.8)	5 (4.04; 5.9)
Activity limitation	5.6 (4.9; 6.1)	6.3 (5.3; 6.7)	6.6 (5.4; 6.7)*# $p_{3-2} = 0.043$ $p_{3-1} = 0.005$	5.3 (5.1; 5.6)	5.4 (5.1; 6.5)	5.7 (4.9; 6.05)
Emotional sphere	5.3 (3.8; 6.6)	5.4 (5.0; 5.6)	6.4 (5.2; 6.4)* $p_{3-1} = 0.021$	4.8 (3.8; 6.0)	4.8 (3.8; 6.4)	6.5 (5.2; 6.7)
Environmental influence	4.9 (4.0; 5.75)	5.75 (4.0; 6.0)	6.25 (4.0; 7.0)* $p_{3-1} = 0.009$	5 (4.5; 6.5)	4.75 (4.25; 6.25)	6.9 (5.5; 8.0)

* significant differences compared with the parameters before the therapy ($p < 0.05$).
# significant differences compared with the parameters 2 months after the start of the therapy.

Table 3

The level of total serum IgE in the course of therapy with autologous activated T-lymphocytes, *Me* (Q_{25}; Q_{75})

Parameter	Comparison groups of patients with allergic BA					
	T cell therapy, *n* = 12			Standard treatment, *n* = 7		
	Point 1 (before the treatment)	Point 2 (after 2 months – 5 injections)	Point 3 (after 7 months – 10 injections)	Point 1 (before the treatment)	Point 2 (after 2 months – 5 injections)	Point 3 (after 7 months – 10 injections)
lgE (IU / ml)	231 (58; 3,000)	163 (65; 1,721)*	145.5 (72; 1,357)* $p_{2-1} = 0.017$ $p_{3-1} = 0.033$	84 (32; 125)	90 (13; 326)	75 (11; 184)

* significant differences compared with the parameters before the treatment ($p < 0.05$)

According to spirometry data, in the group of patients with allergic BA receiving immunotherapy, there was significant improvement in forced expiratory volume in one second (FEV1), vital capacity (VC), and forced vital capacity (FVC) and a trend toward an increase in the Tiffeneau index ($p = 0.059$) by the end of the treatment. The results are presented in Table 4. In the control group receiving standard treatment, a significant increase in VC after 2 months was observed; no significant changes by the end of the follow-up were noted.

Table 4

Characteristics of the external respiration function in the course of therapy with autologous activated T-lymphocytes, *Me* (Q_{25}; Q_{75})

Parameter	Comparison groups of patients with allergic BA					
	T cell therapy, $n = 12$			Standard treatment, $n = 7$		
	Point 1 (before the treatment)	Point 2 (after 2 months – 5 injections)	Point 3 (after 7 months – 10 injections)	Point 1 (before the treatment)	Point 2 (after 2 months – 5 injections)	Point 3 (after 7 months – 10 injections)
VC	105 (99; 116)	110 (10; 113)	115 (103; 121)* $p_{3-1} = 0.033$	103 (93; 109)	111 (103; 117)* $p_{2-1} = 0.018$	112 (95; 114)
FVC	109.5 (103; 121)	112 (108; 117)	118 (109; 122)* $p_{3-1} = 0.014$	103 (100; 112)	110 (97; 120)	103 (95; 115)
FEV1	94 (88.5; 102.5)	99 (88; 114)	99.5 (93.5; 112.5)* $p_{3-1} = 0.028$	96 (93; 100)	99 (92; 109)	96 (95; 114)
Tiffeneau index	91 (78; 98)	93 (86; 102)	97 (84; 102)	97 (93; 99)	96 (89; 98)	103 (89; 106)

* significant differences compared with the parameters before the treatment, $p < 0.05$.

Based on the data obtained, we can speak of good tolerability and clinical efficacy of immunotherapy with autologous activated T-lymphocytes in patients with allergic BA.

DISCUSSION

The main advantages of cell therapy include safety and the absence of systemic side effects, since the patient is injected with their own cells with a slight modification. The study showed safety and good tolerability of immunotherapy with T-lymphocytes in BA; previously, safety of this was shown in atopic dermatitis [9].

An important criterion of therapy effectiveness is parameters of asthma control according to the ACQ-5 questionnaire. According to the comparative characteristics of the GINA, Goal, and ACQ-5 rating scales, there is reason to believe that the use of the ordinal ACQ-5 scale is a more appropriate and preferred tool for assessing changes in asthma control in clinical trials [10]. An increase in the asthma control level from uncontrolled to partially controlled BA according to GINA is clinically significant during treatment. The quality of life assessment in patients with BA is also a criterion of therapy effectiveness; an improvement in the parameters in all 4 categories is usually observed by the end of treatment, the changes are significant.

Immunoglobulin E is the main participant of type I hypersensitivity, according to which allergic reactions occur, including BA. The severity of clinical manifestations of allergy may depend on the quantitative parameter of allergen-specific IgE. Upon repeated contact with the allergen, which binds to allergen-specific IgE already produced during sensitization, degranulation of mast cells with release of preexisting early mediators of allergic inflammation occurs along with *de novo* synthesis of lipid mediators – products of arachidonic acid metabolism. A decrease in the IgE level indicates a positive effect of immunotherapy in patients with allergic BA.

Regular monitoring of lung function is especially important for patients with BA, as symptoms of the disease are difficult to detect until airflow obstruction becomes severe. The main parameters of spirometry, reflecting the degree of pulmonary obstruction, are FVC and FEV1. The use of immunotherapy with T-lymphocytes had a positive effect on the lung function in patients with allergic BA. Autologous activated T-lymphocytes are non-toxic and have practically no side effects, which makes immunotherapy a promising approach to BA treatment.

CONCLUSION

In our study, safety and good tolerability of immunotherapy with activated T-lymphocytes in patients with BA has been shown. The therapy was effective in relation to the allergic form of the disease. This was manifested through significant improvements in the degree of asthma control according to the ACQ-5 questionnaire and the quality of life parameters according to the AQLQ(S)

questionnaire, a significant decrease in the total IgE level, and significant improvement in the lung function by the end of the treatment.

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The effect of an aminoguanidine derivative on adjuvant arthritis in rats

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ABSTRACT

Aim. To study anti-inflammatory, analgesic, and possible ulcerogenic effects of a novel aminoguanidine derivative in adjuvant arthritis (a model of rheumatoid arthritis) in rats.

Materials and methods. The experiments were carried out on 42 outbred male Sprague Dawley rats. After modeling arthritis (starting from day 7 after the administration of complete Freund's adjuvant), intramuscular injections of the aminoguanidine derivative (code LIS-M) at a dose of 2.5, 5, and 10 mg / kg or the reference drug diclofenac at a dose of 4 mg / kg were performed once a day for 22 days. The volume of the inflamed limb was measured twice a week, pain threshold was measured every week. After finishing the administration of the compounds, the levels of interleukin (IL) 1, IL-6, and tumor necrosis factor-α (TNFα) were measured in rat plasma, the ankle joint was histologically studied, and the gastric mucosa was studied to detect damage, ulcers, and scarring.

Results. The aminoguanidine derivative, an inhibitor of inducible nitric oxide synthase, was more effective at the dose of 10 mg / kg than diclofenac at the dose of 4 mg / kg. It had anti-inflammatory and analgesic effects in the joint affected by complete Freund's adjuvant, promoted restoration of the histologic structure in the synovial membrane and articular cartilage, and reduced the plasma concentration of IL-1, IL-6, and TNFα by 1.4–1.5 times. The LIS-M compound did not damage the gastric mucosa in rats with adjuvant arthritis.

Conclusion. The aminoguanidine derivative LIS-M exerts potent anti-inflammatory and analgesic effects in adjuvant arthritis in rats (a model of rheumatoid arthritis). LIS-M has no ulcerogenic effect on the gastric mucosa in rats.

Keywords: aminoguanidine derivative, diclofenac, adjuvant arthritis, anti-inflammatory effect, effect on the gastric mucosa, rats

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. The study was approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 5591 of 23.10.2017) and IPHAR LLC (Protocol No. 191 of 10.07.2017).

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Влияние производного аминогуанидина на течение адъювантного артрита у крыс

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РЕЗЮМЕ

Цель исследования – изучить при адъювантном артрите у крыс (модель ревматоидного артрита) противовоспалительное, анальгетическое и потенциальное ульцерогенное действие нового соединения – производного аминогуанидина.

Материалы и методы. Эксперименты проводили на 42 аутбредных самцах крыс стока Sprague Dawley. После развития артрита, начиная с 7-х сут после введения адъюванта Фрейнда, животным в течение 22 сут 1 раз/сут внутримышечно вводили производное аминогуанидина (шифр – LIS-M) в дозах 2,5; 5 и 10 мг/кг или препарат сравнения диклофенак в дозе 4 мг/кг. Объем воспаленной конечности измеряли 2 раза/нед, болевую чувствительность – еженедельно. После завершения введения веществ в плазме измеряли концентрацию интерлейкина (ИЛ) 1, ИЛ-6 и фактора некроза опухоли α (ФНО-α), гистологически изучали ткани заплюсневого сустава, с помощью бинокулярной лупы оценивали состояние слизистой оболочки желудка на наличие дефектов, язв и рубцов.

Результаты. Ингибитор индуцируемой синтазы оксида азота – производное аминогуанидина – в дозе 10 мг/кг эффективнее диклофенака в дозе 4 мг/кг оказывало в суставе, поврежденном адъювантом Фрейнда, противовоспалительное и анальгетическое действие, способствовало восстановлению гистологической структуры синовиальной оболочки и суставного хряща, в 1,4–1,5 раза уменьшало в плазме концентрацию ИЛ-1, ИЛ-6 и ФНО-α. Соединение LIS-M не повреждало слизистую оболочку желудка крыс с адъювантным артритом.

Заключение. Производное аминогуанидина LIS-M оказывает выраженное противовоспалительное и анальгетическое действие при адъювантном артрите у крыс α модели ревматоидного артрита. LIS-M лишен ульцерогенного действия на слизистую оболочку желудка крыс.

Ключевые слова: производное аминогуанидина, диклофенак, адъювантный артрит, противовоспалительное, анальгетическое действие, влияние на слизистую оболочку желудка, крысы

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Rheumatoid arthritis is usually treated with immunosuppressive drugs and glucocorticoids and, at earlier stages, with nonsteroidal anti-inflammatory drugs (NSAID) [1, 2]. NSAID inhibit cyclooxygenase-2 and reduce prostaglandin production in the inflamed tendon sheath. Prostaglandin E_2 at a low concentration is unable to activate the nuclear factor kB, which inhibits its activating effect on nitric oxide (NO) synthase, induced during inflammation. This results in reduced production of aggressive proinflammatory cytokines, such as prostaglandins and NO, in the joints [3]. In normal conditions, these compounds regulate many functions in the body, in particular, they exert a gastroprotective effect. Nitric oxide increases production of protective mucosa in the stomach, improves blood flow, and reduces leucocyte migration to the ulceration site [4, 5]. Therefore, development of a selective inhibitor of inducible nitric oxide synthase (iNOS), able to inhibit NO synthesis at the inflammation site without adverse ulcerogenic effect, is promising. According to prior studies, a new compound, an aminoguanidine derivative codenamed LIS-M, has these properties.

The aim of the study was to investigate the anti-inflammatory, analgesic, and potential ulcerogenic effects of the aminoguanidine derivative LIS-M in adjuvant arthritis (a model of rheumatoid arthritis) in rats.

MATERIALS AND METHODS

The aminoguanidine derivative LIS-M is ({[3-(4-nitrophenyl amino)indole-2-yl] methylene} amino)guanidine methanesulfonate (Fig. 1) synthesized at IPHAR LLC (Tomsk, Russian Federation). LD_{50} of LIS-M in intramuscular administration to male rats is 382.6 mg / kg.

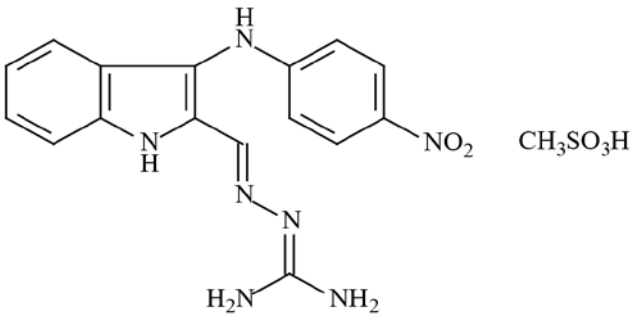

Fig. 1. Structural formula of LIS-M.

Studies were performed in the R&D center of IPHAR LLC using 42 outbred male Sprague Dawley rats weighing 230–250 g (6 groups, 7 animals per group). The animals were obtained from the laboratory animal facility of the R&D center and kept in plastic cages at 18–26 °C, relative humidity 45–65%, 10–11 air changes per hour (ACPH), and 12 / 12 h lighting regime. The study was approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 5591 of 23.10.2017) and R&D center (Protocol No. 191-OFI of 10.07.2017) and performed in accordance with the European Convention for the Protection of Laboratory Animals (Strasbourg, 1986) and principles of Good Laboratory Practice (GOST 33044–2014, Decree of the Ministry of Health of Russia No. 199n of 01.04.2016).

Adjuvant arthritis was induced by administering 0.1 ml of complete Freund's adjuvant (CFA) (Sigma, USA) under the plantar aponeurosis of the hind paw [6]. Starting from day 7 after CFA administration (after arthritis has developed), intramuscular injections of the LIS-M compound at a dose of 2.5, 5, and 10 mg / kg or the reference drug diclofenac (Sandoz, Germany) at a dose of 4 mg / kg were performed once a day for 22 days [7]. Both compounds were dissolved in 1% aqueous solution of polyvinylpyrrolidone. The animals of the control group received the equivalent volume of the solvent.

The volume of the inflamed paw was measured using a plethysmometer (Ugo Basile, Italy) before CFA administration and twice a week for 29 days after the start of the experiment. The difference between the volume of the inflamed paw in rats receiving LIS-M or diclofenac and those from the control group was expressed as a percentage [6]. Pain sensitivity in the inflamed limb, caused by sensitized mechanoreflexes, was measured once a week using von Frey filaments (Ugo Basile, Italy). Sharp withdrawal of the paw in response to sensitization with a Frey filament of a certain size was registered as a positive response, the result was expressed in grams. After the last injection of LIS-M at a dose of 10 mg / kg and diclofenac at a dose of 4 mg / kg, the levels of interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α) were measured by enzyme-linked immunosorbent assay (Vector-Best, Russian Federation).

After finishing the administration of the compounds, the animals were sacrificed in a carbon dioxide chamber. The tissues of the ankle joint, formed by the lower leg bones, tarsus, and the proximal end of the tarsal bones, were studied histologically in paraf-

fin-embedded sections stained with hematoxylin and eosin. The sections were studied using the Carl Zeiss microscope (Germany) at ×100 magnification. The degree of damage to the joint was assessed using a 5-point scale [8].

The score for the maximum severity of the pathology in one animal by all parameters (hyperplasia within the tendon sheath, inflammatory infiltrate in the periarticular tissue, joint effusion, joint space narrowing, pannus formation) was 25. The gastric mucosa of the animals was assessed for defects, ulcers, and lesions using a binocular loupe (Micromed, Russian Federation) at ×10 magnification.

Normality testing in the groups was performed using the Shapiro – Wilk test. The results of measuring the paw volume, pain threshold, and inflammation marker concentration were presented as the mean and the standard error of the mean ($M \pm m$). The one-way ANOVA method with subsequent Tukey test was used to access the intergroup differences in the variables. The results of the histological examination were presented as the total score per group, as well as the median and the interquartile range (Me (Q_1; Q_3)). The Kruskal – Wallis test was used to assess the intergroup differences in the baseline score. The differences between the groups were statistically significant at $p \leq 0.05$. Statistical processing of the data was performed using Statistica 10.0 software (StatSoft, USA).

RESULTS AND DISCUSSION

On the day of CFA administration, hyperemia and swelling of the periarticular tissues were observed in the rats. By day 7, the volume of the limb in which CFA had been injected increased by 3.1 times, reached the maximum by day 10, and was 4.4 times larger than in the intact animals by the end of the study. After 3 injections of LIS-M at the dose of 10 mg / kg, the volume of the inflamed limb decreased by 35%, after 10 injections – by 53%, and after 21 injections – by 73%, compared with the control group ($p < 0.05$). LIS-M had no anti-inflammatory effects at the dose of 2.5 and 5 mg / kg. Diclofenac at the dose of 4 mg / kg reduced limb swelling by 54% after 3 injections, and by 65% – by day 21 of the study ($p < 0.05$). LIS-M at the dose of 10 mg / kg and diclofenac at the dose of 4 mg / kg had equal anti-inflammatory effects ($p > 0.05$) (Fig.2).

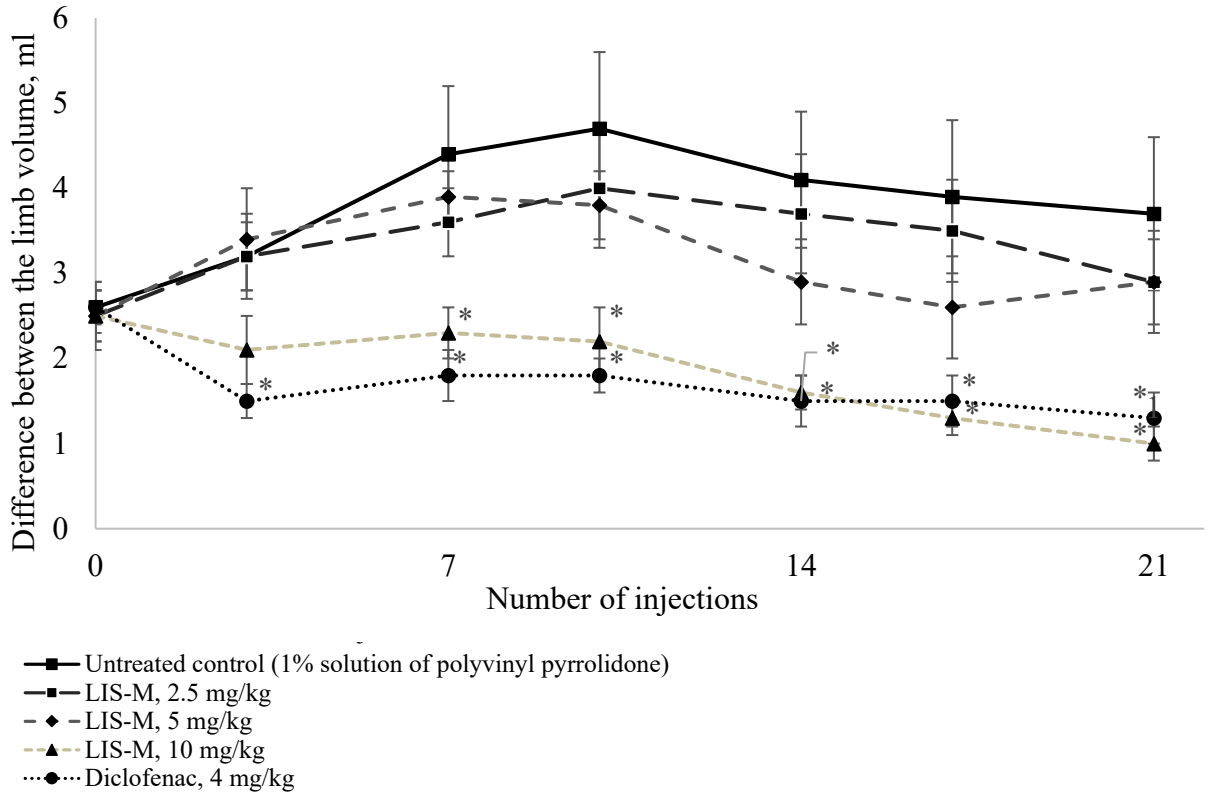

Fig. 2. Anti-inflammatory effect of LIS-M (2.5, 5, 10 mg / kg) and diclofenac (4 mg / kg) in adjuvant arthritis: * difference compared with the untreated control group, $p < 0.05$

Adjuvant arthritis resulted in a considerable increase in pain sensitivity in the inflamed limb: on day 7, it grew by 2.7 times, was increasing throughout the study, and was 3.8 times higher than that in the intact animals by day 21. LIS-M at the dose of 10 mg / kg and diclofenac at the dose of 4 mg / kg had analgesic effects after 7 days of treatment. 21 days after the start of the test compound administration, LIS-M had completely normalized pain sensitivity. With diclofenac administration, pain sensitivity reduced, compared with the values in adjuvant arthritis, but was still considerably higher than in the intact animals. The compound LIS-M had no analgesic effects at the dose of 2.5 and 5 mg / kg.

On day 28 after CFA administration, the concentration of proinflammatory cytokines in the blood plasma was elevated: IL-1 – by 2.5 times, IL-6 – by 5 times, TNF-α – by 3 times. After 21 injections of LIS-M at the dose of 10 mg / kg, the level of IL-1, IL-6, and TNF-α decreased by 1.4–1.5 times, while diclofenac at the dose of 4 mg / kg reduced their concentration less significantly, by 1.2 times ($p < 0.05$) (Table 2).

Table 1

Pain sensitivity in animals with adjuvant arthritis receiving the aminoguanidine derivative LIS-M or diclofenac, g, $n = 7$, $M \pm m$

Group	Number of injections			
	0	7	14	21
Intact animals, $n = 7$	25.5±2.1	26.3±1.7	26.1±1.4	27.3±1.0
Animals with adjuvant arthritis who received:				
1% solution of polyvinylpyrrolidone (control), $n = 7$	11.3±2.1*	4.2±1.1*	6.2±1.6*	7.2±1.4*
LIS-M, 2.5 mg / kg, $n = 7$	12.9±3.6*	5.8±1.2*	6.6±2.0*	8.1±1.0*
LIS-M, 5 mg / kg, $n = 7$	9.8±2.9*	5.7±0.7*	6.4±0.6*	8.5±1.7*
LIS-M, 10 mg / kg, $n = 7$	9.4±2.6*	15.2±4.1*^	19.9±4.0*^	26.0±1.4^#
Diclofenac, 4 mg / kg, $n = 7$	10.3±2.4*	14.9±3.0*^	16.2±3.3*^	14.7±3.6*^

* $p < 0.05$ compared with the intact animals; ^ $p < 0.05$ compared with the animals with adjuvant arthritis receiving 1% solution of polyvinylpyrrolidone on the corresponding day of the experiment (control); # $p < 0.05$ compared with the animals receiving diclofenac.

Table 2

Cytokine concentration in the blood plasma of rats with adjuvant arthritis after 21-day administration of the aminoguanidine derivative LIS-M or diclofenac, pg / ml, $M \pm m$

Experiment conditions	IL-1	IL-6	TNF-α
Intact animals	35.6 ± 4.4	25.3 ± 4.9	50.3 ± 5.5
Animals with CFA-induced arthritis who received:			
1% solution of polyvinylpyrrolidone (control), $n = 7$	87.8 ± 5.4*	127.7 ± 5.4*	148.5 ± 6.1*
LIS-M, 10 mg / kg, $n = 7$	61.8 ± 4.8*^	85.8 ± 3.8*^	101.8 ± 2.4*^
Diclofenac, 4 mg / kg, $n = 7$	76.3 ± 4.7*	102.5 ± 6.0*	122.1 ± 4.8*

* $p < 0.05$ compared with the intact animals; ^ $p < 0.05$ compared with the diclofenac group.

The histological examination of the ankle joint tissue demonstrated that adjuvant arthritis resulted in proliferative synovitis and inflammation of the periarticular tissues, joint space narrowing, formation of intraarticular pannus, and damage to the articular cartilage. Damage to the joint slightly decreased in the group of animals receiving LIS-M at the dose of 2.5 mg / kg. LIS-M at the dose of 5 mg / kg reduced hyperplasia within the tendon sheath and joint effusion and prevented joint space narrowing. LIS-M at the dose of 10 mg / kg and diclofenac at the dose of 4 mg / kg prevented synoviocyte proliferation, joint effusion, joint space narrowing, and pannus formation (Table 3). LIS-M lowers the NO concentration in the inflamed joint, which inhibits synthesis of collagen and proteoglycans and normalizes joint microstructure [9, 10].

Intramuscular administration of the aminoguanidine derivative LIS-M at the dose of 2.5, 5, and 10 mg / kg in rats with adjuvant arthritis had no ulcerogenic effect: no ulceration or erosion in the gastric mucosa was observed. Diclofenac at the dose of 4 mg / kg caused bleeding, ulcers, and erosions in the gastric mucosa.

Table 3

Severity of histologic changes in the ankle joint in rats with adjuvant arthritis after 21-day administration of the aminoguanidine derivative LIS-M or diclofenac, total score, *Me* (Q_1; Q_3)

Articular tissue damage	Animals with CFA-induced arthritis who received				
	1% solution of polyvinylpyrrolidone, $n = 7$	LIS-M 2.5 mg / kg, $n = 7$	LIS-M 5 mg / kg, $n = 7$	LIS-M 10 mg / kg, $n = 7$	Diclofenac, 4 mg / kg, $n = 7$
Hyperplasia within the tendon sheath	13 2 (1; 3)	8 1 (0; 2)	7* 1 (1; 1)	4* 0 (0; 1)	5* 1 (0; 1)
Inflammatory infiltrate in the periarticular tissue	29 4 (4; 4)	27 4 (4; 4)	23 3 (3; 3)	22 3 (3; 3)	25 4 (3; 4)
Joint effusion	10 1 (0; 1)	4* 0 (0; 1)	1* 0 (0; 0)	0* 0 (0; 0)	0* 0 (0; 0)
Joint space narrowing	16 2 (2; 2)	11 2 (1; 2)	6* 1 (0; 1)	4* 1 (0; 1)	5* 1 (0; 1)
Pannus formation	18 2 (2; 3)	13 2 (1; 2)	10 1 (1; 2)	5* 1 (0; 1)	5* 1 (0; 1)

* $p < 0.05$ compared with the animals with CFA-induced arthritis treated with 1% solution of polyvinylpyrrolidone (control).

CONCLUSION

CFA administration in rats has resulted in progressive swelling and pain hypersensitivity in the injected limb, as well as inflammation and degenerative changes in the tissues of the ankle joint. The levels of proinflammatory cytokines IL-1, IL-6, and TNF-α in the blood plasma increased by 2.5–5 times. The production of these cytokines is stimulated by NO produced in the reaction catalyzed by inducible NO synthase (iNOS) [11]. The aminoguanidine derivative LIS-M, a selective iNOS inhibitor, at the dose of 10 mg / kg was more effective in treating arthritis and alleviating pain than diclofenac at the dose of 4 mg / kg. LIS-M decreased the level of cytokines by 1.4–1.5 times compared with the untreated control, while diclofenac reduced their level by 1.2–1.3 times. An important advantage of LIS-M is the absence of ulcerogenic effects on the stomach. This compound does not inhibit the activity of cyclooxygenase and constitutive isoforms of NO synthase and does not disrupt synthesis of gastroprotective prostaglandins and NO.

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The effect of systemic melatonin administration on the intensity of free radical damage to lipids and proteins in the burn wound in the dynamics of experimental thermal injury

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ABSTRACT

Aim. To assess the effect of melatonin (MT) on the content of lipid peroxidation (LPO) and protein oxidation (PO) products in the tissue homogenate from the burn wound in experimental thermal injury (TI).

Materials and methods. Third-degree (IIIA) TI with a relative area of 3.5% was modeled on male Wistar rats via contact of the skin with boiling water. Intraperitoneal administration of MT (10 mg / kg) was performed once a day for 5 days. On days 5, 10, and 20, LPO products in the heptane and isopropanol phases of lipid extraction and PO products were determined in the tissue homogenate from the burn wound.

Results. The content of secondary and end products of LPO in the heptane phase and end products in the isopropanol phase increased in the wound. The content of primary and secondary PO products of neutral nature increased on days 5, 10, and 20, and the level of secondary PO products of neutral nature elevated on days 10 and 20. Administration of MT reduced the content of LPO end products in the heptane phase, secondary and end products of LPO in the isopropanol phase, and the total amount of PO products due to primary and secondary products of neutral nature.

Conclusion. In the 20-day follow-up, LPO and PO products accumulated in the burn wound. The administration of MT at a total dose of 50 mg / kg led to reduction and partial restoration of the content of LPO and POM products, which can limit secondary alterations and accelerate healing of the burn wound.

Keywords: melatonin, thermal injury, lipid peroxidation, protein oxidative modification

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Conformity with the principles of ethics. The study was approved by the local Ethics Committee at South Ural State Medical University (Protocol No. 10 of 15.11.2019).

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Влияние системного применения мелатонина на интенсивность свободно-радикальной деструкции липидов и белков ожоговой раны в динамике экспериментальной термической травмы

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РЕЗЮМЕ

Цель работы – исследовать влияние мелатонина (МТ) на содержание продуктов перекисного окисления липидов (ПОЛ) и окислительной модификации белков (ОМБ) в гомогенате ожоговой раны при экспериментальной термической травме (ТТ).

Материалы и методы. На самцах крыс линии Wistar моделировали ТТ степени IIIА площадью 3,5% контактом кожи с кипящей водой в течение 12 с. МТ применяли внутрибрюшинно (10 мг/кг), 1 раз/сут в течение 5 сут. В гомогенате ожоговой раны на 5, 10 и 20-е сут определяли содержание продуктов ПОЛ в гептановой и изопропанольной фазах липидного экстракта, продуктов ОМБ.

Результаты. В ожоговой ране увеличивалось содержание вторичных и конечных продуктов ПОЛ в гептановой фазе, конечных продуктов в изопропанольной фазе липидного экстракта; увеличивалось содержание первичных и вторичных продуктов ОМБ нейтрального характера на 5, 10 и 20-е сут, вторичных продуктов нейтрального характера – на 10-е и 20-е сут. Применение МТ снижает содержание конечных продуктов ПОЛ в гептановой фазе, вторичных и конечных продуктов ПОЛ в изопропанольной фазе липидного экстракта; суммарное количество продуктов ОМБ за счет первичных и вторичных продуктов нейтрального характера.

Заключение. В динамике 20-суточного наблюдения при ТТ кожи в ожоговой ране накапливаются продукты ПОЛ и ОМБ, применение МТ в суммарной дозе 50 мг/кг приводит к снижению и частичному восстановлению содержания продуктов ПОЛ и ОМБ, что может ограничивать вторичную альтерацию, ускорять заживление ожоговой раны.

Ключевые слова: мелатонин, термическая травма, перекисное окисление липидов, окислительная модификация белков.

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INTRODUCTION

Despite a wide range of drugs used to treat thermal injury (TI), their efficacy in the clinical setting does not always satisfy burn specialists, and side effects of the drugs often limit their use. When searching for new drugs, special attention is paid to endogenous regulators of homeostasis with pleiotropic effects [1–4]. The effectiveness of oxytocin, growth hormone, insulin, testosterone, and others has been proven in TI, and additional data are required to substantiate their use [5]. The skin is the largest organ with intense free radical oxidation (FRO), products of FRO formed in the skin have local and distant effects [6].

Oxidative stress is registered not only in the lesion, but also in the heart, lungs, kidneys, muscles, and

other organs [7, 8]. It is of particular interest to study aldehyde- and ketone-containing protein carbonyl derivatives in the TI focus – protein oxidation (PO) and lipid peroxidation (LPO) products as markers of FRO and the effectiveness of antioxidants [9]. Most data on the content of LPO and PO products in TI were obtained for plasma and internal organs, but not for a burn wound [7, 8, 10].

Melatonin (MT) is evolutionally one of the most ancient molecules with the original function of an antioxidant; its sources are the pineal gland, retinal ganglion cells, and the gastrointestinal tract [11]. Skin cells synthesize MT; its metabolites are found in keratinocytes, melanocytes, and dermal fibroblasts [12]. In the experiment, when the skin is damaged, MT accumulates in the epidermis, protecting the mitochondria [13]. MT receptors, including MT1 (Mel1a), MT2 (Mel1b), and RORα are found in keratinocytes, dermal fibroblasts, hair follicle cells, and melanocytes [14]. MT participates in the regulation of sleep – wake rhythms, has antioxidant, pro- and anti-inflammatory, and anti-apoptogenic effects, and regulates cell proliferation and differentiation [15]. These and other MT effects attract attention in terms of fundamental research due to homeostasis regulation and participation in the disease pathogenesis and in terms of applied research – due to potential use for prevention and treatment of diseases.

The aim of this study was to assess the effect of MT on the content of LPO and PO products in the tissue homogenate from the burn wound in experimental TI.

MATERIALS AND METHODS

The study involved 120 male Wistar rats weighing 200–240 g in the experimental biological clinic (vivarium) of the South Ural State Medical University. The experiment was carried out in strict compliance with the requirements for the care and maintenance of animals in accordance with the conclusion of the Ethics Committee (Protocol No. 10 of 15.11.2019). The animals were randomly divided into groups: group 1 ($n = 20$) – intact; group 2 ($n = 36$) – with TI; group 3 ($n = 32$) – with TI exposed to MT.

To simulate third-degree (type A) TI with a relative area of 3.5%, the interscapular area was immersed in water at 98–99 °C for 12 s. The burn depth was verified by morphological methods. Zoletil-100 (international nonproprietary name: tiletamine hydrochloride; Virbac Sante Animale, France) at a dose of 20 mg / kg was used for anesthesia. In groups 2 and 3, an aseptic bandage was applied to the wound every day for 20 days after TI. MT (FLAMMA S.P.A., Italy) was used intraperitoneally at a daily single dose of 10 mg / kg for 5 days. The levels of LPO and PO products were assessed in the tissue homogenate from the burn wound on days 5, 10, and 20.

To prepare a 10% tissue homogenate, the burn wound was excised, after that about 40 mg of the tissue was immersed in a cooled buffer solution, dried, and then homogenized at a temperature of 2–4 °C in 0.4 ml (1 : 10) of a cooled 0.1 M phosphate buffer (pH 7.4). A SF-56 spectrophotometer (LOMO-Spectr, St. Petersburg) was used to determine the content of LPO products in the tissue homogenate [16]. Optical density was measured in heptane and isopropanol lipid extraction at 220 nm (isolated double bonds), 232 nm (conjugated dienes, CD), 278 nm (ketodienes and conjugated trienes, KD and CT), and 400 nm (Schiff bases, SB). The relative content of LPO products was expressed in units of oxidation indices (u.o.i): E_{232}/E_{220} (CD), E_{278}/E_{220} (KD and CT), and E_{400}/E_{220} (SB).

The PO products in the tissue homogenate were determined according to the reaction between protein carbonyl derivatives and 2,4-dinitrophenylhydrazine with the following registration of aldehyde dinitrophenylhydrazine (ADNPH) and ketone dinitrophenylhydrazine (KDNPH) in the ultraviolet (UV) region of the spectrum and visible light region [17]. The results were expressed in units of optical density per 1 mg of protein (c.u. / mg). IBM SPSS Statistics 19 software was used for statistical processing of the data presented as the median and the interquartile range (Me (Q_{25}; Q_{75})). The significance of differences was assessed using the Kruskal – Wallis and Mann – Whitney tests. The differences were considered statistically significant at $p < 0.05$.

RESULTS

On day 5 of TI, the content of KD, CT, and SB increased in the heptane and isopropanol phases of lipid extraction in the wound (Table 1). On days 10 and 20 of the experiment, the content of KD, CT, and SB increased in the heptane phase of the burn wound, while only SB increased in the isopropanol phase. No significant changes were observed in the content of CD in the heptane and isopropanol phases on days 5, 10, and 20, as well as in the content of KD and CT in the isopropanol phase on days 10 and 20. In the dynamics of TI, the content of SB in the heptane and isopropanol phases was lower on day 10 ($p < 0.01$) than on day 5, and on day 20 it was higher ($p < 0.01$) than on day 10.

The total amount of PO products increased on days 5, 10, and 20 (Table 2). This is due to an increase in the total content of ADNPH on days 5, 10, and 20, the content of KDNPH on days 10 and 20, and the total amount of PO products in the UV spectrum region on days 5, 10, and 20. The total amount of PO products in the visible light region decreased on day 10 of TI. On day 5, an increase in ADNPH was revealed in the UV part of the spectrum. On day 10, an increase in ADNPH and KDNPH was observed in the UV region, with a decrease in ADNPH and KDNPH in the visible light region. On day 20, ADNPH increased in the UV region of the spectrum and in the visible light region, an increase in KDNPH was noted in the UV region of the spectrum.

In dynamics, the total amount of PO products and KDNPH, as well as the amount of KDNPH in the UV region of the spectrum is greater on days 10 and 20 ($p < 0.01$) than on day 5. The total amount of PO products and the amount of ADNPH in the UV region of the spectrum on day 10 is greater ($p < 0.01$) than on days 5 and 20; the amount of ADNPH and KDNPH in the visible light region is lesser on day 10 ($p < 0.01$) than on days 5 and 20.

Table 1

The effect of melatonin (MT) on the content of LPO products in the burn wound with TI, *Me* (Q_{25}; Q_{75})

Parameter	Group 1 (intact), $n = 20$	Group 2 (TI), day 5, $n = 16$	Group 2 (TI), day 10, $n = 20$	Group 2 (TI), day 20, $n = 21$	Group 3 (TI + MT), day 5, $n = 13$	Group 3 (TI + MT), day 10, $n = 10$	Group 3 (TI + MT), day 20, n =16
CD (h.), u.o.i.	0.920 (0.863; 0.975)	0.889 (0.834; 0.966)	0.891 (0.836; 0.944)	0.927 (0.873; 0.951)	0.891 (0.885; 0.908)	0.893 (0.881; 0.915)	0.906 (0.887; 0.931)
KD and CT (h.), u.o.i.	0.049 (0.013; 0.088)	0.123 (0.112; 0.141)*	0.115 (0.101; 0.141)*	0.126 (0.092; 0.155)*	0.134 (0.094; 0.140)*	0.133 (0.086; 0.141)*	0.089 (0.085; 0.095)* #
SB (h.), u.o.i.	0 (0; 0.011)	0.018 (0.013; 0.031)*	0.009 (0.003; 0.018)*	0.025 (0.015; 0.056)*	0.013 (0.012; 0.014)* #	0 (0; 0.002)#	0.002 (0.001; 0.004)#
CD (i.) u.o.i.	0.601 (0.596; 0.622)	0.594 (0.570; 0.732)	0.580 (0.568; 0.614)	0.613 (0.590; 0.647)	0.538 (0.534; 0.545)#	0.556 (0.550; 0.558)*#	0.562 (0.558; 0.569)*#
KD and CT (i.), u.o.i.	0.217 (0.209; 0.228)	0.259 (0.200; 0.213)*	0.210 (0.169; 0.264)	0.224 (0.211; 0.263)	0.209 (0.195; 0.228)*#	0.209 (0.200; 0.220)	0.199 (0.173; 0.213)*#
SB (i.), u.o.i.	0 (0; 0.011)	0.030 (0.015; 0.04)*	0.007 (0.004; 0.026)*	0.034 (0.016; 0.039)*	0.009 (0.007; 0.015)*#	0.007 (0.004; 0.009)*	0.011 (0.009; 0.016)*#

* statistically significant differences ($p < 0.05$) with group 1, # with group 2 (here and in Table 2).
Note: extracts: h. – heptane, i. – isopropanol.

Table 2

The effect of melatonin on the content of PO products in the burn wound in TI, *Me* (Q_{25}; Q_{75})

Parameter	Group 1 (intact), $n = 20$	Group 2 (TI), day 5, $n = 16$	Group 2 (TI), day 10, $n = 20$	Group 2 (TI), day 20, $n = 21$	Group 3 (TI + MT), day 5, $n = 13$	Group 3 (TI + MT), day 10, $n = 10$	Group 3 (TI + MT), day 20, n =16
ADNPH uv, c.u. / mg	29.85 (24.69; 32.84)	51.49 (48.03; 55.81)*	60.50 (52.95; 93.13)*	52.08 (35.14; 82.61)*	44.44 (35.14; 49.70) *#	49.82 (47.09; 55.59)*#	39.81 (32.79; 53; 80) *#
ADNPH vs, c.u. / mg	6.93 (5.32; 8.71)	6.91 (5.72; 9.75)	3.53 (2.09; 5.07)*	8.36 (7.06; 15.36)*	5.29 (3.97; 6.67)	7.68 (5.24; 9.16)	8.69 (6.25; 22.08)*
KDNPH uv, c.u. / mg	8.19 (7.37; 10.59)	7.79 (7.34; 9.43)	15.19 (9.05; 25.63)*	15.27 (11.44; 31.38)*	6.48 (3.42; 7.86)	12.11 (9.21; 13.06)*#	6.22 (4.16; 8.39)#
KDNPH vs, c.u. / mg	0.89 (0.69; 1.14)	0.91 (0.69; 1.41)	0.50 (0.35; 0.66)*	1.09 (0.72; 1.67)	0.79 (0.41; 1.09)	0.74 (0.43; 1.00)	1.82 (1.44; 4.14)*#
S PO, c.u. / mg	47.83 (41.94; 55.40)	66.87 (60.56; 76.11)*	79.30 (62.59; 122.34)*	82.04 (55.79; 35.89)*	55.79 (51.53; 64.65)*#	71.03 (67.38; 72.93)*#	66.05 (56.70; 74;87)*#
S_{ADNPH}, c.u. / mg	38.54 (30.64; 41.39)	59.19 (52.29; 62.31)*	65.04 (54.51; 96.45)*	65.04 (42.19; 97.89)*	47.78 (42.19; 57.09)*#	57.71 (54.26; 62.64)*	54.92 (50.75; 65.43)*#
S_{KDNPH}, c.u. / mg	10.12 (8.23; 11.31)	8.81 (8.09; 10.67)	15.49 (9.56; 26.11)*	16.99 (12.18; 33.88)*	6.75 (5.05; 8.83)*	13.21 (10.29; 13.88)	8.74 (5.95; 12.49)#
S uv, c.u. / mg	38.47 (34.05; 45.31)	59.10 (53.57; 68.66)*	74.97 (60.81; 118.45)*	65.17 (48.02; 114.01)*	49.24 (42.57; 57.19)*#	61.32 (59.49; 63.87)*	46.49 (39.24; 58.45)*#
S vs, c.u. / mg	7.87 (6.02; 9.73)	7.81 (6.41; 11.16)	4.05 (2.35; 5.85)*	9.22 (7.77; 17.56)	6.03 (4.50; 7.77)	8.41 (5.66; 10.16)#	10.51 (7.97; 26.29)*

Note: S – total content, vs – visible light region, uv – ultraviolet region of the spectrum.

Under the conditions of MT administration, on day 5 of TI, the amount of SB in the heptane phase decreased; in the isopropanol phase, the amount of CD, KD and CT, and SB decreased (see Table 1). On day 10, the amount of SB decreased in the heptane phase, and the level of CD decreased in the isopropanol phase. By day 20, a fall in the content of KD and CT and SB was recorded in the heptane phase, while the content of CD, KD, CT, and SB decreased in the isopropanol phase. During all periods of observation, MT did not result in complete restoration of LPO parameters in the wound. Significant differences with intact animals persisted in the heptane phase for KD and CT on day 20 of TI, for SB – on days 5, 10, and 20; for KD, CT and SB in the isopropanol phase – on days 5 and 20.

On days 5, 10, and 20, the content of CD in the isopropanol phase was lower than in the skin of the intact animals. Under the conditions of MT application in the wound, on days 5, 10, and 20, the total amount of PO products decreased (Table 2). On day 5, the total amount of ADNPH and PO products in the UV region of the spectrum and the content of ADNPH in the UV spectrum decreased. On day 10, the content of ADNPH and KDNPH decreased in the UV region, and the total content of PO products increased in the visible light region. On day 20, the total amount of ADNPH, KDNPH, and PO products in the UV region and the amount of ADNPH and KDNPH in the UV region of the spectrum decreased; the amount of KDNPH in the visible light region increased. During all periods of observation, the total amount of PO products, the total amount of ADNPH and PO products in the UV region of the spectrum, as well as the amount of ADNPH in the UV spectrum differed from the values in the intact group, which suggests partial recovery of parameter values.

DISCUSSION

In the burn wound with TI, secondary and end LPO products accumulate, which are extracted into the heptane phase mainly concentrating triacylglycerides (non-polar lipids) and LPO end products in the isopropanol phase, which contains mainly membrane phospholipids. The absence of significant changes in the content of primary LPO products in the heptane and isopropanol phases may be a consequence of their excessive formation on day 1 and participation in the formation of secondary and end products. Against this background, the total content of PO products in the burn wound increases due to neutral primary (ADNPH) and secondary (KDNPH) products, reflecting aggregation and fragmentation of protein molecules.

The content of the basic primary and secondary PO products decreases, most likely due to depletion of the reserves of protein products and their possible consumption in the first 5 days. It is believed that the accumulation of primary POM products, early markers, mainly reflects protein aggregation under the influence of $OH^{\cdot}$, while the accumulation of secondary products, late markers, reflects protein fragmentation under the combined action of $OH^{\cdot}$ and $O_2^{\cdot-}$. Protein fragments are resistant to proteolysis, trigger apoptosis and necrosis, and expand the area of alteration. The increase in SB reflects features and sources of their formation as products of non-enzymatic interaction of LPO products with products of free radical-mediated degradation of proteins under conditions of oxidative and carbonyl stress. There are no specific mechanisms for elimination of SB formed in this way; therefore, they are prone to accumulation, which increases their damaging effects. An increase in the content of LPO and PO products in the TI focus reflects activation of FRO under conditions of excessive generation of free radicals and / or a decrease in the activity of antioxidant defense systems.

FRO inducers in TI are NADPH oxidase, MPO of neutrophils and monocytes / macrophages, endothelial xanthine oxidase, NO-synthase of monocytes / macrophages, and mitochondrial complex I [18, 19]. A decrease in the activity of antioxidant defense systems may be due to their consumption for inactivation of excess free radicals, a decrease in the level of zinc and copper in the body (components of superoxide dismutase), and deficiency of selenium (a component of glutathione peroxidase) [20]. FRO and LPO and PO products formed in the skin in TI are associated with *in situ* cytotoxic effects, damage to internal organs, and possible advancement to systemic inflammatory response syndrome (SIRS) in the context of the OxInflammation concept. They are involved in skin repair due to activation of matrix metalloproteinases, modification of extracellular matrix components, and activation of stem cells [6, 21–23].

The use of MT in TI leads to a decrease in the content of LPO end products in the heptane phase, as well as primary, secondary, and end products in the isopropanol phase of the tissue homogenate. Apparently, a pronounced decrease in the level of primary LPO products in the isopropanol phase reflects MT-dependent limitation of early LPO stages and shielding of phospholipids due to predominant oxidation of pro-

teins. Firstly, the antioxidant effect of MT entering the burn wound from the systemic circulation through passive diffusion, as well as via glucose transporters and oligopeptides, may be due to direct absorption of reactive oxygen species (ROS) [24]. Secondly, it may be due to an increase in the synthesis of glutathione and the activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and hemoxidase-1 and a decrease in the activity of NOS [13]. Finally, the antioxidant effect of MT is realized by maintaining the mitochondrial membrane potential and increasing oxidative phosphorylation and production of ATP, and not ROS [25].

CONCLUSION

In experimental TI, an increase in the content of secondary and end products of LPO in the heptane phase was noted in the wound; the LPO end products increased in the isopropanol phase of lipid extraction; the total content of PO products increased due to primary and secondary products of a neutral nature. The use of MT reduced mainly the content of LPO end products in the heptane phase and secondary and end products of LPO in the isopropanol phase, as well as reduced and partially restored the amount of primary and secondary PO products of a neutral nature. In the TI focus, decreased damage to proteins and lipids limited secondary alteration, reduced the time of vascular and exudative reactions, promoted early activation of regeneration, and accelerated wound healing.

The results obtained expand the understanding of the role of changes in the redox state in the pathogenesis of TI and are a prerequisite for studying FRO in the skin to designate LPO and PO products as markers and predictors of complications and the effectiveness of therapy. LPO- and PO-limiting effect of MT suggests further study of the mechanism of MT action and the effectiveness of its use.

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Experience in the use of lung ultrasound in patients of the respiratory hospital of Siberian State Medical University with COVID-19 pneumonia

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ABSTRACT

Aim. To evaluate the possibility of using lung ultrasound for diagnosing COVID-19 pneumonia in patients of the respiratory hospital of Siberian State Medical University (SSMU).

Materials and methods. An analysis of lung ultrasound data was carried out in 39 patients (17 men and 22 women aged 33–78 years) with COVID-19 pneumonia. Lung ultrasound was performed in all patients in addition to radiography performed at the prehospital stage and in 15 patients who underwent computed tomography (CT) of the lungs.

Results. In the majority (61.6%) of cases, during the ultrasound examination, COVID-19 pneumonia manifested itself as interstitial lung disease. The white lung phenomenon and a combination of the aforementioned interstitial changes were recorded with the same frequency (5.1%), while pulmonary consolidation in addition to interstitial changes was visualized in 10.2% of cases. Interstitial lung disease was bilateral in 83.3% of patients and unilateral in 16.7% of cases. The inferior lobes of the lungs were affected in 60.0% of cases, middle lobe – in 30.0% of cases, and superior lobes – in 15.0% of patients. The ultrasound examination detected changes in the lungs in 32 patients, while radiographic changes were present in 35 cases. Bilateral inflammation was more often detected by radiography than by ultrasound. When comparing the data of lung ultrasound and CT, the agreement between the methods was found in 66.7% of cases, and the discrepancy between the findings of the two methods was observed mainly in patients with a large number of affected segments of the lungs and localization of the disease in the superior lobes according to CT.

Conclusion. Lung ultrasound is a valuable tool that can be used to stratify risk in patients at any stage of diagnosis and treatment in the context of the COVID-19 pandemic due to availability, speed of implementation, and the absence of a need for patient transportation.

Keywords: COVID-19 pneumonia, lung ultrasound, radiology

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Conformity with the principles of ethics. All patients signed an informed consent to examination. The study was approved by the local Ethics Committee at SSMU.

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Опыт применения ультразвукового исследования легких у пациентов респираторного госпиталя СибГМУ с коронавирусн пневмонией

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РЕЗЮМЕ

Цель. Изучить возможность использования ультразвукового исследования (УЗИ) легких в диагностике пневмонии COVID-19 у пациентов респираторного госпиталя СибГМУ.

Материалы и методы. Проведен анализ данных УЗИ легких у 39 пациентов (17 мужчин и 22 женщины в возрасте 33–78 лет) с пневмонией, вызванной SARS-CoV-2. УЗИ легких выполнено всем пациентам дополнительно к рентгенографии (РГ), проведенной на догоспитальном этапе, и 15 пациентам с компьютерной томографией (КТ) легких.

Результаты. В большинстве случаев (61,6%) при УЗИ пневмония проявлялась интерстициальным синдромом, с одинаковой частотой (5,1%) регистрировались феномен «белого легкого» и сочетание перечисленных интерстициальных изменений, в 10,2% визуализировалась консолидация легочной ткани дополнительно к интерстициальным изменениям. Интерстициальный синдром в 83,3% носил двусторонний характер, в 16,7% – односторонний. Поражение нижних отделов легких выявлено в 60,0% случаев, средних – в 30,0%, верхних – в 15,0%. При УЗИ изменения в легких были диагностированы у 32 пациентов и 35 пациентов методом РГ. Двусторонний воспалительный процесс чаще выявлялся при РГ, чем при УЗИ. При сравнении данных УЗИ и КТ легких совпадение установлено в 66,7% случаев, а расхождение результатов двух методов наблюдалось у пациентов с большим числом пораженных сегментов легких и локализацией процесса в верхних долях легких по КТ.

Заключение. Проведенное УЗИ легких показало, что этот метод визуализации должен быть применен для стратификации риска у пациентов на любом этапе диагностики и лечения в условиях пандемии COVID-19 в силу доступности, быстроты выполнения, отсутствия необходимости в транспортировке больных.

Ключевые слова: пневмония, COVID-19, ультразвуковое исследование легких, лучевая диагностика

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Все пациенты подписали информированное согласие на проведение исследования. Исследование одобрено локальным этическим комитетом СибГМУ.

Для цитирования: Поровский Я.В., Беспалова И.Д., Сорокина Т.В., Диш А.Ю., Канев А.Ф., Кощавцева Ю.И., Чуяшенко Е.В., Шульга О.С., Балабанова А.А. Опыт применения ультразвукового исследования легких у пациентов респираторного госпиталя СибГМУ с коронавирусной пневмонией. *Бюллетень сибирской медицины.* 2022;21(1):96–102. https://doi.org/10.20538/1682-0363-2022-1-96-102.

INTRODUCTION

In March 2020, the World Health Organization declared COVID-19 (COrona VIrus Disease 2019) caused by the SARS-CoV-2 virus a pandemic [1]. Rapid spread of the virus required significant restructuring of the healthcare system, changes in the working conditions for doctors, and obligatory widespread implementation of the most essential methods for examining a patient's condition, such as measuring arterial blood oxygen saturation and diagnostic radiology methods for detecting COVID-19 pneumonia [2], mostly computed tomography (CT) of the lungs, which has the greatest sensitivity in its diagnosis [3–5].

Lung ultrasound (US) for diagnosing pneumonia and viral infections in the lungs previously occupied a

modest place in clinical practice and was more often used in situations when plain radiography and CT of the lungs were not available (pregnant women, lack of technical capabilities) [6, 7].

To date, the theoretical foundations of lung US have been significantly enriched, and, therefore, clinical application of the method has become more objective for use in the context of the COVID-19 pandemic [8]. Studies [9–11] indicate a significant role of lung US for the diagnosis of interstitial lung disease [12], acute respiratory distress syndrome (ARDS) [13], pleural disorder, and lung inflammation of any etiology [14], i.e. the main manifestations of lung damage in COVID-19. Currently, domestic evidence base for lung US in patients with COVID-19 is being compiled in the context of a growing number of COVID-19 cases among the population and forced restriction on the use of classical methods of lung examination (palpation, percussion, auscultation) by therapists due to a high risk of viral contamination.

The aim of the study was to evaluate the possibility of using lung US for diagnosing COVID-19 pneumonia in patients of the respiratory hospital of Siberian State Medical University (SSMU).

MATERIALS AND METHODS

To implement the program on combating the novel coronavirus infection at Siberian State Medical University, from May 16 to September 30, 2020, a separate building of Advanced Therapy Clinics was allocated and converted into a respiratory hospital for treating COVID-19 patients. Lung US was included in examination of patients at the respiratory hospital, in contrast to other hospitals in the Tomsk region providing similar medical care.

To investigate the capabilities of lung US and the potential for applying the method in the diagnosis of COVID-19 pneumonia, 39 patients (17 men and 22 women) aged 33–78 years (average age 59.4 ± 15.4 years) were included in the study; they were admitted to the respiratory hospital of Siberian State Medical University.

Inclusion criteria were a verified diagnosis of novel coronavirus infection in oropharyngeal swabs, diagnostically significant blood concentration of IgM and IgG to the viral antigen, the presence of clinical signs of respiratory infection, and a signed informed consent to participate in the study.

Exclusion criteria included concomitant lung pathology (neoplasms, tuberculosis, pneumoconiosis) and somatic symptom disorders (diffuse connective tissue diseases, severe heart failure), which could affect the radiographic lung pattern.

The severity of the patient's condition was assessed using the National Early Warning Score (NEWS), a protocol for assessing the severity of a patient's condition [15]. It included assessment of the respiratory rate, blood oxygen saturation level, body temperature, systolic blood pressure, heart rate, and changes in the level of consciousness.

In 4 patients, the NEWS score was equal to or more than 7, the condition of these patients was assessed as severe, requiring mechanical ventilation (MV). In 12 patients, the NEWS score ranged from 5 to 6, their condition corresponded to moderate severity. In 23 patients with the score of 4 or less, the condition was considered mild.

Lung US was performed on a Mindray M 7 ultrasound machine in a sitting position. In order to reduce the time spent in the red zone of the hospital, the protocol of lung US was simplified. The following anatomical approaches were sequentially used: posterior surface of the chest: 1) right and left lower zones: paravertebrally at the level of IX – X ribs; 2) right and left upper zones: paravertebrally at the level of the scapular spine. Lateral sections of the chest: 1) right and left lower zones (along the midaxillary line to the intersection with the horizontal line drawn through the epigastric angle); 2) right and left upper zones (along the midaxillary line at the entrance to the armpit). Anterior sections of the chest: 1) right and left lower zones (along the right midclavicular line at the horizontal line drawn through the epigastric angle); 2) right and left upper zones (along the right midclavicular line at the level of II – III ribs).

To assess the identified radiographic changes in the examined patients, we used the terminology and descriptive characteristics presented by the Consensus Statement of the RASUDM (Russian Association of Ultrasound Diagnostic Specialists in Medicine) [7]. The following parameters were analyzed: the state of the pleural line, registration of B-lines in various variants, and the presence of signs of pulmonary consolidation and pleural effusion.

A prerequisite for including the results of lung US of patients in the analysis was performance of radiography (RG) and, in some cases, CT of the lungs. Chest X-ray at the prehospital stage was performed in all 39 patients and repeated in 4 individuals; CT of the lungs was performed in 15 cases additionally after radiography.

Chest X-ray included plain radiography of the lungs and lateral chest views. For the period of functioning of the respiratory hospital at Siberian State Medical University, this method was the most accessible and often primary, and sometimes it was the only technique for visualizing changes in the lungs of patients with suspected COVID-19 pneumonia. In case of an ambiguous X-ray pattern or when the condition of the patients aggravated, they additionally underwent multi-slice computed tomography (MSCT). MSCT of the chest was performed with 1.25 mm reformatted slice thickness and subsequent image analysis in the maximum intensity projection (MIP) and volume rendering technique (VRT). In 2 cases, MSCT was supplemented with lung densitometry for greater objectivity in assessing the density of the lung parenchyma.

Quantitative data were presented as $X \pm \sigma$, where X is the mean, and σ is the standard deviation. Qualitative data were presented as absolute and relative frequencies, *n* (%).

RESULTS AND DISCUSSION

Following lung US, normal lung tissue was visualized in 7 patients in the form of typical reverberation artefacts – thin lines located parallel to the pleura (A-lines). In this case, the entire complex of images (pleural line, A-lines) shifted in rhythm with respiration (the phenomenon of lung sliding).

Signs of interstitial lung disease ("I") were detected in 24 (61.6%) patients, that is, in the majority of the studied individuals. In this case, more than 3 B-lines were detected in 2 scanning areas. The formation of B-lines (hyperechoic bands) that originated from the pleural line and gradually expanded due to the entry of exudate into the interalveolar space is pathognomonic for interstitial lung disease. In 2 (5.1%) cases, with progression of interstitial lung disease, the B-lines became coalescent, up to a continuous hyperechoic area ("E", the white lung phenomenon). In the same number of cases, a combination of the listed interstitial changes was noted (Table 1).

The progressive course of the disease was characterized by transition from purely interstitial pneumonia to mixed interstitial – parenchymal disease with the appearance of pulmonary consolidation ("C") – airless lung tissue with signs of inflammatory exudate. In this case, the US pattern was characterized by the disappearance of the pleural line and the presence of a hypoechoic area of irregular shape, along the border with which lung tissue with characteristic B-lines was visualized. In 4 cases, different combinations of signs of interstitial lung disease, white lung, and pulmonary consolidation were visualized. A small volume of pleural effusion in addition to the major pulmonary changes was detected in 9 patients.

Table 1

US signs characteristic of lung damage in COVID-19

Parameter	*n* (%)
Visualization of normal lung tissue (N)	7 (18.0)
Signs of interstitial lung disease (I)	24 (61.6)
White lung phenomenon (E)	2 (5.1)
Combination of the white» lung phenomenon and interstitial lung disease	2 (5.1)
Combination of the white lung phenomenon interstitial lung disease, and pulmonary consolidation (C)	4 (10.2)

In 20 patients (83.3%), the US pattern of interstitial lung disease was bilateral, in 4 (16.7%) – unilateral. The inferior lobes of the lungs were affected in 11 cases (60.0%), middle lobe – in 6 cases (30.0%), and superior lobes – in 3 cases (15.0%).

The previous results of the authors' study showed high sensitivity of similar identified radiographic changes for interstitial and alveolar – interstitial lung diseases in acute respiratory distress syndrome (ARDS) [16] and lung damage in the influenza A (H1N1) pandemic in 2009 [17].

The use of US for the diagnosis of lung damage in the COVID-19 pandemic is described in the studies by foreign authors who demonstrated high sensitivity of the method combined with moderate specificity for the diagnosis of coronavirus infection [18, 19]. In addition, a number of researchers assessed the predictive accuracy of scales based on lung US findings in relation to the outcome of the disease [20], the effectiveness of specific treatment methods [21], the length of stay in the intensive care unit, and the need for respiratory support in COVID-19 [22].

Chest X-ray detected changes in the lung tissue in 35 patients, and US – in 32 patients. The architecture of the lungs in most patients was described as local or diffuse increased attenuation in the pulmonary interstitial stroma, less often – as a ground glass opacity. In the US protocol, the most typical changes included manifestations of interstitial lung disease and the white lung phenomenon in the corresponding anatomical zones. According to the RG data, the development of viral and bacterial pneumonia, in addition to the described interstitial changes, was characterized by

the appearance of areas of pulmonary consolidation, which complied with the US findings.

Repeated lung US in the studied group of patients was performed in single cases. Thus, with a combined pattern of interstitial lung disease, the white lung phenomenon, and pulmonary consolidation diagnosed by US, repeated US performed 5 days later typically demonstrated positive changes – the presence of only interstitial lung disease. This was confirmed by RG carried out at runtime, indicating resolution of pulmonary consolidation with persisting signs of viral lesions. On the contrary, in 2 cases, with negative dynamics of the radiographic lung pattern (in the context of probable ARDS) manifested through a significant increase in the attenuation of the pulmonary pattern due to the interstitial component, repeated US revealed signs of diffuse bilateral interstitial lung disease in all scanning areas and in all lung segments.

Bilateral localization of the process was more often detected by X-ray than by ultrasound – in 34 and 29 patients, respectively. However, in two cases, interstitial lung disease was present according to lung US in patients with ambiguous X-ray data. Probably, these discrepancies are associated with the dynamics of the pathological process, the study of patients at different time intervals, better diagnostic capabilities of US in subpleural localization of changes, and the presence of a respiratory artifact in patients with dyspnea.

Using CT, changes in the lungs were additionally diagnosed in 4 patients with negative X-ray presentation. The agreement between the data of CT and lung US was observed in 10 (66.7%) cases out of 15 patients who underwent CT. Similar changes in the lungs were identified by CT and US with lower lobe localization of the process, and the discrepancy between the two methods was observed mainly in patients with a large number of affected lung segments and localization of the inflammation in the superior lobes of the lungs according to CT. It is worth noting that the results of CT were in line with lung densitometry data and US findings, which confirmed the absence of areas of pulmonary consolidation in 2 patients.

A priori, clinical observations indicate that in diseases that cause drastic morphological changes in the lung tissue (pneumonia, tuberculosis), repeated use of radiology techniques for providing real-time, visual control over the state of the affected tissue is not relevant, since the dynamics of pathological changes during treatment is determined by a combination of physical research methods.

On the contrary, COVID-19 pneumonia often develops rapidly, with short survival for patients, therefore, even minimal changes in the lung tissue detected during follow-up are important for patient routing and determining further treatment strategy. Evaluation of the dynamics in US signs allows to make a right decision on prescription of antibiotic and glucocorticoid therapy. It is possible that in the future, the accumulated experience in US in patients with different clinical courses of COVID-19 (phenotypes L and H) will contribute to the diagnosis of lung recruitment maneuvers and earlier and justified transition to the prone position, as well as to the use of oxygen therapy and high positive end-expiratory pressure (PEEP) during mechanical ventilation, i.e. to preservation of the only possibility of prolonging life in some patients.

CONCLUSION

The analysis of US changes in the lungs in patients with verified coronavirus infection SARS-CoV-2 and their comparison with the data of RG and CT of the lungs indicate the possibility of using the method in the diagnosis of COVID-19 pneumonia in a tense epidemiological situation.

In the vast majority of cases, in patients with COVID-19 pneumonia, the ultrasound pattern of the lungs was characterized by interstitial lung disease. Less frequently, diffuse lesions of the lung tissue (the white lung phenomenon) and a combination of interstitial changes (interstitial lung disease and the white lung phenomenon) with pulmonary consolidation were recorded. The obtained and presented data of lung US will help doctors to improve their understanding of radiographic changes in COVID-19 pneumonia. The use of this lung imaging technique should be expanded and brought closer to the patient at any stage of diagnosis and treatment in the context of the COVID-19 pandemic due to the information content, availability, speed of implementation, and the absence of a need for patient transportation.

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Authors contribution

Porovskiy Ya.V. – conception and design, substantiation of the manuscript, analysis and interpretation of data, critical revision of the article for important intellectual content, drafting of the article. Bespalova I.D. – analysis and interpretation of data, critical revision of the article for important intellectual content, drafting of the article, editing of the article, final approval of the manuscript for publication. Sorokina T.V., Dish A.Ju. – analysis of data, editing of the article. Kanev A.F., Koshchavtseva Yu.I. – data search and analytics, interpretation of data. Chuyashenko E.V., Shoulga O.S., Balabanova A.A. – carrying out of the research, analysis of data.

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Флавоноиды как потенциальные ингибиторы коронавируса SARS-CoV-2: исследование *in silico*

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РЕЗЮМЕ

Введение. Вирус SARS-CoV-2 (Severe Acute Respiratory Syndrome CoronaVirus 2) обладает одним из крупнейших геномов, который кодирует 16 неструктурных белков (NSP: Non-Structural Protein), необходимых для репликации и преодоления защитных механизмов организма-хозяина. Флавоноиды представляют интерес в качестве объектов исследования при разработке препаратов для комплексной терапии COVID-19 (Corona Virus Desease 2019). Представители этой группы характеризуются широким спектром биологической активности и высоким профилем безопасности.

Цель работы – провести виртуальный скрининг флавоноидов на возможность ингибирования жизненно важных белков коронавируса SARS-CoV-2.

Материалы и методы. Структуры белков SARS-CoV-2: ADP-связывающего домена NSP3, основной протеазы NSP5, РНК-зависимой-РНК-полимеразы NSP12, эндорибонуклеазы NSP15 получены из Protein Data Bank (PDB). Структуры 163 флавоноидов различных групп, взяты из базы данных ZINC. Процессинг моделей белков осуществляли в программе AutoDockTools, а лигандов – в Raccoon | AutoDock VS. Виртуальный скрининг и ре-докинг проводили в AutoDock Vina.

Результаты. В ходе валидации установлено совпадение конформации нативных лигандов в исходной структуре и при ре-докинге, что позволяет судить о применимости методики виртуального скрининга. Флавоноиды взаимодействовали с ключевыми аминокислотными остатками во всех исследованных белках. Наилучшую энергию аффинитета продемонстрировали 3,7-дигидроксифлавон и 6*S*-кокцинеон Б, обладающий мультимодальным эффектом.

Заключение. Полученные результаты могут быть использованы в разработке фитопрепаратов для комплексной терапии COVID-19.

Ключевые слова: SARS-CoV-2, COVID-19, флавоноиды, молекулярный докинг, виртуальный скрининг, кокцинеон Б

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Flavonoids as potential inhibitors of SARS-CoV-2 infection: *in silico* study

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ABSTRACT

Background. SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) has one of the largest genomes. It encodes 16 non-structural proteins that are necessary for replicating and overcoming host defense mechanisms. Flavonoids are of interest as research objects in developing drugs for comprehensive COVID-19 therapy. This group of compounds is characterized by a wide range of biological activity and a high safety profile.

Aim. To perform virtual screening of flavonoids for possible inhibition of proteins of the SARS-CoV-2 infection.

Materials and methods. Structural proteins of SARS-CoV-2 infection, such as ADP-binding domain NSP3, main protease NSP5, RNA-dependent RNA-polymerase NSP12, and endoribonuclease NSP15, were obtained from Protein Data Bank (PDB). Flavonoid structures were obtained from the ZINC database. Protein models were processed using AutoDockTools software, and ligands were processed in Raccoon | AutoDock VS. Virtual screening and re-docking were performed in AutoDock Vina.

Results. Validation showed agreement between native and re-docked conformations, indicating the applicability of the virtual screening method. Flavonoids interacted with the key amino acid residues in all the studied proteins. The highest binding energy was demonstrated by 3,7-dihydroxyflavone and 6S-coccineone B, the latter having a multimodal effect.

Conclusion. The results of the study may be used for the development of phytomedicines for comprehensive therapy for COVID-19.

Keywords: SARS-CoV-2, COVID-19, flavonoids, molecular docking, virtual screening, coccineone B

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

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INTRODUCTION

Coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) belongs to the *Betacoronavirus* genus, which is a member of the *Coronaviridae* family. SARS-CoV-2 is characterized as an enveloped, positive-sense, single-stranded (+RNA) RNA virus. It has one of the largest genomes among the entire domain, which includes about 30,000 nucleobases. This allows to assume that it has a wide range of biological targets [1]. The genome encodes two overlapping polyproteins that contain 16 non-structural proteins (NSP). Some of them are involved in the replication and life cycle of the virus, while others are necessary to overcome host defense mechanisms [2].

Flavonoid compounds are of particular interest in the development of new drugs for COVID-19 (CoronaVirus Disease 2019) treatment. They are characterized by antiradical [3], antiviral [4], capillary protective [5], and anti-inflammatory effects [5, 6].

The aim of the study was to perform virtual screening of flavonoids for possible inhibition of proteins of the SARS-CoV-2 infection.

MATERIALS AND METHODS

To search for antiviral drugs, we selected four biological targets of SARS-CoV-2 with a resolution of at least 2.5 Å. These targets are presented in the Protein Data Bank (PDB) (date of access: 25.04.2020): ADP-binding domain NSP3 (PDB ID: 6W6Y), main

protease NSP5 (PDB ID: 6LU7), RNA-dependent RNA polymerase NSP12 (PDB ID: 7BV2), endoribonuclease NSP15 (PDB ID: 6VWW). The protein characteristics are presented in Table 1. Processing of protein structures for virtual screening was performed in AutoDockTools Version 1.5.6 (The Scripps Research Institute; USA) [7]. During the processing, water and ligand molecules were removed, missing hydrogen atoms and partial atomic charges were added according to the Gasteiger partial charge calculation method.

The coordinates of native ligands were chosen as centers for constructing GRID maps with dimensions of 25 × 25 × 25 Å (see Table 1). The center of the active site in endoribonuclease NSP15 was determined according to the literature [8].

The structures of 163 flavonoids were obtained from the ZINC database. Preparation of ligands for the study was carried out by adding partial charges in the Raccoon | AutoDock VS version 1.0 (The Scripps Research Institute; USA) [9]. The processing of native ligands for validation re-docking was performed by a similar method.

Table 1

SARS-CoV-2 proteins included in the study

Biological target	Resolution of the resulting protein, Å	Native ligand	GRID-map center, Å
ADP-binding domain NSP3	1.45	AMP	X: 10.567 Y: -8.238 Z: 17.980
Main protease NSP5	2.16	N3 inhibitor	X: -12.149 Y: 14.097 Z: 69.719
RNA-dependent RNA polymerase NSP12	2.50	Remdesivir metabolite	X: 90.089 Y: 93.714 Z: 102.212
Endoribonuclease NSP15	2.20	–	X: -52.239 Y: 30.584 Z: 31.357

Re-docking of native ligands and virtual screening of all compounds were carried out with the AutoDock Vina 1.1.2 software (The Scripps Research Institute; USA) [10] using the Lamarckian genetic algorithm (LGA). The results of molecular modeling were visualized in the Discovery Studio Visualizer v19.1.0.18287 (BIOVIA; USA). To describe the distribution of virtual screening results, the binding energies of flavonoids were chosen that bind better than 10, 50, and 90% of the compounds in the sample.

RESULTS

Positions of ligands during the re-docking almost repeated the geometry in the initial protein structures, which allows to suggest the validity of the research method. The results of the virtual screening of the compounds were ranked according to the scoring function value in the AutoDock Vina 1.1.2 software in the form of binding energy. The obtained data were compared with the binding energy of native ligands. The activity threshold was a scoring function value of 7.1 kcal / mol, as a marker of reversibility of the protein – ligand complex.

In general, the lowest binding energy in the studied flavonoids was noted for endoribonuclease NSP15: successful docking took place for 20 compounds. In contrast, RNA-dependent RNA polymerase NSP12 bound to all 163 virtual structures. ADP-binding domain NSP3 and main protease NSP5 had medium binding energy: 111 and 108 flavonoids, respectively. Within the samples of ligands that were successfully docked into the active centers of biological targets, the median binding energies were –7.4; –7.4; –8.9, and –7.3 kcal / mol for the ADP-binding domain NSP3, main protease NSP5, RNA-dependent RNA polymerase NSP12, and endoribonuclease NSP15, respectively (Table 2).

Table 2

Distribution of flavonoid binding affinity to biological targets

Value characterizing the sample	Binding energy, kcal / mol			
	ADP-binding domain NSP3	Main protease NSP5	RNA-dependent RNA polymerase NSP12	Endoribonuclease NSP15
X_{10}*	–7.1	–7.1	–7.4	–7.1
X_{50}*	–7.4	–7.4	–8.9	–7.3
X_{90}*	–7.8	–7.9	–10.2	–7.6

*X_{10}, X_{50}, X_{90} – binding energy of flavonoids, where 10, 50, and 90% is a proportion (%) of compounds with binding energy that is less in modulus.

The results of virtual screening for the leader compounds are presented in Table 3. Flavonoids were shown to form non-covalent bonds with the same amino acid residues as native ligands. For example, for endoribonuclease NSP15, the following amino acid residues were involved: His 235, His 250, Lys 290, Ser 294, Thr 341, and Tyr 343. The best scoring function values were obtained for 6S-coccineone B and 3,7-dihydroxyflavone. The docking results with the leader compounds are shown in the Figure.

Table 3

Results of re-docking of native ligands and docking of 3 leader compounds into the active sites of SARS-CoV-2 proteins

Ligand	Scoring function (binding energy), kcal / mol	Protein – ligand interaction
ADP-binding domain NSP3		
AMP	–8.7	H-bonds: Ala 21, Asp 22, Ile 23, Val 49, Ile 131. Hydrophobic: Ala 38, Gly 48, Ala 50, Ala 52, Pro 125, Leu 126, Gly 130, Phe 132, Ala 154, Val 155, Phe 156, Leu 160. π-stacking: Ile 23, Val 49.
6*S*-coccineone B	–8.3	H-bonds: Ala 21, Gly 130. Hydrophobic: Asp 22, Ile 23, Gly 48, Pro 125, Leu 126, Pro 136, Ala 154, Phe 156, Leu 160, Leu 164. π-stacking: Val 49, Ala 52, Ala 129, Val 155.
6*R*-coccineone B	–8.0	H-bonds: Gly 130. Hydrophobic: Ala 21, Asp 22, Ile 23, Pro 125, Pro 136, Ala 154, Asp 157. π-stacking: Gly 48, Val 49, Ala 52, Leu 126, Ala 129, Val 155, Phe 156, Leu 160.
7,8-dihydroxyflavone	–8.0	H-bonds: Ile 23, Gly 48. Hydrophobic: Ala 21, Asp 22, Gly 130, Pro 136, Ala 154, Phe 156, Leu 160. π-stacking: Val 49, Ala 52, Leu 126, Ala 129, Val 155.
Main protease NSP5		
N3 inhibitor	–8.4	H-bonds: Phe 140, Asn 142, Gly 143, His 163, His 164, Glu 166, Gln 189, Thr 190. Hydrophobic: Thr 24, Thr 25, Thr 26, Met 49, Tyr 54, Ser 144, Cys 145, Met 165, His 172, Asp 187. π-stacking: His 41, Leu 141, Pro 168, Ala 191. Covalent: Cys 145.
6*S*-coccineone B	–8.5	H-bonds: Ser 144, Cys 145. Hydrophobic: Thr 25, Thr 26, His 41, Phe 140, Leu 141, Asn 142, Gly 143, His 163, His 164, Met 165, Glu 166. π-stacking: Leu 27, Cys 145.
Scutellarein	–8.1	H-bonds: Leu 141, Ser 144, Cys 145, Glu 166. Hydrophobic: His 141, Met 49, Phe 140, Asn 142, His 163, His 172, Arg 188, Gln 189, Thr 190. π-stacking: Met 165.
2-(1,3-benzodioxol-5-yl)-6-hydroxy-4*H*-chromen-4-one	–8.1	H-bonds: His 41, Phe 141, His 163, Asp 187. Hydrophobic: Pro 52, Tyr 54, Leu 141, Ser 144, His 164, Met 165, Glu 166, His 172, Arg 188, Gln 189. π-stacking: Cys 145, Met 49, His 41.
RNA-dependent RNA polymerase NSP12		
Remdesivir metabolite	–8.3	H-bonds: U(T) 10, U(P) 20, Asp 760. Hydrophobic: Lys 545, Val 557, Cys 622, Asp 623, Ser 682, Thr 687, Ala 688, Ser 757, Ser 759. π-stacking: A(11) 11, U20. Covalent: U(P) 20. Coulombic: Mg 101, Mg 1004.
3,7-dihydroxyflavone	–10.4	H-bonds: U(T) 12, U(P) 20, Gly 590. Hydrophobic: A(T) 11, A(P) 19, Val 588, Thr 591, Ser 592, Trp 598, Met 601, Ala 688, Gln 815. π-stacking: Ile 589, Lys 593, Leu 758, Cys 813.
2-(2,5-dimethoxy-phenyl)-3-hydroxy-chromen-4-one	–10.3	H-bonds: Gly 590, Thr 591. Hydrophobic: U(T) 12, A(T) 14, A(P) 19, U(P) 20, Ser 592, Phe 594, Trp 598, Met 601, Phe 812, Gln 815. π-stacking: A(T) 13, Ile 589, Lys 593, Leu 758, Cys 813.
Pinobanksin 3-*O*-propanoate	–10.3	H-bonds: U(P) 20, Gly 590. Hydrophobic: U(T) 12, A(T) 14, U(P) 18, A(P) 19, Thr 591, Ser 592, Phe 594, Trp 598, Gln 815. π-stacking: A(T) 13, Ile 589, Lys 593, Leu 758, Cys 813.
Endoribonuclease NSP15		
6*S*-coccineone B	–7.9	H-bonds: His 250, Val 292, Tyr 343. Hydrophobic: Lys 290, Tyr 343. π-stacking: His 235, Gly 248, Cys 293, Thr 341, Leu 346.
6*R*-coccineone B	–7.6	H-bonds: Val 292, Tyr 343. Hydrophobic: His 235, Gly 247, His 250, Cys 293, Thr 341, Leu 346. π-stacking: Lys 296, Tyr 343.
Calycosin	–7.6	H-bonds: His 235, Lys 290, Ser 294, Tyr 343. Hydrophobic: Gly 248, His 250, Cys 293, Trp 333, Glu 340, Leu 346. π-stacking: Trp 333, Thr 341, Tyr 343.

Note: the scoring values obtained as a result of three repetitions were identical. The native ligand is in italics.

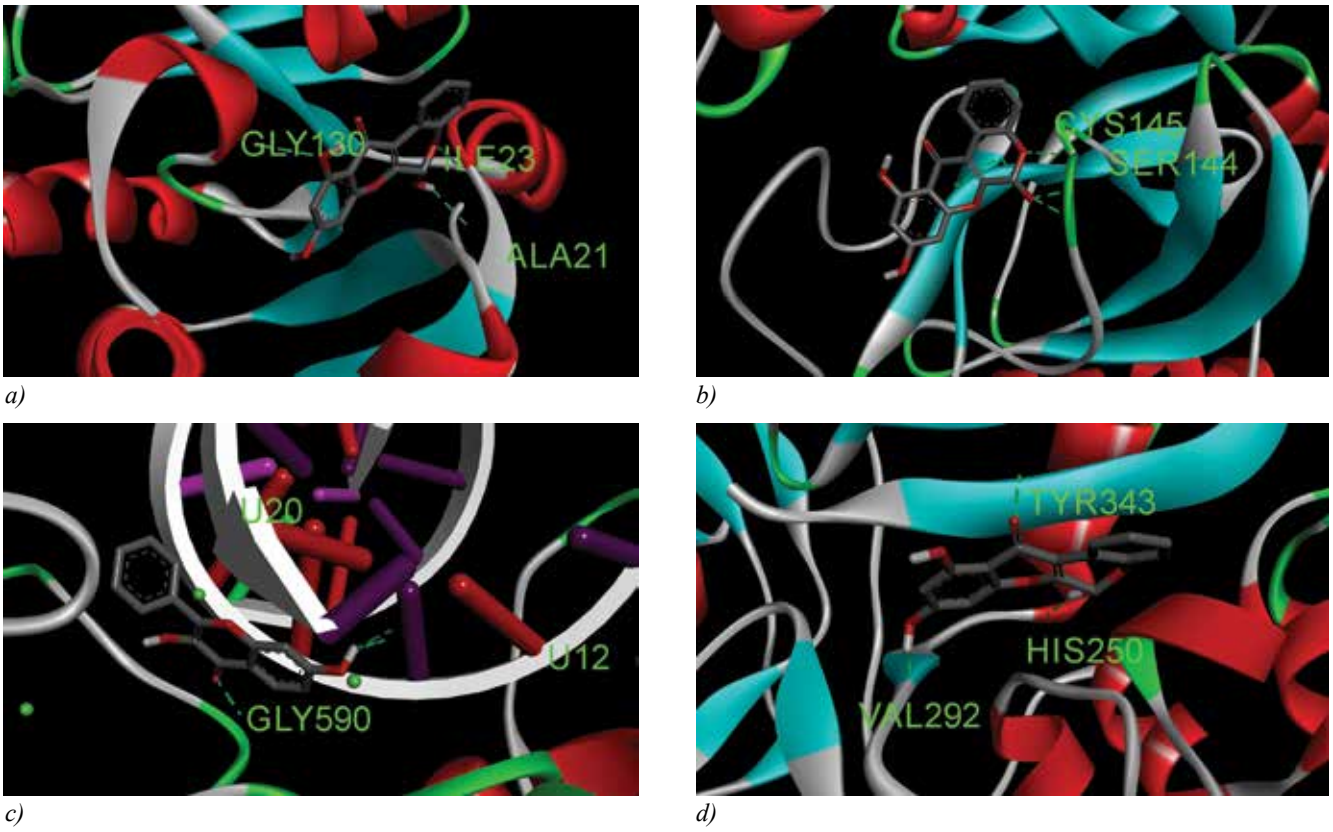

Figure. Docking: *a)* 6*S*-coccineone B into ADP-binding domain NSP3, *b)* 6*S*-coccineone B into main protease NSP5, *c)* 3,7-dihydroxyflavone into RNA-dependent RNA polymerase NSP12, *d)* 6*S*-coccineone B into endoribonuclease NSP15. Hydrogen bonds are shown as green dotted lines

It is interesting to note that the spatial structure of the ligand affects the qualitative and quantitative characteristics of docking. Thus, for 2*R*,3*R*-dihydroquercetin, better binding to main protease NSP5 was found compared with its other stereoisomers.

DISCUSSION

The median flavonoid binding energies to the ADP-binding domain NSP3, main protease NSP5, and endoribonuclease NSP15 had similar values (Table 2), which can be explained by belonging of these proteins to a class of hydrolases. In contrast, the median value of binding affinity to RNA-dependent RNA polymerase NSP12 belonging to a class of transferases was higher than that of the three above-mentioned proteins (Table 2).

The interaction of these compounds with the ADP-binding domain NSP3 can presumably block the ability of coronavirus to hide from host defense mechanisms [11]. Inhibition of main protease NSP5 and RNA-dependent RNA polymerase NSP12 can lead to prevention of assembly of new virions. The interaction of flavonoids with the uridylate-specific site of endoribonuclease NSP15 presumably blocks protein interference with the host innate immune response [12]. COVID-19 affects the lungs and is accompanied by inflammation [13]. Given a wide spectrum of pharmacological activities of flavonoids, which have proven to be effective capillary protectors [5] and anti-inflammatory agents [6], and their multitarget antiviral effect, these natural compounds can find application in the treatment of this disease.

CONCLUSION

In the course of the study, flavonoids were found to have a virucidal effect on SARS-CoV-2. One of them, 6S-coccineon B, is able to show high activity against several biological targets of SARS-CoV-2. The obtained results can be used to develop phytomedicines for comprehensive therapy of COVID-19.

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Remote monitoring of chronic noncommunicable diseases: potential in the COVID-19 pandemic

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ABSTRACT

Aim. To review the current progress in the use of remote health monitoring (RHM) technologies for chronic noncommunicable diseases (CNCD).

To search for data, we used Web of Science, Scopus, Russian Science Citation Index, Academic Search Complete (EBSCO), Cochrain, and PubMed databases. The date range was 5–10 years. The importance of development of RHM technologies and their further study was shown to confirm the evidence of effect of certain RHM systems.

New approaches to the integration of the medical community into the international telemedicine strategy are considered. It was established that RHM can potentially decrease treatment costs and reduce the burden on medical organizations. The review analyzes the experience in using RHM in patients with cardiovascular diseases, as well as respiratory and endocrine disorders. The review also summarizes and systematizes the findings of studies on assessing the effectiveness of RHM technologies in clinical practice, including their use in the COVID-19 pandemic.

It is noted that despite high interest of the scientific community in the study of RHM technologies, unambiguous results demonstrating the effectiveness of such developments in clinical practice have not been presented.

Keywords: chronic noncommunicable diseases (CNCD), remote health monitoring, telemedicine, bronchial asthma, chronic obstructive pulmonary disease (COPD), implantable cardioverter defibrillator (ICD), implantable loop recorder, implantable pacemakers, diabetes, telemonitoring

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Удаленный мониторинг хронических неинфекционных заболеваний: потенциал в условиях пандемии COVID-19

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РЕЗЮМЕ

Цель: анализ актуального опыта использования существующих технологий удаленного мониторинга (УМ) хронических неинфекционных заболеваний (ХНИЗ).

Для поиска были использованы базы данных Web of Science, Scopus и Российского индекса научного цитирования, Academic Search Complete (EBSCO), библиотеки PubMed и Cochrain. Глубина поиска – 5–10 лет. Показана значимость развития технологий УМ и их дальнейшего изучения для подтверждения доказательности конкретных методов УМ.

Рассмотрены новые подходы к интеграции медицинского сообщества в международную повестку телемедицины. Установлено, что использование УМ потенциально способно снизить финансовые затраты на лечение пациентов и уменьшить нагрузку на медицинские организации. Проанализированы результаты применения УМ состояния пациентов с патологией сердечно-сосудистой системы, болезнями дыхательной системы, с заболеваниями эндокринной системы. Обобщены и систематизированы результаты исследований, посвященных оценке эффективности применения конкретных технологий УМ в клинической практике, в том числе в условиях пандемии новой коронавирусной инфекции SARS-CoV-2.

Отмечено, что несмотря на высокую заинтересованность научного сообщества в изучении технологий УМ, однозначных результатов, демонстрирующих эффективность разработок в клинической практике, в настоящее время не представлено.

Ключевые слова: ХНИЗ, удаленный мониторинг, УМ, телемедицина, бронхиальная астма, ХОБЛ, имплантируемый кардиовертер-дефибриллятор, ИКД, имплантируемый петлевой регистратор, имплантируемые кардиостимуляторы, сахарный диабет, телемониторинг

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

Current progress in medicine makes it possible to successfully combat many pathological conditions, and synthesis of biomedicine with innovative technologies allows to diagnose many pathologies at early stages. However, despite advances in understanding the etiology and pathogenesis of many diseases, as well as rapid development of pharmacology, mortality from chronic noncommunicable diseases (CNCDs) is currently estimated by the World Health Organization (WHO) at 41 million people, which accounts for 71% of all deaths in the world. Of them, 17 million deaths occur before the age of 70 and are premature [1].

One of the factors contributing to the spread of CNCDs is the problem of their treatment only in the exacerbation phase. Patient parameters are not monitored during remission, and, therefore, a doctor is not able to timely adjust the backbone therapy and treatment strategy. In accordance with the above-men-

tioned, as well as with the current conditions of a novel coronavirus infection SARS-CoV-2 (COVID-19) pandemic, uncontrollable increase in the disease incidence, and high burden on the healthcare system, innovative digital remote health monitoring (RHM) technologies are gaining momentum. The use of RHM technologies can potentially decrease treatment costs due to remote diagnosis and treatment, as well as reduce the burden on medical organizations and risks of complications from chronic diseases [2–4].

The unprecedented spread rate of COVID-19 requires changes in a usual lifestyle, in particular, reducing to a minimum contacts with people and observing the self-isolation regime [5, 6]. Recommendations on reducing the number of physical contacts between patients and healthcare professionals are defined by the European Center for Disease Prevention and Control (ECDC) and WHO as medical distancing [7, 8]. The need to comply with the ECDC and WHO recommendations increases the importance of new digital technologies and demand of the healthcare system for them.

Nevertheless, a significant barrier to introduction of RHM into medical practice is a small number of randomized clinical trials in this area, as well as a lack of systematic reviews on this topic, which casts doubt on the evidence of effect of RHM methods for controlling CNCDs and the possibility of their use in the post-COVID-19 era [9–11].

The aim of this study was to systematize and review the current progress in the use of RHM technologies and assess their effectiveness for patients in clinical trials, including their use in the COVID-19 pandemic.

MATERIALS AND METHODS

This review includes randomized clinical trials published from 2010 to 2020. The Web of Science, Scopus, Russian Science Citation Index, Academic Search Complete (EBSCO), Cochrain, and PubMed databases were used for the search. Key words, such as “telemedicine”, “telemonitoring of CNCDs”, “remote health monitoring”, “cost analysis”, “m-health”, and “SABA monitoring”, were used as search markers. A total of 5,556 publications were found, after the initial screening, 864 publications were included in the analysis, of which 86 were included in the comparative analysis. In addition, the review includes earlier studies that make it possible to assess the historical perspective of the effectiveness of RHM technologies for assessing the condition of patients with CNCDs.

PATIENTS WITH CARDIOVASCULAR DISEASES

In the Russian Federation, according to the statistical report of the WHO (2017), CNCDs account for 86% of all deaths, most of which are related to cardiovascular diseases (60%) [12, 13]. Currently, implantable pacemakers (IPM) and implantable cardioverter defibrillators (ICDs), which correct heart rhythm disorders, as well as implantable loop recorders, which perform remote electrocardiogram (ECG) monitoring, play an essential role in the diagnosis and treatment of chronic cardiovascular diseases.

Despite high diagnostic efficiency of these devices, there is a need for regular visits to a doctor to analyze the operation of a device and assess the clinical condition of a patient. Currently, such visits can be avoided due to IPM and ICD equipped with a RHM system and clinically tested in a number of countries [14].

A group of scientists from the BIOTRONIK global medical device company carried out a multicenter, prospective, randomized trial in 2010 aimed at safely reducing the number of standard checks of office devices. In this study, a group of patients (n = 977) were implanted with an IPM with the RHM function, while another group of patients (n = 473) were implanted with an ICD without RHM. According to the results of the study, 3,099 out of 3,316 possible planned follow-up observations were performed in the first group, compared with 1,354 out of 1,526 observations in the control group (93.5% versus 88.7% within 12 months, $p < 0.001$), which indicates more careful adherence to routine health checks in the group with RHM. It is important to note that the average number of doctor’s visits (planned and unplanned) was 2.1 per patient per year for the RHM group and 3.8 for the control group. Thus, it was shown that RHM reduces the total number of doctor’s visits by 45% and at the same time contributes to more effective detection of asymptomatic abnormalities [15]. Similar results were obtained by Adamson et al. (2012) at the Oklahoma Heart Hospital [16]. Later, Ching et al. (2016) in their experiment proved the effectiveness of RHM in patients with ICD, as well as permanent IPM and implantable defibrillators for cardiac resynchronization therapy [17].

L. Guedon-Moreau et al. in 2013 confirmed and supplemented the data obtained in the study by BIOTRONIK. Participants of the study with ICD equipped with the RHM system (n = 221) were invited for a face-to-face examination only at the 1st, 3rd, and

27th months after ICD implantation. Participants from the control group (n = 212) were additionally invited at the 9th, 15th, and 21st months after ICD implantation. The group of patients whose ICD transmitted data to the doctor reduced the burden on the hospital to 1.46 visits per patient per year, in contrast to the control group with 2.23 visits per patient per year [15, 18].

The group of researchers led by P. Mabo (2010, 2012) also demonstrated a decrease in the number of outpatient observations per patient per year in the group with RHM (0.51 ± 0.71 (95% confidence interval (CI): 0.43–0.59) versus 1.15 ± 1.07 (95% CI: 1.03–1.27)) compared with the control group. Their results also showed high efficiency of RHM in identifying various disturbances in the ICD operation. In the COMPAS (Cardiovascular Outcomes for People Using Anticoagulation Strategies) study (n = 494) conducted by the same group of scientists, it was shown that the use of the RHM system may decrease the interval between the onset of cardiac events and a doctor's examination by 117 days for patients with IPM compared with traditional follow-up ($p < 0.001$)[19].

H. Versteeg et al. in 2019 conducted one of the first multicenter, randomized trials in parallel groups on the efficiency and safety of ICD, assessing the effect of implantation and RHM on the quality of life (QOL) in patients during 2 years of post-implantation follow-up. Patients with ICD were randomized into two groups. The first group was experimental (n = 300) and included RHM with annual examination and consultation in a medical organization. The second group (n = 295) implied registration of ICD data without RHM in a medical organization for 3–6 months during 2 years after implantation. QOL and well-being were assessed using the Kansas City Cardiomyopathy Questionnaire (KCCQ) and the Florida Patient Acceptance Survey (FPAS). The authors found that the mindset of patients in the first two years of the post-implantation period can completely replace meetings with medical professionals. The results of the study showed an insignificant statistical difference in the QOL and well-being of patients after ICD implantation in different post-implantation periods (3.3 points on both scales, (beta –6.41; 95% CI: p = 0.001) [20].

G.H. Crossley et al. (2011) demonstrated that clinical decision-making in patients with RHM is reduced on average by 17.4 days and amounts to 4.6 days ($p < 0.001$), whereas standard follow-up observations allow to make decisions on average only 22 days (p <0.001) after the emergence of disorders in the cardiovascular system [21].

In addition to IPM and ICD, there are systems that allow for remote monitoring of systolic blood pressure parameters in patients with cardiovascular diseases. For example, Abbott Corporation (Abbott, Illinois, USA) launched a CardioMEMS Heart Failure Sensor (CardioMEMS HF Sensor) on the US market in 2014. CardioMEMS HF Sensor is a diagnostic system for monitoring heart failure which is implanted into the pulmonary artery (PA) and monitors systolic blood pressure, accumulating data on the functions of the cardiovascular system on the server [22].

P.B. Adamson et al. (2011) implanted this sensor in 550 patients with NYHA (New York Heart Association Functional Classification) functional class III chronic heart failure (CHF). The results showed that if the doctor was granted access to PA pressure readings remotely, the hospitalization rate decreased by 33% compared with the control follow-up. The authors also pointed out that the decrease in the hospitalization rate was associated with a possibility of taking preventive measures to eliminate the attack based on daily RHM [23, 24]. After the sensor was introduced in the market, the same team of researchers determined the effectiveness of the device based on data from 2,000 patients [25]. Later in 2018, a retrospective study was conducted in Los Angeles confirming the decrease in the number of hospitalizations (n = 73) and improvement in hemodynamic parameters in patients with an implantable CardioMEMS HF Sensor [26].

A number of studies prove the effectiveness of RHM in patients with essential hypertension. For example, B. McKinstry et al. compared standard blood pressure control in the medical organization and control using RHM (2013). After a six-month follow-up, the difference in systolic and diastolic blood pressure in the control group (n = 201) and the RHM group was 4.3 mmHg (95% CI: 2.0–6.5; p = 0.0002) and 2.3 mmHg (95% CI: p = 0.001), respectively. In a retrospective comparison, it was found that 39% of participants in the RHM group promptly increased the daily dose of the antihypertensive drugs, while in the control group, the proportion of such participants was only 12% (p = 0.0003) [27].

J. Evans et al. (2016) showed that the use of a wireless, wristwatch-based monitoring device that continuously recorded health data in patients over 55 years with the underlying cardiovascular disease reduced hospitalizations to a heart hospital [28, 29].

The effectiveness of RHM in terms of blood pressure control has also been demonstrated by K.L.Margolis et al. (2013). In their study, after a six-month

follow-up in the control group (n = 222), the target blood pressure level was achieved in 30% of patients (95% CI: 23.2–37.8); in the RHM group, the target value was achieved in 57.2% of participants (95% CI: 44.8–68.7). The difference in systolic and diastolic blood pressure between the groups was 10.7 mmHg (95% CI: 14.3–7.7; $p < 0.0001$) and 6.0 mmHg (95% CI: 8.6–13.4; $p < 0.0001$), respectively [30].

E. Piotrowicz et al. (2019) conducted a large, multicenter, prospective, open-label, randomized clinical trial to evaluate the implementation of hybrid comprehensive telerehabilitation (HCTR) in clinical practice. The study involved 850 patients with CHF 6 months after hospitalization with NYHA functional class I, II, and III CHF and left ventricular ejection fraction (LVEF) of 40% or less according to the Simpson's ejection fraction tool. For 9 weeks, the patients underwent a telerehabilitation program (1 week in the medical organization and 8 weeks outside the medical facility), which included RHM, taking medications, and undergoing rehabilitation in the medical organization.

After 26-month follow-up, the average duration of hospital stay in the RHM group (n = 425, 91.3 days) showed a statistically insignificant difference compared with the control group without the implementation of the telerehabilitation program (n = 425, 92.8 days) (95% CI: 0.46–0.53; $p = 0.74$). During the follow-up, the number of deaths after 24 months of the program implementation in the RHM group was 54 (12.5%) versus 52 (12.4%) in the control group (95% CI: 0.70–1.51). There was also no statistically significant difference in the hospitalization rate (95% CI: 0.79–1.13). During the study, cardiorespiratory endurance test parameters for determining peak oxygen consumption were 0.00 ml / kg / min (95% CI: 0.31–0.30; $p < 0.001$) versus 0.95 ml / kg / min (95% CI: 0.65–1.26; $p < 0.001$) in the control group and the RHM group, respectively. The QOL in the patients had been assessed using a non-specific questionnaire for assessing the quality of life (SF-36, The Short Form-36) over 24 months: 1.58 (95% CI: 0.74–2.42, $p = 0.008$) versus 0 (CI 95%: -0.84–0.84, $p = 0.008$) in the experimental and control groups, respectively. Therefore, the implementation of RHM into clinical practice did not contribute to a decrease in the number of days spent in the medical institution per patient and the number of hospitalizations and deaths [31, 32].

Insignificant differences in the hospitalization rate between the control (n = 110) and experimental (n = 223) groups were noted (34.5% versus 39.1%, $p = 0.48$), however, significant improvement in QOL according to SF-36 in the experimental group (2.6 points for physical wellbeing, $p < 0.0001$; 1.69 points for mental health, $p = 0.4$) was demonstrated in the study by Olivari et al. (2018) [33].

J.P. Halcox et al. (2017) conducted a randomized, controlled trial (RCT) on the risk of atrial fibrillation (AF) in 1,001 patients over 65 years of age using a Wi-Fi AliveCor heart monitor with the function of connecting to a mobile device. All studied patients were divided into a control group (n = 501) and an experimental group (n = 500) depending on the parameters of the risk assessment scale for thromboembolic complications in patients with AF. During the first 12 months, AF was diagnosed in 3.8% of patients in the experimental group compared with 1% of patients in the control group (risk ratio (RR) = 3.9; 95% CI: 1.4–10.4, $p = 0.007$). The proportion of patients with thromboembolic complications (acute cerebral stroke, transient ischemic attack, systemic thromboembolism) in the experimental group was 1.2% versus 2% in the control group (RR = 0.61; 95% CI: 0.22–1.69, $p = 0.34$), which confirms the role of RHM in prevention and early diagnosis of complications of cardiovascular diseases [34].

Similar results were obtained by M.J.Reed et al. (2018); they proved the effectiveness of remote monitoring of the risks for AF in emergency care units with the ability to synchronize AliveCor with mobile devices of medical personnel [35]. However, there are studies that have proven the absence of a relationship between a positive outcome of patient treatment and RHM. Thus, the results obtained by J.H.Morgan et al. (2017) in the clinical study of RHM in patients with CHF using an ICD did not confirm the role of RHM in reducing the number of hospitalizations [36–39].

A.P.Vanezis et al. (2018) evaluated the efficiency of remote ischemic preconditioning (RIPC) for restoration of reduced (less than 45%) LVEF in patients after ST-segment elevation myocardial infarction (n = 73) who underwent percutaneous coronary intervention (PCI). The baseline mean value for LVEF in the experimental and control groups (n = 38) was comparable both before and after 4 weeks of the follow-up ($p = 0.952$) [40].

Implantable antiarrhythmic devices (IADs) which are therapeutic and diagnostic systems that collect and transmit statistical information about the health status of a patient with heart rhythm disturbances play a crucial role in RHM of patients with cardiovascular diseases [41]. According to a number of domestic researchers, the number of IADs is growing rap-

idly; currently, about 300 devices are implanted per one million population in the Russian Federation. A review presented by a group of authors led by N.N. Lomidze et al. (2019) reported data on the RHM system "Home Monitoring" manufactured by Biotronik, which is based on RHM of patients with IAD via a mobile phone. The data received from the device are transmitted to a unified service portal with subsequent analysis of the information, which is then remotely transmitted to the attending physician.

A group of researchers from the Vishnevsky National Medical Research Center of Surgery (Moscow) demonstrated the effectiveness of using ICDs (n = 56) and pacemakers (n = 7) manufactured by Biotronik. The average age of the patients was 57.0 ± 11.6 years. Most of the subjects (n = 45) were implanted with ICDs due to the presence of paroxysmal ventricular tachycardia (PVT) or ventricular fibrillation (VF), the rest (n = 11) were implanted with ICDs to prevent sudden cardiac death. Every day, the received data were transmitted to the doctor via the Biotronik "Home Monitoring" system. On average, the follow-up period was 24.5 ± 17.4 months, and the number of critical situations per 1 patient per year according to RHM data was 35.3 ± 33.6 [41].

The research on the attitude of patients to RHM is particularly worth mentioning. I.Timmermans et al. (2018) analyzed patient satisfaction (n = 300) with RHM, as well as their preferences. It was found that 12 months after the implantation, the average patient satisfaction with RHM was 0.8 (interquartile range = 7–10). Of 244 patients, 44% preferred RHM, 16% preferred face-to-face follow-up in the medical organization without RHM, and 40% of patients did not express any preference. In addition, it was found that patients without RHM were much more likely to receive resynchronization therapy (p = 0.018), which confirms the preventive role of RHM. It is worth noting that RHM patients were more likely to report well-being during the study (p = 0.02 and p = 0.017) and were satisfied with the ICD performance [42].

Summarizing the results of the studies on RHM methods in cardiac patients, it can be concluded that currently the use of RHM technologies can improve the patient's condition, but does not always help to reduce mortality, hospitalization rate, and risks of disease exacerbation.

PATIENTS WITH RESPIRATORY DISEASES

According to the WHO estimates, 65 million people worldwide suffer from moderate to severe chronic obstructive pulmonary disease (COPD), and 235 million people suffer from bronchial asthma (BA). Nearly 90% of COPD deaths occur in low- and middle-income countries. In addition, in 2017, more than 120,000 patients with BA, more than 380,000 patients with chronic and unspecified bronchitis, and 95,000 patients with COPD were registered in the Russian Federation [43].

In the study by P.H. Lilholt et al. (2017), during 12-month follow-up, patients from the group with the ability to remotely measure blood pressure, oxygen saturation level, and heart rate (n = 258) were required to regularly fill out the SF-36 questionnaire. Study participants without RHM were included in the control group (n = 316). According to the results of the analysis, the difference between the SF-36 scores in the RHM group and the control group was statistically insignificant and amounted to 0.2 points (95% CI: 0.9–1.3) and 0.4 points (95% CI: 1.0–1.7), respectively [44].

Similar results were demonstrated by P.P. Walker et al. (2018) using the European Quality of Life Questionnaire (EuroQoL EQ-5D). In addition, the difference in the number of exacerbations of COPD in the control and experimental groups (1.74 versus 1.52; p = 0.499), the number of hospitalizations (0.79 versus 0.99, p = 0.276), and the number of patients not hospitalized during the study (71% versus 74%, p = 0.599) were statistically insignificant. Nevertheless, the followed-up patients previously hospitalized with the exacerbation of COPD showed a 53% decrease in the hospitalization rate (p = 0.017) compared with the control group [45]. A number of studies with a similar design also demonstrated that there is no significant difference in the clinical presentation between the standard care COPD group and the RHM COPD group, and the number of hospitalizations and exacerbations also changes insignificantly [46–48].

A. Farmer et al. (2017) conducted six-month monitoring of patients with COPD, which revealed that in the control group (n = 166) and the group with RHM (n = 110), the differences in the clinical presentation according to the scores of the St.George's Respiratory Questionnaire for patients with COPD (SGRQ-C) were insignificant (p = 0.69 and p = 0.49). However, the use of RHM contributed to a decrease in the number of physical examinations by a doctor in the RHM group compared to the control group (4 versus 5.5; p = 0.06), as well as in the number of hospitalizations [RR = 0.83; 95% CI: 0.56–1.24, p = 0.37] [49].

A slight difference in the parameters between the control and RHM groups in the studies described above may be due to a high level of medical care for patients with COPD in the countries where the studies were conducted, which may reduce the positive effect of RHM technologies. It should be noted that modern equipment for remote measurement of spirometry parameters requires further study and improvement, which is confirmed in the paper by V.I.Sirichana et al. (2014) [50].

Data from a number of studies prove that RHM systems in BA are potentially capable of improving symptom control and tracking patient's medication intake [51]. M.A.Barrett et al. (2017) demonstrated the effect of RHM of the use of β-adrenergic agonists on BA control. The authors used an inhaler with a sensor that remotely monitored the frequency of inhalations, as well as spotted the location of the patient. The study included 95 participants who used the sensor for at least 60 days, 30 of which were the control period, the data on the frequency of inhalations were not transmitted to the doctors and participants themselves. According to the results of the study, the number of inhalations per patient was 0.27 per day, which is 39% (0.44) smaller than in the control period. For the participants who completed the study within 12 months (n = 35), the proportion of asymptomatic days was 95%, which is 23% more than the baseline value. Throughout the follow-up, asthma control significantly improved, which was associated with regular assessments and discussions of certain attacks that provoked the need for inhalations with the doctor [52].

R.C.Merchant et al. (2016) obtained similar results on RHM of the use of inhaled drugs. After 12 months of monitoring in the control group (n = 247), the average number of seizures decreased by 0.31 versus 0.41 (p < 0.001) in the RHM group, and the number of days without seizures increased by 17% versus 21% (p = 0.01), respectively [53]. Kew et al. (2016), however, demonstrated an insignificant difference between face-to-face and remote forms of BA control in terms of the frequency of exacerbations, BA control, and the quality of life in patients [54].

In the study by J.C. de Jongste et al. (2009), children with BA were randomly divided into two groups: in the first group, the participants used a device for monitoring airway inflammation, which recorded the amount of nitric oxide during exhalation; the second group was the control group. In addition, each participant in both groups recorded their asthma attacks in an electronic diary. As a result of the three-month study, a decrease in the dose of the inhaled corticosteroid (400 mg versus 200 mg; p < 0.0001) and an increase in the number of asymptomatic days in both groups were noted. In addition, the forced expiratory volume improved from 88% (AV (average volume) of 13% for group 1; AV of 15% for group 2) to 95% (AV of 14% for groups 1 and 2). There was no significant difference between the groups. The authors of the study concluded that only the electronic diary contributed to such improvements, while monitoring of inflammatory markers did not affect the improvement of the patients' condition [55].

PATIENTS WITH ENDOCRINE DISEASES

Another important group of CNCDs includes pathologies of the endocrine system. Currently, according to the International Diabetes Federation, over 425 million people suffer from diabetes mellitus (DM) worldwide [56]. Taking into account the peculiarities of the course and complications of this disease, RHM of patients can be of great importance in the work of an endocrinologist [57].

M.L. Michaud et al. (2018) compared standard control of health parameters in patients with type 2 diabetes (T2D) in the medical organization and control with the use of RHM. Study participants (n = 955) for three months measured blood pressure, blood glucose level, and body weight daily with the ability to upload data to a unified server. In addition, they contacted healthcare professionals on a weekly basis for dietary adjustments, self-management counseling, and compliance assessments. Prior to study initiation, the mean glycosylated hemoglobin (HbA_{1c}) value in the participants was 7.92%, and after the end of the study, it was 7.09% (p < 0.001). Besides, the number of participants with HbA_{1c} > 9% decreased from 213 to 93 (p < 0.001) [58].

The effectiveness of RHM in terms of controlling HbA_{1c} levels has been demonstrated by A. Steventon et al. (2014). In their study, after 12-month follow-up in the RHM group (n = 300), the HbA_{1c} level decreased by 0.21% (2.3 mmol / l) versus 0.1% in the control group (95% CI: 0.04–0.38; p = 0.013). The design of the project involved the use of a glucometer with a RHM function by patients from the RHM group, as well as completion of an online questionnaire about their wellbeing [59].

Similar results were obtained in the study by S.H. Wild et al. (2016), where in the RHM group (n = 146), the HbA_{1c} level decreased by 5.6 mmol / l (95% CI: 2.38–8.81, p = 0.0007) and reached 57. 4 mmol / l.

In the control group (n = 139), the HbA_{1c} level was 67.8 mmol / l. The analysis of the results also showed a decrease in systolic blood pressure by 3.06 mmHg (95% CI: 0.56–5.56 mmHg; p = 0.017) and diastolic blood pressure by 2.17 mmHg (95% CI: 0.62–3.72 mmHg; p = 0.006) in the RHM group [60].

The effectiveness and safety of RHM in patients with T2D was studied by J.Y. Jeong et al. (2018). Study participants were divided into a control group without RHM (n = 113) and two groups with RHM, which received follow-up for 24 weeks. In the groups with RHM, a glucometer and a bioimpedance body composition analyzer with the function of remote data processing were used. According to the results of the study, all three groups showed a decrease in the level of HbA_{1c} (-0.66% ± 1.03% in the control group, –0.66% ± 1.09% in the group with RHM and –0.81% ± ± 1.05% in the group with remote consultations, $p < 0.001$), whereas in the groups with RHM the decrease in the HbA_{1c} level was statistically insignificant [61].

Similar results were obtained in the study by C. Dario et al. (2017), where after 12-month follow-up, the difference in the HbA_{1c} level between the RHM group and the control group was 0.01 (-0.26 ± 0.92 versus -0.27 ± 0.99, respectively, p = 0.76). The authors also noted that RHM made it possible to reduce the number of doctor's visits of the participants ($p < 0.0001$) and the number of hospitalizations (p = 0.02) [62].

Summarizing the results of the presented studies, it can be stated that today the advantage of using RHM for controlling and diagnosing the health status of patients with T2D is not obvious and requires further study.

DISCUSSION

Therefore, despite high interest of the scientific community in the study of RHM technologies, there are currently no unambiguous results demonstrating the effectiveness of RHM systems in clinical practice.

Positive results on the use of RHM were demonstrated by the authors who studied the advantages of RHM in diseases of the cardiovascular system, which can have direct practical application in cardiology centers and at local visits to a cardiologist. These studies show significant improvements in patients' health status and a decrease in the number of hospitalizations. In addition, RHM of the condition of a patient with cardiovascular diseases makes it possible to create unified databases with the results of monitoring patients with ICD and IPM, which will contribute to accumulation of medical data and maintenance of personal electronic health records [63]including resource management, medical process management and care delivery. A great number of the Internet users in Russia has a significant impact on integration of Internet technologies into all areas of public life, including health care. Purpose is to identify most perspective directions for development of Internet technologies in (domestic.

It should be noted that the effectiveness of RHM implementation in patients with cardiovascular diseases requires further study and systematization of methods for processing data obtained using the RHM systems. The latter is supported by a number of studies, including the work by G. Pounds et al. (2017), devoted to the analysis of the efficiency and labor costs of medical personnel in evaluating data obtained from implantable loop recorders, as well as the results of a randomized, prospective, multicenter, economic trial within the EuroEco project in the work by H. Heidbuchel et al. (2015) [64, 65].

Some researchers in the field of endocrinology agree that glucometers with the ability to remotely transmit physiological data are not significantly more effective than standard devices, where patients are forced to independently control their blood glucose level [61, 62]. Similar ambiguity is also observed in the works by authors who studied the effectiveness of RHM in COPD [46–48]. According to a number of described studies, the introduction of RHM technologies in work with BA patients can lead to positive results [52, 53]. Remote monitoring of the number of inhalations and maintenance of an electronic patient diary improve disease control and reduce the number of seizures [55].

At the same time, in such studies, factors indicating the unsafe use of RHM technologies are overlooked. In addition, the authors of most of the works report a decrease in the number of face-to-face meetings with doctors when using RHM, which in the long term results in a decrease in the burden on medical institutions. It is worth noting that the ambiguity of the impact of RHM technologies can also be determined by the level of classical medical care for patients with chronic diseases in a particular country, and high quality of standard follow-up can reduce the positive impact of RHM on patient's health.

It is especially important to assess the potential of applying RHM in the context of the COVID-19 pandemic and the possibility of using RHM technologies

in the post-COVID-19 era. Isolated studies on the use of specific RHM technologies for patients with COVID-19 and a lack of systematic reviews on the use of RHM in a pandemic cast doubt on the evidence of effect of RHM methods and require further study [9, 10, 66–69].

Special attention should be paid to the economic component of the effectiveness of RHM implementation in practical medicine. It is noted that most of the scientific publications on RHM are considered as a promising area of preventive medicine that helps to overcome the costs of the healthcare system, reduce the burden on the budget, and accelerate adaptation of healthcare to modern conditions of a market economy. There is a number of studies confirming the economic feasibility of introducing RHM into practical medicine [70, 71]. J. P. Hummel et al. (2019) conducted a large study (n = 15,254) analyzing the economic model for RHM implementation in patients with ICD. The data obtained indicated a decrease in the readmission rate and, as a consequence, a decrease in costs by 554 USD per patient per year in the RHM group (n = 5,348). It is worth noting that the total costs of visits to doctors and outpatient services were higher in the RHM group (47,515 versus 42,792 USD), but the average cost per patient per year was lower (6,232 versus 6,244 USD), which confirms high economic feasibility of introducing RHM into clinical practice [72].

A similar study by A. Capucci et al. (2017) demonstrated an assessment of economic benefits from the introduction of RHM in patients with ICD after acute coronary syndrome. As a result of 12-month follow-up of patients in the RHM group (n = 457) compared with the control group (n = 401), the readmission rate was 0.16 / year versus 0.27 / year (RR = 0.59, p = 0.0004). At the same time, the annual cost of treatment per patient was 817 euros in the control group versus 604 euros in the RHM group (p = 0.0004) [70].

Despite the differences in the obtained results and polarity of the conclusions, the study of the effectiveness of RHM implementation into clinical practice continues, and protocols of new studies are being published in international journals [31, 73, 74]. The COVID-19 pandemic, as well as a number of economic, geographic, and social factors dictate the need for the medical community to actively integrate into the international telemedicine strategy and develop RHM technologies in the Russian Federation.

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Ulcerative colitis: focus on colonic mucosal resistance

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ABSTRACT

In recent decades, following cooperation between scientists in various specialties, new unique data on the pathogenesis of ulcerative colitis have been obtained. The role of an impaired immune response to antigens of gut microbiota in genetically predisposed individuals under the effect of certain environmental factors was proven. Assessing the interaction between the colonic mucosa and gut microbiota will help to understand the mechanisms of ulcerative colitis and develop new treatment strategies for the disease.

This review presents modern views on the pathogenesis of ulcerative colitis with a focus on the imbalance between local protective and aggressive factors of the gastric and intestinal mucosa. The structure and role of the epithelial barrier both under normal conditions and in ulcerative colitis are considered in detail.

The aim of this review was to summarize the data on resistance of the colonic mucosa and its damage in ulcerative colitis.

Keywords: ulcerative colitis, microbiota, epithelial barrier, mucin, tight junction proteins, epithelial cells, immunoglobulins A

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Язвенный колит: в фокусе резистентность слизистой оболочки толстой кишки

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РЕЗЮМЕ

В результате кооперации ученых различных специальностей в последние десятилетия получены новые уникальные данные о патогенезе язвенного колита, доказано участие нарушенного иммунного ответа по отношению к антигенам собственной кишечной микрофлоры у генетически предрасположенных лиц под воздействием определенных факторов внешней среды. Оценка взаимодействия слизистой оболочки толстой

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кишки и микробиоты кишечника поможет понять механизмы развития язвенного колита и разработать новые стратегии лечения.

В обзоре мы представляем современные взгляды на патогенез язвенного колита, сосредоточив внимание на нарушении равновесия между местными факторами защиты и агрессии слизистой оболочки желудочно-кишечного тракта. Подробно рассматриваем строение и роль эпителиального барьера как в норме, так и при язвенном колите.

Целью обзора является обобщение данных литературы о резистентности слизистой оболочки толстой кишки и ее повреждении при язвенном колите.

Ключевые слова: язвенный колит, микробиота, эпителиальный барьер, муцин, белки плотных контактов

Конфликт интересов. Авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Modern lifestyle has a significant impact on the microbial composition of the intestine and leads to a change in the diversity of the gut microbiota in ulcerative colitis (UC), decrease in the resident flora, and a rise in the number of opportunistic and pathogenic bacteria. A combination of aggressive factors (imbalance of the gut microbiota composition, the presence of aggressive gut metabolites) leads to impaired intestinal permeability and disruption of its mucosal barrier. This is normally determined by the state of tight junctions, as well as the amount and quality of mucin that protects the epithelium.

The dynamic interaction between the anatomical and functional elements of the mucoepithelial barrier in the gastrointestinal tract (GIT) is arranged in such a way that, on the one hand, they create a semipermeable barrier that provides absorption and transport of nutrients, and, on the other hand, they regulate passage of proinflammatory molecules, microorganisms, toxins, antigens, and pathogens from the luminal to the internal environment of the body and provide development of immune responses to penetration of pathogenic agents, as well as immune tolerance in relation to food components and commensal bacteria.

The functioning of the protective mucosal barrier is based on the unity of physical, biochemical, and immunological interactions of its structures [1]. Given complex organization and regulation of the intestinal mucosal barrier, it is necessary to determine the most essential protective elements in the pathophysiology of UC.

PRE-EPITHELIAL PROTECTION

Pre-epithelial protection (mucous bicarbonate barrier) is a layer of mucous gel in combination with substrates secreted by the surface epithelium. Previously, the pre-epithelial mucus layer was characterized only by the ability to move chyme through the digestive tract due to its moisturizing and lubricating effects, as well as to protect the epithelium from the aggressive effects of antigens, acids, and enzymes. With the advent of modern research methods, scientists began to study such characteristics of the intestinal mucosal barrier as its composition, secretion, and destruction, as well as the role of various external factors in changing its permeability, structure, and chemical composition [2]. Most scientists agree that an increase in the thickness of the mucus layer is associated with an increase in its protective functions, but there is also an opinion that a thick layer of parietal mucus is a favorable environment for opportunistic and pathogenic bacteria [3].

The main components of the pre-epithelial protection are mucins, or highly glycosylated glycoproteins, which, due to their specific properties, protect

the internal environment of the body from bacteria and damaging agents [4]. Mucins are divided into 2 types: membrane-bound (transmembrane) (MUC1, MUC3, MUC4, MUC13, MUC15, MUC17, MUC20, and MUC21) and secreted (secretable, gel-forming) (MUC2, MUC5AC, MUC5B, MUC6, and MUC20). This component of the mucosal barrier is represented in the small intestine by a single, loose, and permeable layer, and in the large intestine – by a denser, double layer [5]. The double layer of the colonic mucus is subdivided into a dense inner layer that is firmly attached to the epithelial cells and is impermeable for bacteria. This layer is composed of transmembrane mucins and is called glycocalex. An increase in the permeability of this layer facilitates easier penetration of bacteria in the epithelial cells.

Transmembrane mucins MUC3, MUC4, MUC12, MUC13, and MUC17 are expressed in both unchanged and altered mucous membranes. Membrane-bound mucins act as sensors of the luminal environment in the interaction between the host and the microbe [6]. Transmembrane mucins consist of two subunits: a large extracellular subunit and a smaller subunit, which consists of extracellular, transmembrane, and cytoplasmic domains. The extracellular subunits of these mucins rise above the plasma membrane to a height of about 1 μm [7]. The main transmembrane mucins of the mucosal barrier are MUC3, MUC12, and MUC17. MUC1 is synthesized during the development of pathological conditions, for example, cancer and infectious diseases of the GIT [8]. MUC1 functions as a regulator of the Toll-like receptor (TLR)-initiated innate immune response, which is an example of cellular signaling by transmembrane mucins [9]. There is conflicting evidence from research findings on MUC16. In most works, the authors indicated its absence in the colon [10] in both healthy and sick patients, while J. Yamamoto-Furusho et al. indicated the expression of MUC16 and MUC20 in the colonic mucosa, which was associated with histologic remission in patients with UC [11].

The gel-forming mucins MUC2, MUC5AC, MUC5B, and MUC6 are the main components of the mucous layer which provide its viscoelastic properties. MUC2 is the major universal mucin which is secreted in all parts of the GIT and plays the key role in keeping microbes at a distance from the epithelial surface. MUC2 regulates intestinal homeostasis and tolerance to food components through dendritic cells and intestinal epithelial cells, and the MUC2 receptor complex suppresses inflammatory responses in dendritic cells [12].

In inflammatory bowel disease (IBD), impairment of mucin synthesis and subsequent emergence of mucosal barrier dysfunction are observed [13]. A decrease in glycosylation and sulfation [14], as well as an increase in sialylation, reduce the effects of mucin in patients with UC and prevent maintenance of an effective intestinal barrier function, especially in relation to bacterial translocation [15]. Healthy colonic mucus is sufficiently sulfated, which provides increased resistance to bacterial and enzymatic degradation. The study by D. Boltin et al. demonstrated that sulfation occurred to a lesser extent in patients with UC [16]. In addition, in the colon affected by UC, a decrease in the number of goblet cells, MUC2 expression, and mucus layer thickness in comparison with the control group of healthy people was noted [17]. In this pathology, a decrease in the content of sulfates in MUC2 is identified, but a compensatory increase in the expression of this mucin in the active phase of the disease leads to an overall unchanged level of sulfates in the colon [18].

MUC1 and MUC5AC, which usually cannot be found in the colon of healthy people, were identified in the scrapings from the resected part of the colon in patients with UC [19]. In patients with UC, a specific increase in MUC1 expression and a decrease in MUC2 expression at the sites of crypt abscesses and erosive ulcerative lesions were observed [20]. A decrease in the expression of the *MUC9* [21] and *MUC20* genes and increased expression of the *MUC16* gene [22] were also noted both in the active phase of the disease and at the remission stage in UC patients compared with the control group. Sialylation and sulfation increase tissue resistance to degradation. An increase in sialylation of mucin oligosaccharides was detected in rectal biopsies of patients with UC [23].

In a large-scale study by S. van Der Post et al., the basic composition of the intestinal mucosal barrier was identified. It consists of gel-forming and transmembrane proteins that form the mucosal barrier in healthy people and in patients with UC in remission. Several of these proteins were reduced in UC patients, including the major structural compo-

nents, MUC2 and IgGFc-binding protein FCGBP, as well as other goblet cell products, including calcium-activated chloride channel regulator 1 (CLCA1) and zymogen granule protein 16 (ZG16). Scientists suggest that the disease may be preceded by insufficient replenishment and increased destruction of goblet cells in response to sequential microbial attacks, which initiates a new episode of UC [24].

In the unchanged gastric and intestinal mucosa, the localization of mucins coincides with the distribution of trefoil peptides. Trefoil factors (TFF1–3) are a group of peptides synthesized and secreted by the epithelium of the mucous membrane [25]. A combined effect of TFF and mucin enhances the protection of the mucous membrane from ulcerogenic agents, prevents the penetration of protons through the mucus, and increases its viscosity [26]. The structural domain of TFF is presented in the form of a clover leaf, which contributes to their resistance to proteolytic degradation [27]. Each TFF interacts with mucin differently. The most viscous mucus is in the stomach and upper duodenum (to protect against acid and enzymes), which coincides with the localization of TFF2. Formation of intercellular contacts in the epithelial layer is mediated by E-cadherin, which interacts with β-catenin, leading to destabilization of intercellular connections and possible cell migration [28]. If the cell is not attached to the matrix, it is vulnerable to apoptosis [29].

It was found that TFF3 has a pronounced antiapoptotic (anoikis-resistant) effect on enterocytes through activation of NF-κB [30] and epidermal growth factor (EGF) [31]. In the study by R. Nakov et al. [32], it was demonstrated that the level of serum TFF3 correlates with the intensity of clinical manifestations, endoscopic presentation, and the content of fecal calprotectin in patients with UC.

The mucous bicarbonate barrier is the basis for the interaction between gut microbiota and the host organism. In a healthy organism, this interaction has a form of partnership, and the pre-epithelial barrier is a favorable environment for microorganisms, which, in turn, regulate its state [33]. There is an assumption that the proportion of bacteria that destroy the mucus layer increases when the diet is poor in dietary fiber. It means that under these conditions, the mucus layer becomes an energy source for gut microbiota instead of fiber, which results in gradual destruction of the mucus layer [34].

The protective function of mucus is also determined by its interaction with the immunity. The parietal mucus layer contains a resistin-like molecule β (RELMβ), Fc-γ binding protein FCGBP, secretory immunoglobulins A, and antibacterial substances (defensins, lysozyme, and ribonuclease). Immunoglobulin A (IgA) is one of the most common antibodies in the mucosal secretion, which neutralizes pathogenic bacteria and maintains the commensal microflora through several mechanisms. The discovery of IgA at the end of the 50s of the XX century played a significant role in the development of immunology [35]. Firstly, it created the basis for transformation of early concepts of tissue immunity, elaborated by the outstanding immunologist A.M. Bezredko, a student of I.I.Mechnikov, in 1929. It was A.M. Bezredko who defined tissue immunity as formation of resistance of an individual organ to infection without formation of protective antibodies. He believed that local resistance is provided by cells accustomed to weakened or killed microorganisms.

The interest in the study of the immune mechanisms associated with IgA has not waned since its discovery. Its protective functions are realized at the surfaces of mucous membranes that are in contact with the environment. In 1993, A.V. Kononov formulated a concept of local secondary sIgA deficiency in mucous membranes during their chronic inflammation. He proposed a scheme for the morphogenesis of chronic inflammation, taking into account the interactions between local immunity, microcirculation, and epithelium [36]. Despite abundance of experimental and theoretical data, many issues concerning the nature of the interaction and the physiological function of IgA and Fc-binding ligands are still unclear and require further study.

Pattern recognition receptors (PRRs), which include Toll-like receptors (TLRs) and NOD-like receptors (NLRs), play an essential role in mucin synthesis. PRRs are activated by pathogen-associated molecular patterns (PAMPs) and microbe-associated molecular patterns (MAMPs), which leads to induction of the NF-kB family of transcription factors and development of an immune response of varying severity [37]. TLR1, -2, -4, -5, -6 (extracellular sensors) and NLR1, -2 and TLR9 (cytosolic sensors) are expressed in epithelial cells and have complementary effects, promoting both innate and adaptive immunity.

TLRs are a family of 11 transmembrane receptors that are located at the cell surface and in intracellular endosomes. The profile (level and localization) of TLR expression differs in different parts of the GIT. It was found that TLR2, -4, -5, -7, and -9 are either minimally or not expressed in the epithelium of the small intestine, and expression of TLR2, -4, and -5 can be found in the colon. At the same time, TLR3 expression is at the same level both in epithelial cells of the small intestine and in the colon [38]. Examples of MAMPs include lipopolysaccharides (found in the outer membrane of gram-negative bacteria), lipoteichoic acid (present on the wall of gram-positive bacteria), peptidoglycan (bacterial cell wall component), and flagellin (the main structural component of bacterial flagella). They all function as PRR ligands. MUC2 expression increases, when TLR is activated by lipopolysaccharides, lipoteichoic acid, and flagellin [39].

NLRP6 inflammasome, when stimulated with the TLR-2/1, TLR-4, and TLR-5 ligands, is activated in goblet cells located in the colonic crypts. NLRP6 inflammasome acts as a sensor of cellular stress, triggers an inflammatory cascade, and plays the key role in maintaining the intestinal barrier, protecting against infection, and regenerating the mucous membrane [40]. TLR-initiated cascades stimulate complex MUC2 exocytosis and mucin secretion in adjacent goblet cells via intercellular signals. The increased secretion of MUC2 may thus facilitate expulsion of bacteria from the upper part of the crypts [41]. Unlike transmembrane TLRs, NLRs are a family of innate intracellular receptors [42]. Activation of NOD1 and NOD2 by such ligands as bacterial peptidoglycans ultimately leads to activation of NF-kB transcription factors and triggers immune responses [43].

EPITHELIAL PROTECTION

Epithelial protection requires a contiguous layer of cells including five cell types: enterocytes, goblet cells, enteroendocrine cells, Paneth cells, and undifferentiated epithelial cells. Enterocytes are the most common type of cells, forming an effective barrier to protect the internal environment and controlling selective absorption of ions, nutrients, and other components from the luminal environment. Goblet cells are located between enterocytes; they are responsible for secretion of mucus. Enteroendocrine cells produce gastrointestinal hormones, peptides, and neurotransmitters [44]. Dysregulation of the epithelial barrier with changes in paracellular permeability due to altered intercellular junctions is probably one of the primary factors in the pathogenesis of IBD [45].

The paracellular space is sealed by tight junctions (TJs), which regulate the flow of water ions and small molecules, building a dynamic intestinal barrier [46]. TJs are composed of two types of proteins: 1) transmembrane proteins, which include occludin, claudins, tricellulin, and junctional adhesion molecules (JAMs); 2) peripheral membrane proteins of zonula occludens cells (ZO-1, ZO-2, and ZO-3). Some TJs have properties of increased barrier permeability, while others form channels and pores that are selective in size and / or charge [47]. Adherens junctions and desmosomes are mainly involved in communication between adjacent epithelial cells [48]. Dysfunction of TJs leads to disruption of the intestinal barrier integrity. The intestinal barrier function is influenced by changes in pH, osmotic pressure, and cytoskeleton function [49]. TJs can be damaged by various pathogens with a subsequent increase in epithelial permeability and bacterial translocation.

Occludin and adhesion proteins regulate the integrity of TJs, and tricellulin ensures transport of macromolecules. Claudins are mainly responsible for the intestinal barrier function and are represented by a family of 27 members that modulate paracellular ion transport depending on the charge and size [50]. According to their functions, claudins can be divided into two groups: pore-forming claudin-2, -7, -12, -15, -16 and claudin-1, -3, -4, -5, -8, -14, -18, -19, which reduce the permeability of the epithelium. The expression of TJs differs throughout the GIT and depends on functional needs of its segments. At a finer structural level, it also depends on localization on cell membranes [51].

Claudin-1 and claudin-2 are capable of initiating the formation of TJ filaments on fibroblasts that lack tight junctions [52]. Claudin-2 controls transport of monovalent cations, such as Na+, to the interstitium and reduces paracellular transepithelial resistance, enhancing transepithelial water flow [53], as opposed to claudin-1, -3, -4, -5, and -8, which “tighten” the epithelium [54]. Another important property of claudin-2 is that it directly reduces the barrier function of claudin-1 and claudin-4 [50].

In UC, a decrease in the expression of claudin-1, claudin-4, and occluding and activation of claudin-2 are registered [55]. The greatest protective effect in IBD was demonstrated by claudin-1, -3, -4, -5,

and -8 [56]. In patients with UC, both in the active phase of the disease and in its remission, increased expression of claudin-2 and a decrease in the expression of occludin and ZO-1 were observed compared with the healthy controls. The expression of ZO-1 was significantly higher in patients with UC in remission, compared with patients in the active phase of the disease. Expression of ZO-1 and occludin had a negative correlation with C-reactive protein and erythrocyte sedimentation rate (ESR).

L.S. Poritz et al. [57] found an increase in the claudin-1 / occludin ratio in colon biopsies in patients with UC compared with samples from healthy controls and patients with Crohn's disease. In another study, it was demonstrated that claudin-1 was elevated in the colon of UC patients compared with the control group, but did not correlate with the severity of the disease [58]. In biopsies obtained from the sigmoid colon of patients with UC, there was a tendency to an increase in claudin-12 expression [59].

The physiological role of zonulin has not been fully established, but there is no doubt that it also regulates TJs. Excessive production of zonulin can lead to an excessive increase in the permeability of the epithelial layer [60]. The study of zonulin in the blood, as a rule, is associated with diagnosis for suspected leaky gut syndrome and increased permeability of the epithelium in the examined person. Elevated serum zonulin levels have been reported in celiac disease, non-celiac gluten sensitivity, irritable bowel syndrome, and IBD [61], compared with healthy controls. However, the study [62] demonstrated that serum zonulin is not a reliable marker of increased intestinal permeability in the examined individuals.

Finally, the third level in the complex structure of the epithelial barrier is represented by intermediate filaments, catenins, cadherins, and desmosomes. One desmosome is rather small, therefore, several desmosomes can usually be seen at the site of contact between two cells [63].

SUBEPITHELIAL PROTECTION

The subepithelial layer is represented by the lamina propria of the mucous membrane. The lamina propria contains cells of innate and adaptive immunity that secrete IgA, cytokines, chemokines, and proteases and are involved in immune defense mechanisms of the body. The subepithelial immune complex provides regulation, trophism, and kinetics of the skin epithelium and realizes nonspecific and specific immune responses. Immune cells respond immediately and synchronously to invading pathogens.

Neutrophils are some of the first cells to reach the site of inflammation and limit the invasion of microorganisms through phagocytosis [64]. Macrophages are able to determine the shape and size of possible targets, cooperate in performing functions, exhibit high proteolytic and weak antigen-presenting activity, play a primary role in maintaining tissue homeostasis, and patrol tissues [65]. Regulatory T cells play a crucial role in maintaining immune homeostasis, since they are able to suppress activation of various immune cells involved in GIT inflammation and induce immune tolerance to antigens from the diet or commensal flora [66].

Since the discovery of dendritic cells by R. Steinmann and Z. Cohn, they have been called natural adjuvants of the immune response. Due to the presence of multiple outgrowths in the cytoplasmic membrane, dendritic cells have a large surface area, which allows them to actively recognize the patterns of microbes and dead cells, soluble molecules, and other cells of the body and activate primary and secondary B-cell- and T-cell-dependent immune responses (memory cells). Mast cells are located close to the nerves and are activated by neural mediators. They are also involved in several types of neuroinflammatory responses.

Submucosal neurons control secretion and absorption of nutrients into the local circulation, while Meissner's plexus neurons coordinate smooth muscle contractions [67]. A network of millions of enteric sensory neurons, interneurons, and motor neurons is capable of producing a variety of neurotransmitters and neuropeptides [68].

When MAMPs / PAMPs are activated, an immediate inflammatory response to foreign microorganisms is initiated. This interaction helps to identify foreign molecules by antigen-presenting cells, such as dendritic cells and macrophages. The cells then migrate to the peripheral site where they present antigens to T-cells with subsequent production of proinflammatory cytokines, such as interferon-gamma (IFN-γ), chemokines, and antimicrobial peptides, to protect the intestinal barrier. Given a close relationship between inflammation and increased permeability, markers of inflammation are often considered as surrogate markers of intestinal permeability.

Therefore, the content of α1-antitrypsin is often estimated in combination with fecal myeloperoxidase and calprotectin [69] as markers of subclinical intestinal inflammation [70]. Researchers propose using α1-antitrypsin fecal clearance as one of the laboratory markers of Crohn's disease intensity [71]. Another surrogate marker for mucosal repair, serum lipocalin-2, is expressed by intestinal epithelial cells in response to proinflammatory stimuli, such as cytokines or TLR activation. Serum lipocalin-2 in combination with metalloproteinase correlates with UC intensity [72].

PHYSIOLOGICAL SIGNIFICANCE OF GUT MICROBIOTA

Under normal conditions, the GIT is inhabited by gut microbiota that maintains its integrity. Gut microbiota is in symbiosis with its host, comprises more than 100 trillion microbes, and contains at least 150 times more genes than the human genome [73]. The composition of the gut microbiota in each person is stable, individual, and adapted precisely to the person's needs. The indigenous microbial flora maintains the morphology of the gastric and intestinal mucosa.

Shotgun metagenomic sequencing of gun microbiota revealed 1,952 unclassified bacterial species in addition to 553 species previously cultivated from the human intestine [74]. Microorganisms do not just exist, but interact, build complex relationships, and are characterized by a complex hierarchical structure with various interspecies relationships. Due to their coexistence in the same territory, they compete with each other for nutritional components, parasitize, adapt to each other or, developing together, enhance each other's functions (synergy, symbiosis, antagonism, parasitism, etc.). The gut microbiota produces enzymes involved in metabolism of carbohydrates, lipids, and nucleic acids and synthesis of vitamins, short-chain fatty acids (SCFAs), antimicrobial substances, hormones, and amino acids. The gut microbiota is also involved in immunomodulation, detoxification, and evacuation function of the GIT [75].

Four types of bacteria represent the colonic microbiota: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Bacteroidetes and Firmicutes are predominant types in adults [76]. The diversity of bacteria is higher in the contents of the intestinal lumen than in the parietal mucus layer [77] due to the facultative microbiota supplied with food. The number of bacteria changes even during the day. The colon contains 70% of all microorganisms in the human ecosystem. The predominant microorganisms are obligate anaerobes and their content in this part of the digestive tract exceeds the number of aerobes by 1,000 times [78]. In addition to bacteria, the colonic microbiota of a healthy person consists of viruses, fungi, archaea, and protists, which are an equally important component of the intestinal ecosystem [79, 80]. Together with the host organism, the gut microbial community forms some kind of a "superorganism" that performs many functions.

Changes in the microbiota can cause disturbances in the intestinal motor function and sensitivity. In addition, altered composition of the gut microbiota contributes to motor dysfunction and visceral hypersensitivity [81].

The microbial flora of the colon is in direct contact with the apical membrane of colonocytes and forms microcolonies in the mucous layer, which diversity depends on the composition of the chyme. Dietary fibers, sugars, and proteins that are not digested by enzymes of the macroorganism in the small intestine are fermented by the microbiota. The main products of dietary fiber fermentation are SCFAs (acetate, propionate, butyrate) [82]. SCFAs take part in the regulation of intestinal motility, control over inflammatory responses, maintenance of glucose levels, and blood circulation in the intestinal wall. In addition, they have an anticarcinogenic effect. Physiological effects of SCFAs are related to their interaction with G-protein-coupled receptors. These include GPR41, GPR43, and GPR109A receptors, which are exposed on immunocompetent cells, colonocytes, and adipocytes [83]. Butyrate activates the GR-P109A receptor and suppresses inflammation in the colon. Acetate and propionate activate the GPR43 cell surface receptor and induce chemotaxis of neutrophils [84].

In IBD patients, on the one hand, the proportion of microorganisms with anti-inflammatory activity, such as Firmicutes and Bacteroides, decreases. On the other hand, the proportion of proinflammatory bacteria, which include the Proteobacteria type, increases. In addition, in IBD, the total number of microorganisms increases, however, their diversity, on the contrary, decreases [85]. The pathogenetic mechanisms of the Western diet that provoke the emergence of UC remain unknown. Scientists suggest a direct effect of the Western diet on the composition of the colonic microbiota and indirect effects through

production of microbial metabolites, changes in the local immune response, and impaired barrier function of the colonic mucosa [86]. Undoubtedly, the Western diet has a significant impact on the qualitative and quantitative intraspecies diversity of the gut microbiota [87].

CONCLUSION

The protective barrier in the gastric and intestinal mucosa is a dynamic structural and functional system (Figure). The first line of immune defense is aimed at preventing penetration of antigens into the mucous membrane and eliminating foreign antigens with subsequent activation of the antigen-specific immune response. Innate immunity provides a response through recognition of PAMPs and MAMPs and results in activation of acquired immunity. The two major PRR systems are TLRs and NOD molecules. In IBD, Paneth cells are found in the colon following an increased need for antimicrobial protection. Under normal conditions, these cells are present only in the small intestine. After presentation of antigens to T-helpers and macrophages, naive T cells (Th0) are differentiated into Th1 and Th2 cells. Differentiation of Th0 into Th1 is accompanied by production of proinflammatory cytokines, while differentiation into Th2 cells promotes production of anti-inflammatory cytokines.

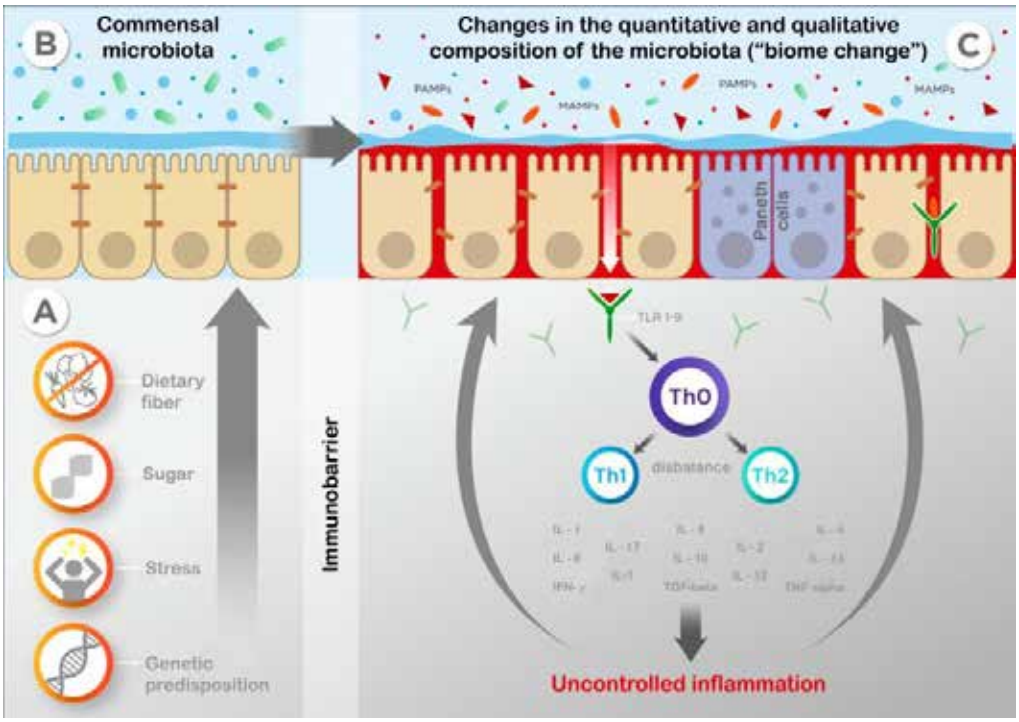

Figure. Simplified diagram of stages of colonic mucosa damage in ulcerative colitis: *B* – under normal conditions, the intestinal barrier function is determined by the state of tight junctions of the epithelium, as well as the quantity and quality of mucin that protects the epithelium. In UC, a combination of genetic factors and certain environmental factors (*A*) leads to impaired permeability of the intestinal mucosa and changes in the gut microbiota, thus impairing the intestinal barrier function (*C*)

Each level of protection has a complex logical organization. Modern research methods make it possible to study structural composition of the mucosal barrier and its interaction with the gut microbiota. Studying the structural and functional capabilities and understanding the mechanisms of coexistence and functioning of the gut microbiota and intestinal mucosal barrier are necessary for every practicing physician. New, effective methods for treating diseases that were earlier considered resistant to therapy are emerging. Further study of the interaction between the mucosal barrier and gut microbiota will help to understand the development mechanisms of chronic inflammatory diseases and develop targeted treatment strategies through restoration of barrier function and integrity.

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Molecular genetic markers of QT interval duration and sudden cardiac death: literature review

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ABSTRACT

The study of sudden cardiac death (SCD) and its etiopathogenesis in cardiology practice remains one of the most pressing public health problems. In Western countries, SCD accounts for 20% of the total mortality and 50% of mortality associated with cardiovascular diseases. Considering the electrical instability in the myocardium as one of the main reasons for the development of life-threatening arrhythmias (ventricular tachycardia / ventricular fibrillation) and SCD, one should be aware of such provoking factors as ischemic heart disease, myocarditis, valvular heart disease, pharmacological influences, cardiomyopathy, and channelopathy. An increase or decrease in the duration of the QT interval, which reflects the work of ion channels, as well as ventricular depolarization and repolarization, increases the risk of SCD.

The aim of this review was to study and analyze the available literature data on the relationship of molecular genetic markers with the duration of the QT interval.

Currently, there is a number of genetic studies that allow to identify a large number of mutations and polymorphisms of known genes that affect the variability of the QT interval, showing their significance in risk stratification of sudden arrhythmic death and choosing the right tactics for managing, preventing, and treating patients, thus reducing the risk of SCD. The predictive value of genetic testing is the highest for long QT syndrome (LQTS), for which a gene-specific risk profile has been established, and lower for other channelopathies. A large amount of genetic data may be a promising approach to quantifying the risk of SCD, especially at a young age, which will be facilitated by further study of this problem.

Keywords: sudden cardiac death, duration of the QT interval, long QT syndrome, short QT syndrome, single nucleotide polymorphism, molecular genetic marker

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Молекулярно-генетические маркеры длительности интервала QT и внезапная сердечная смерть: обзор литературы

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РЕЗЮМЕ

Изучение внезапной сердечной смерти (ВСС) и ее этиопатогенетических факторов в кардиологической практике остается одной из наиболее актуальных проблем здравоохранения. В западных странах ВСС составляет 20% общей летальности и 50% летальности, связанной с сердечно-сосудистыми заболеваниями. Рассматривая электрическую нестабильность миокарда в качестве одной из главных причин развития жизнеугрожающих аритмий (желудочковая тахикардия/фибрилляция желудочков) и ВСС, следует помнить о таких провоцирующих факторах, как ишемическая болезнь сердца, миокардит, клапанные пороки сердца, фармакологические влияния, кардиомиопатии и каналопатии. Увеличение или уменьшение длительности интервала QT, который отражает работу ионных каналов, процессы деполяризации и реполяризации миокарда желудочков, повышает риск ВСС.

Цель данного обзора – изучение и анализ имеющихся данных литературы о взаимосвязи молекулярно-генетических маркеров с длительностью интервала QT.

На сегодняшний день существует ряд генетических исследований, позволяющих идентифицировать большое количество мутаций, полиморфизмов известных генов, оказывающих влияние на вариабельность интервала QT, показывая их значимость в стратификации риска внезапной аритмогенной смерти, выборе верной тактики ведения, профилактики и лечения пациентов, уменьшая вероятность ВСС. Прогностическая ценность генетического тестирования наиболее высока для синдрома удлиненного интервала QT (LQTS), для которого установлен ген-специфический профиль риска, и в меньшей степени определена при других каналопатиях. Большой объем генетических данных может стать многообещающим подходом для количественной оценки риска ВСС, особенно в молодом возрасте, чему способствует дальнейшее изучение данной проблемы.

Ключевые слова: внезапная сердечная смерть, длительность интервала QT, синдром удлиненного интервала QT, синдром укороченного интервала QT, однонуклеотидный полиморфизм, молекулярно-генетический маркер

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

The study of sudden cardiac death (SCD) and its etiopathogenetic factors is of particular interest in cardiology practice. In Western countries, SCD accounts for 20% of the total mortality and 50% of mortality associated with cardiovascular diseases [1]. According to the epidemiological register, the following parameters of annual SCD prevalence among all age groups are noted: Australia – from 34.6 to 99.4 per 100,000 population, which corresponds to the parameters in New Zealand; China – 41.8 per 100,000 population; Japan – 14.9 per 100,000 population; South Korea – 20.1 per 100,000 population; USA – from 50 to 100 per 100,000 per 100,000 population; Europe – 84.0 per 100,000 population per year, respectively [2].

To date, a number of studies have been carried out in the Russian Federation on the prevalence of SCD in various regions. In 2011, the results of a large-scale, cross-sectional study "REZONANS" (Ryazan, Voronezh, Khanty-Mansiysk) including 285,736 patients with coronary heart disease (CHD) were published, according to which the incidence of SCD was 156 per 100,000 male population and 72 per 100,000 female population per year, which is 2.3 and 2.8 times higher than the rates registered in medical institutions [3].

In 2016, R.M. Linchak et al. published data from the "GERMINA" register on the structure and frequency of SCD among the working-age population of the Bryansk region for 2012 [4]. The study involved 417,740 people aged 25–64 years. According to the results, the frequency of SCD was 25.4 per 100,000 population, of which about 85% were males and 15% were females. In the structure of the overall and cardiovascular mortality, the share of SCD was 2.9% and 7.3%, respectively [4].

It should be noted that the incidence of SCD increases with the age of the population included in the study. This is especially noticeable after 45 years, which is associated with an increased risk of developing CHD. Middle-aged men have a 4 times increased risk of SCD compared with women of the same age. However, as the age increases, this difference between the sexes decreases, and disappears at the age of ≥ 85 years [5, 6].

Among people under 35 years of age, the highest frequency of SCD is observed in the age group of 0–5 years. The above age-specific characteristics of the SCD prevalence, taking into account gender, are described by C.X. Wong et al. [2]. It is known that people of African American descent have a higher level of SCD compared to people of Hispanic or Caucasian descent [6]. According to the results of the study by J. Ghobrial et al., the average age in the groups of people of African American and Asian descent with a SCD episode was less than that recorded in the group of Hispanic descent [7]. In the same groups, a lower socioeconomic level and a lower survival rate after performed cardiopulmonary resuscitation were observed. Among concomitant diseases, diabetes mellitus, arterial hypertension and end-stage renal failure were more common ($p < 0.001$) [7].

The main cause of SCD is considered to be electrical instability in the myocardium as a possible consequence of CHD, acquired valvular heart disease, cardiomyopathy, drug toxicity, and hereditary channelopathies (Fig. 1) [2, 5, 8, 9]. Accordingly, the prognostic factors of SCD in men and women, first of all, are risk factors for CHD, including arterial hypertension, diabetes mellitus, dyslipidemia, obesity, smoking, etc. [5, 10, 11].

According to the Oregon SUDS study, 58% of subjects aged 5–34 years who experienced community-acquired SCD had at least one risk factor for cardiovascular disease. Moreover, the prevalence of obesity among these young people was 39% [12]. Subsequently, data were published on the relationship of other comorbidities, such as atrial fibrillation, chronic kidney disease, obstructive sleep apnea, depression, anxiety disorder, psychosis, as well as physical activity and other lifestyle factors with the risk of SCD [13–15].

A burdened family history of SCD is an important predictor of SCD development [16]. As a rule, in 5% of SCD cases, no cardiovascular pathology is detected in survivors after successful resuscitation or during autopsy of the deceased [9]. In children and people under 35 years of age, CHD accounts for a much smaller proportion of deaths, with hypertrophic cardiomyopathy, coronary artery anomalies, myocarditis, arrhythmogenic right ventricular cardiomyopathy, and primary ion channelopathies accounting for a significant proportion. SCD can often be the first manifestation of the disease in a family. Clinical and genetic assessment of survived family members plays a key role in the diagnosis of underlying heart disease, relying on the fact that most hereditary heart diseases are characterized by autosomal dominant inheritance, which, in turn, provides a 50% chance of verifying the same disease substrate among survived family members [11].

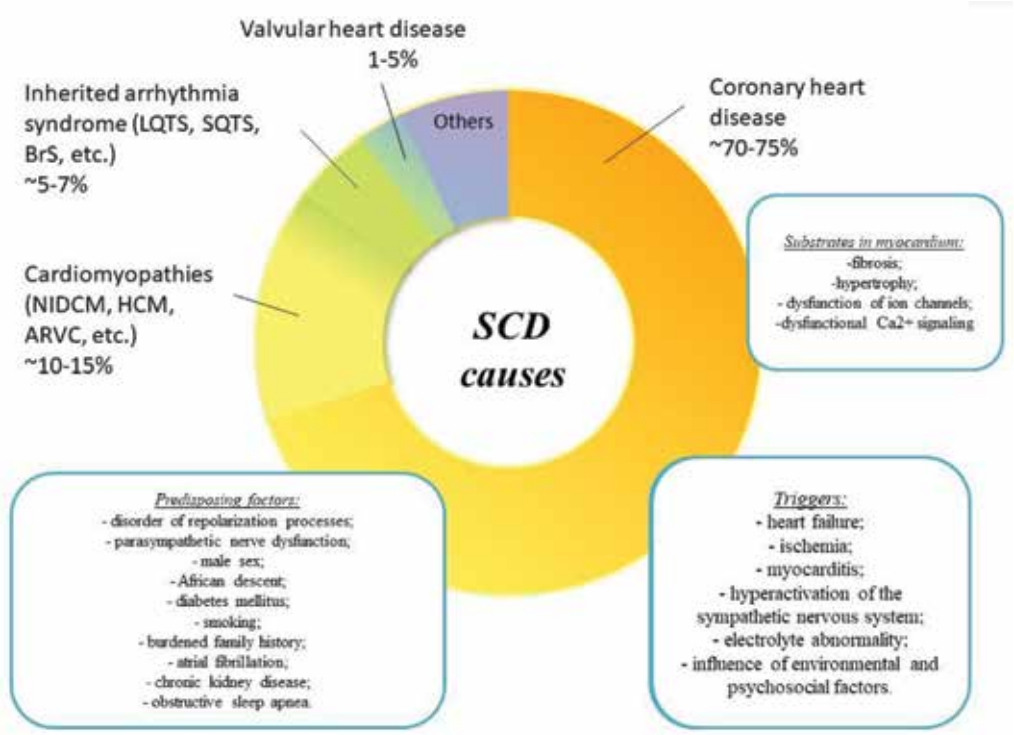

Fig. 1. Main causes of and risk factors for sudden cardiac death.

Focusing on the arrhythmogenic nature of SCD, one should be aware of the following primary nosologies: long and short QT syndromes, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, the molecular and genetic aspects of which are considered as alternative criteria for SCD risk stratification along with electrophysiological and ultrasound parameters, MRI diagnostic parameters (areas of ischemia, foci of fibrosis, ejection fraction, etc.), such biomarkers as B-type natriuretic peptide, troponin, galectin-3, and soluble ST2 [1]. A molecular and genetic analysis reveals predictors in 65% of cases of long QT syndrome (LQTS), in 20% of cases of Brugada syndrome (primary electrical diseases), and in 20–52% of cases of cardiomyopathies [17–20].

Thus, the aim of this literature review was to study and analyze the available literature data on the relationship of molecular and genetic factors with QT interval duration.

LONG QT SYNDROME

QT interval is an electrocardiographic parameter reflecting depolarization and repolarization of the ventricular myocardium, the electrophysiological basis of which is the state of the ion channels in the cardiomyocyte membrane. The balance between calcium, potassium, and sodium ion channels determines the duration of the action potential in cardiomyocytes (Fig. 2) [21–23].

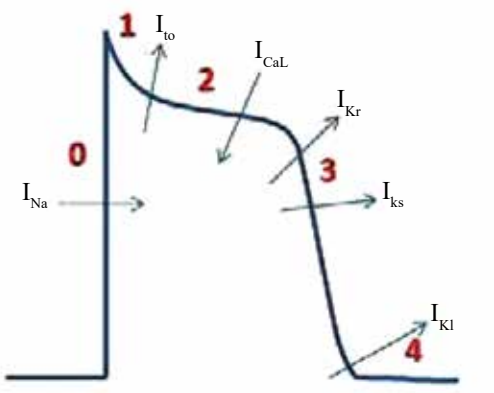

Fig. 2. Action potential and transmembrane ion currents

A decrease in repolarizing outward K^+ currents (mainly I_{Ks}, I_{Kr}, I_{K1}) or an increase in depolarizing inward Na^+ or Ca^{2+} currents (mainly I_{Na} and I_{Ca}) in cells can lead to lengthening of the QT interval, which is a pathophysiological substrate for LQTS [23, 24]. The QT interval duration primarily depends on the cardiac cycle duration. Therefore, to assess this interval, the Bazett formula is used and the term “corrected QT”

or "QTc" is introduced [21]. The Bazett formula has been repeatedly criticized. However, other calculation methods such as Framingham, Fredericia, and Hodges formulas have not become widespread [25, 26].

Prolonged QTc, defined as a QTc value > 450 ms in men and > 460 m in women in lead II or V5 on a standard 12-lead ECG, predisposes to functional re-entry, torsades de pointes form of ventricular tachycardia, and SCD. Otherwise unexplained, baseline QTc ≥ 500 ms should be associated with inherited LQTS. In addition, a drastic increase in QTc (ΔQTc > 60 ms) indicates an increased risk of torsades de pointes form of ventricular tachycardia / SCD in drug-induced LQTS [21].

LQTS is the most common hereditary ion channelopathy characterized by a prolonged QT interval on a 12-lead electrocardiogram and an increased risk of malignant arrhythmias in patients without structural heart disease [27, 28]. Clinical symptoms of LQTS include palpitations, syncope, and convulsions, more often as a consequence of torsades de pointes form of adrenergic tachycardia [29].

LQTS can be diagnosed if the following criteria are met (HRS / EHRA / APHRS, 2013):

1) The risk of LQTS is > 3.5 points on the Schwartz scale and there are no secondary causes of QT interval prolongation;

2) A characteristic pathogenic mutation in one of the LQTS genes;

3) QTc interval according to Bazett formula ≥ 500 ms on repeated 12-lead ECGs and no secondary causes of QT interval prolongation.

However, already in 2015, the updated LQTS criteria were published in the clinical guidelines of the European Society of Cardiology, which used QTc values ≥ 480 ms or a risk assessment on the scale of more than 3 points [30]. As mentioned above, genetic screening plays a key role in the LQTS diagnosis, which makes it possible to identify a pathogenic mutation and determine a further patient management strategy. Currently, there are 17 different LQTS subtypes associated with monogenic mutations in 15 autosomal dominant genes (Table) [29].

Table

The main LQTS subtypes according to P.J. Schwartz et al. [31]

LQTS type	Gene	Locus	Mutation frequency among LQTS cases (%)	Effect
LQT1	*KCNQ1*	11p15.5	40–55	$\downarrow K_{v7.1}$
LQT2	*KCNH2*	7q35–36	30–45	$\downarrow K_{v11.1}$
LQT3	*SCN5A*	3p21–24	< 1	$\downarrow Na_{v1.5}$
LQT4	*ANKB*	4q25–27	< 1	↓Ankyrin B
LQT5	*KCNE1*	21q22.1	< 1	↓MinK
LQT6	*KCNE2*	21q22.1	< 1	↓MiRP1
LQT7	*KCNJ2*	17q23	< 1	↓Kir2.1
LQT8	*CACNA1C*	12p13.3	< 1	↑L-type calcium channel
LQT9	*CAV3*	3p25	< 1	↓Caveolin 3
LQT10	*SCN4B*	11q23.3	< 1	↓Sodium channel – β4
LQT11	*AKAP9*	7q21–22	< 1	↓Yotiao
LQT12	*SNTA1*	20q11.2	< 1	↓Syntrophin α1
LQT13	*KCNJ5*	11q24	< 1	↓Kir3.4
LQT14	*CALM1*	14q32.11	< 1	Calmodulin 1 (dysfunctional Ca^{2+} signaling)
LQT15	*CALM2*	2p21	< 1	Calmodulin 2 (dysfunctional Ca^{2+} signaling)
JLN1	KCNQ1	11p15.5	< 1	$\downarrow K_{v7.1}$
JLN2	KCNE1	21q22.1–22.2	< 1	↓MinK

Causal gene mutations are detected in approximately 70% of patients with LQTS [32]. Approximately 75% of all pathogenic variants are found in the genes *KNCQ1, KCNH2,* and *SCN5A*, which are responsible for the LQTS 1–3 subtypes in patients with the Schwarz.scale score of ≥ 4, while in 25% of cases, the genotype remains unidentified after extensive genetic testing based on various panels [27, 33, 34]. It is possible that a small proportion of patients with genotype-negative LQTS may have an as yet unknown Mendelian disorder, but the existence of a different, more complex inheritance pattern in this subgroup cannot be denied (Fig. 3).

Thus, a genome-wide association study (GWAS) comparing cases of rare arrhythmia syndrome with a control group has shown that it can identify mod-

current, I_{Kr} current. LQT2 mutations in the hERG potassium channel entail amplitude reductions; they are loss-of-function mutations that decrease I_{Kr} amplitudes and prolong repolarization. On the electrocardiogram, it is recorded in the form of a bifurcated or notched T wave, which has low amplitude and is asymmetric. Potential triggers include emotional stress. A more aggressive phenotype is observed in patients with mutations in the pore region (S5–loop–S6).

LQT3 is caused by mutations in the *SCN5A* gene located at position 3p21–24. The *SCN5A* gene encodes $Na_V1.5$, an α-subunit of the voltage-dependent Na^+

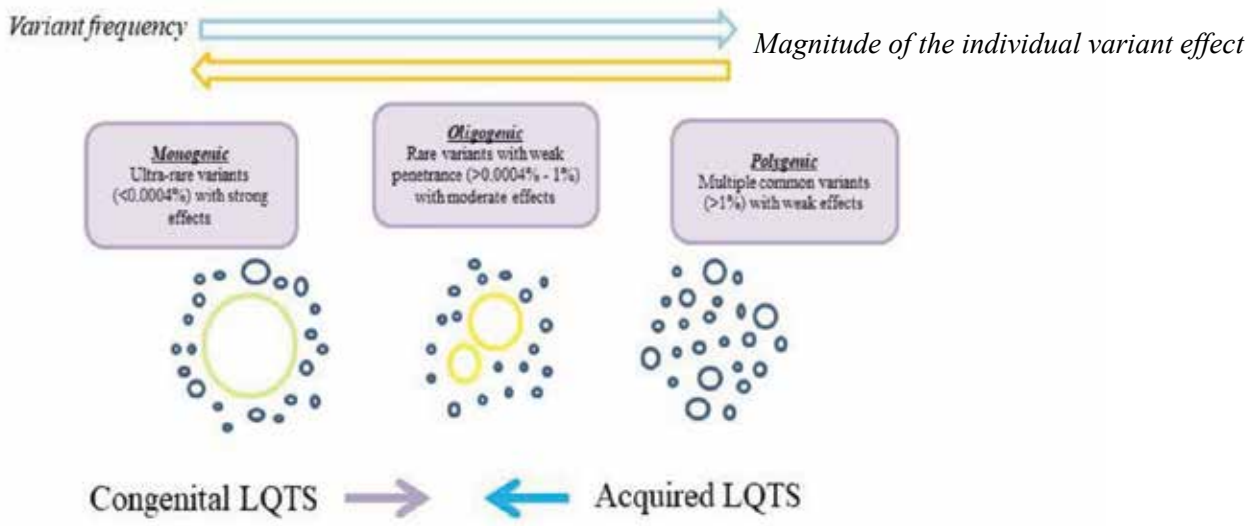

Fig. 3. Genetic models underlying acquired and congenital forms of long QT syndrome.

ulators of susceptibility to the disease and suggest its polygenic etiology. In their case-control study, N. Lahrouchi et al. identified the important role of common genetic variation in LQTS susceptibility and confirmed the complex (polygenic) architecture of genotype-negative LQTS, which was consistent with an earlier publication by J.R. Giudicessi et al. [34, 35].

For LQT1–3, the phenotype – genotype correlation is evident. In about 85% of LQTS cases, a patient with a positive genotype carries a mutation inherited from one of the parents, and in the remaining 15%, a *de novo* mutation is appropriate. Approximately 50% of patients with the LQTS genotype are asymptomatic throughout their lives, while 10–50% of these patients do not show any obvious prolongation of the QT interval. Complex mutations (≥ 2 mutations) are found in 10% of patients with a positive genotype. As a rule, the clinical manifestations of the disease in such patients are more severe [33].

LQT1 is the most common subtype and is verified in > 40% of individuals with LQTS. The main substrate is the loss of / decrease in the function of the *KCNQ1* gene located at the 11p15.5 locus, encoding the α-subunit of the voltage-gated potassium channel, K_V 7.1. K_V 7.1 consists of 4 α-subunits, which, together with the β-subunits of KCNE1, generate a slowly activated delayed rectifier potassium current (I_{Ks}). I_{Ks} physiologically increases due to sympathetic influences in order to adapt the QT interval to a certain heart rate (HR). With a decrease in I_{Ks}, the QT interval is not shortened, which leads to the development of arrhythmia. On the electrocardiogram of a patient with LQT1, a wide and symmetrical T wave is recorded against the background of a prolonged QTc interval. Accordingly, the main trigger for syncope or SCD in LQT1 is exercise. The incidence of life-threatening events is the lowest in LQT1 compared with LQT2 or LQT3. Heterozygous KCNQ1 mutations cause dominant Romano – Ward syndrome of LQT1 and are the most common LQTS genotype. Homozygous mutations in KCNQ1 or complex heterozygous mutations can cause autosomal recessive Jervell and Lange – Nielsen syndrome.

LQT2 is the second most common subtype, affecting 30% of individuals with LQTS. LQT2 is caused by mutations in the *KCNH2* or *hERG* gene located at position 7p35–36, which encodes voltage-gated pores that form the α-subunit of the K_V 11.1 potassium channel. α-subunits form a complex with the transmembrane protein KCNE2, homologous to KCNE1, thereby generating a fast component of the delayed rectifier

channel and a mediator of the I_{Na} depolarizing current. Mutations in this gene, present in 10% of genetically diagnosed LQTS patients, increase the duration of the action potential plateau phase by increasing late depolarizing currents. The trigger for this subtype is sleep (bradycardia). On the electrocardiogram, LQT3 is recorded as a prolonged isoelectric interval and a relatively normal T wave.

Another 6 relatively rare forms of LQTS are associated with a defect in ion channels due to mutations in the corresponding genes:

– *KCNE1* (LQT5) encodes the β1 subunit of the voltage-gated potassium channel K_V 7.1; participates in the generation of current I_{Ks};

– *KCNE2* (LQT6) encodes the β2 subunit of the voltage-gated potassium channel K_V11.1; participates in the generation of current I_{Kr};

– *KCNJ2* (LQT7 or Andersen – Tawil syndrome) encodes the Kir2.1 potassium channel as the mediator of the inward rectifier current, I_{Kl};

– *CACNA1* (LQT8 or Timothy syndrome) – α1C-subunit of the voltage-gated Ca^{2+} L-type channel, Ca_V1.2, the defect of which increases the inward depolarizing calcium current and leads to prolongation of the action potential plateau phase and a prolonged QT interval;

– *SCN4B* (LQT10) encodes the β4 subunit of voltage-gated Na_V 1.5;

– *KCNJ5* (LQT13) is responsible for the work of the potassium channel, Kir3.4, which is activated by the G-protein.

The following three rare forms of LQTS include causal genes encoding adapter proteins that bind the cell membrane to the cytoskeleton:

– *ANK2* (LQT4) – ankyrin-2, which coordinates the work of Na^+ / K^+-ATPase, Na^+ / Ca^{2+-} exchanger and inositol triphosphate receptor, which leads to abnormal restoration of the initial state of ions;

– *CAV3* (LQT9) – caveolin-3, which regulates ion channels in caveolae, including membrane expression of Na_V1.5 / Kir2.1;

– *SNTA1* (LQT12), encoding α-syntropin, which binds Na_V1.5 channels to the NOS-PMCA4b complex.

Other rare genes of LQTS subtypes are associated with kinase activity, such as *AKAP9* (LQT11), encoding binding of the kinase-9 anchor protein A to the protein kinase A regulatory subunit, resulting in a decrease in I_{Ks}; *CALM1* (LQT14), *CALM2* (LQT15), and *CALM3* (LQT16) are responsible for the calmodulin protein, an important intracellular Ca^{2+} sensor that transmits a signal and modulates Ca_V1.2. A mutation in one of the three genes, even when heterozygous, is sufficient to result in an early and severe form of LQTS with an extremely long QTc interval. A mutation in the *TRDN* gene encoding the triadin protein, known as a regulator of RyR receptors and Ca_V1.2 calcium channels, also increases I_{CaL}. Mutations of two more genes, *TRPM4* and *RYR2,* in individuals with LQTS are mentioned in the literature; their mechanisms of influence require further study [23–35].

SHORT QT SYNDROME

Short QT syndrome (SQTS) is a rare, inherited, autosomal dominant cardiac channelopathy associated with malignant ventricular and atrial arrhythmias. The syndrome was first described in 2000 by Gussak et al. as an inherited disease, when idiopathic persistently short QT intervals were recorded in 4 members of the same family on an electrocardiogram. In the future, the goal of many studies was to search for diagnostic criteria. In 2011, Gollob et al. proposed SQTS criteria based on 4 components, including electrocardiographic data, medical history, family history and genotype.

Genetic testing only detects a causal mutation in < 25% of SQTS cases. According to O. Campuzano et al., no more than 200 cases have been diagnosed worldwide; accordingly, the prevalence of SQTS is estimated at 0.02–0.1% [36, 37]. Currently, according to the European Society of Cardiology 2015 clinical guidelines, the clinical diagnosis of SQTS can be made with a decrease in QTc duration ≤ 340 ms and should be considered at QTc values ≤ 360 ms if one or more of the following criteria are met: a confirmed pathogenic mutation; cases of SQTS detection in the family; family history of sudden cardiac death at the age of < 40; a history of syncope of unknown origin or documented ventricular tachycardia / ventricular fibrillation in the absence of structural heart disease [30].

The pathogenetic mechanisms of SQTS are associated with abnormalities of the heart ion channels that regulate the action potential in cardiomyocytes, affecting the duration of repolarization. Major mutations are associated with increased function in voltage-gated potassium channel subunits (KCNH2, KCNQ1, KCNJ2) and decreased / lost function of voltage-gated calcium channels (CACNA1C, CACNB2B, and CACNA2D).

Analysis of 32 gene variants described in the literature showed that only 9 of them (28.12%) have a decisive pathogenic role. All definitively pathogenic variants are located in KCNQ1, KCNH2 or KCNJ2, encoding potassium channels. Other variants, located

in the genes encoding calcium or sodium channels, are associated with electrical disturbances accompanied by shortened QT intervals, but do not guarantee the development of SQTS.

The only previously known pathogenic variant of CACNA2D1, p. (Ser755Thr), associated with a BrS-like phenotype and a short QT interval, was considered by O. Campuzano et al. as a variant with an ambiguous effect on the phenotype, as it did not show significant changes in ionic current. In the *KCNH2* gene, only four variants play a definitely pathogenic role: p. (Asn588Lys) c. (1764C > A), p. (Asn588Lys) c. (1764C > G), p. (Thr618Ile), and p. (Ile560Thr); 3 variants, p. (Glu50Asp), p. (Ser631Ala), and p. (Trp-927Gly), were classified as likely pathogenic due to a lack of functional data. Two more options in *KCNH2*, p. (Arg1135His) and p. (Arg164Cys), remain indeterminate as both were identified in patients with Brugada syndrome and a shortened QT interval but were not consistent enough with the SQTS diagnosis.

Of the three *KCNJ2* variants associated with SQTS, all three, p. (Met301Lys), p. (Glu299Val), and p. (Asp172Asn), are classified as pathogenic, the negative role of which is confirmed by all currently published data. In *KCNQ1,* one variant, p. (Val307Leu), remains classified as pathogenic, while the variant p. (Phe279Ile) is currently classified as likely pathogenic. Finally, the potentially pathogenic variant p. (Arg370His), found in the *SLC4A3* gene, suggests an association of a new gene with SQTS and represents a previously underestimated mechanism for the development of malignant arrhythmia [37].

There are 8 subtypes of SQTS, SQT 1–8, the development of which is based on the following mechanisms:

1) SQT1, is associated with mutations in the *KCNH2* / *hERG* gene, the role and mutations of which were described above. The hERG channels are uniquely rapidly inactivated depending on the voltage, which promotes a certain contribution of the I_{Kr} current to ventricular repolarization. The linker region of the hERG S5 pore plays a role in inactivating the hERG current. Mutations are mainly associated with a shift in the inactivation peak depending on voltage and an increase in the I_{Kr} current, which leads to a decrease in duration of the QT interval, which is electrophysiologically a substrate both for atrial fibrillation / flutter and for ventricular tachycardia / ventricular fibrillation.

2) SQT2 is caused by mutations in the *KCNQ1* gene, the product of which, together with KCNE1, forms functional proteins, affecting the I_{Ks} current. Mutations in the gene lead to rapid activation or delayed inactivation of potassium channels, which are often the cause of accelerated ventricular repolarization.

3) SQT3 is the result of mutations in the *KCNJ2* gene encoding the Kir2.1 protein, the function enhancement of which affects the duration of the final part of the action potential repolarization.

4) SQT4 and SQT5 include mutations in the *CACNA1C* and *CACNB2b* genes encoding the α1C and β2 subunits of L-type voltage-gated calcium channels that provide the I_{Ca} current. Mutations in *CACNA1C* have been identified as shortening the action potential by slowing down the movement of the α1C subunit towards the membrane. A mutation in *CACNB2b* drastically reduces I_{CaL} without affecting the speed of subunit movement. Both mutations, by decreasing internal I_{Ca} currents, cause transmural and epicardial dispersion of repolarization, resulting in SQTS combined with Brugada syndrome.

5) SQT6 is caused by a mutation in the *CACNA2D1* gene encoding the α2δ1 subunit of L-type voltage-gated calcium channels. The proposed mechanism for reducing I_{CaL} currents through $Ca_V1.2$ turned out to be doubtful, since it was not possible to register shortening of the QT interval in genotype-positive relatives.

6) SQT7 is associated with mutations in the *SCN5A* gene, which encodes the α-subunit of sodium channels, affecting late sodium current, the loss of function of which can affect both depolarization and repolarization. The *R689H* mutation increases late sodium flow, which raises doubts about its isolated effect on the SQT phenotype.

7) SQT8 is associated with mutations in the *SLC4A3* gene, which encodes the AE3 protein, which facilitates transport of Cl^- ions into cardiomyocytes in exchange for HCO_3 transport. According to a number of authors, p.(Arg370His) in the gene should be attributed to a likely pathogenic variant, which leads to a decrease in metabolism and an increase in pH, which induces shortening of the QT interval [23, 37–39].

Conducting a number of genetic studies, including case-control studies using the polymerase chain reaction method, studies using next generation sequencing methods, and genomic association studies, made it possible to identify and study a large number of polymorphisms of known genes that affect the QT interval variability, thereby showing their importance in risk stratification of sudden arrhythmogenic death.

In the course of a meta-analysis, involving 13,685 individuals of European descent from three prospective cohort studies, the Framingham Heart Study (FHS, n = 7,650), the Rotterdam Study (RS, n = 4,606), and the Cardiovascular Health Study (CHS, n = 1,429), C. Newton-Cheh et al. found an association of single-nucleotide polymorphisms (SNPs) of the known genes *NOS1AP* (rs12143842, rs12029454, rs16857031), *KCNQ1* (rs2074238, rs12576239), *KCNE1* (rs1805128), *KCNH2* (rs4725982, rs2968864), and *SCN5A* (rs12053903), which are involved in cardiomyocyte repolarization, with the QT interval duration. Associations at five new loci included 16q21 near *NDRG4* and *GINS3*, 6q22 near *PLN* (rs11756438), 1p36 near *RNF207*, 16p13 near *LITAF*, and 17q12 near *LIG3* and *RIFFL*. Collectively, 14 independent variants at 10 loci accounted for 5.4–6.5% of the QT interval variations [40].

Continuing the search for pathogenic variants in individual cohorts, based on the New Zealand Cardiac Inherited Disease Registry, in 273 patients with LQTS, 4 out of 29 SNPs associated with an increased risk of cardiac events were identified, *NOS1AP* (rs12143842, rs16847548) and *KCNQ1* (rs0798, rs8234). Patients homozygous for the risk allele rs12143842 had an increased risk of sudden cardiac death (odds ratio (-OR) = 10.15; 95% confidence interval (CI) 2.38–43.34, p = 0.045). Several other SNPs showed tendencies toward associations with QT interval duration and clinical events [41].

In a recent GWAS case-control study, separately for the European (1,238 cases vs. 8,219 controls) and Japanese populations (418 cases vs. 1,617 controls), three statistically significant polymorphisms were found: rs12143842 (OR = 1.32; 95% CI 1.21–1.42; $p = 1.09 \times 10^{-11}$), rs179405 (OR = 1.38; 95% CI 1.23–1.54; $p = 1.92 \times 10^{-8}$) in the KCNQ1 intron; and rs17061696 (OR = 1.25; 95% CI 1.15–1.35; $p = 4.33 \times 10^{-8}$) in the KLF12 intron. All 3 loci were previously associated with the QT interval duration, a marker of myocardial repolarization on the electrocardiogram, in the general population. The low-frequency missense variant in *KCNE1*, p.Asp85Asn (rs1805128, OR = 2.78; 95% CI 1.67–3.90; $p = 5.31 \times 10^{-7}$), reached the expected threshold of statistical significance in the European population and had a more pronounced effect in polygenic inheritance of genetically elusive LQTS variants (OR = 7.64; 95% CI 3.66–15.95; $p = 5.99 \times 10^{-8}$) [34].

In 2007, in a population-based study carried out in a cohort of 2,008 apparently healthy subjects, 200 of which had the shortest QT interval and 200 people – the longest, the known variants of the *KCNH2*, *SCN5A*, *KCNQ1*, *KCNE1*, and *KCNE2* genes were analyzed. The minor allele *SCN5A* IVS24 + 116 "A" was more common in the group with the shortest QTc, while the minor alleles *KCNQ1* rs757092 "G" and *KCNH2* rs3815459 "A" were more common in the group with the longest QTc. There were no significant differences for *KCNE1* IVS2–128 G > A and *KCNE2* rs2234916 between the two groups. A genotype analysis showed a twofold increase in the risk of QTc prolongation for carriers of the genotype combining the "C" and "A" alleles of the 2 *KCNE1* SNPs, IVS2–129 C > T (rs2236609) and rs1805127 (G38S), respectively [42]. In the work by S.F. Qureshi et al., the AA genotype and the "A" allele rs1805124 in the *SCN5A* gene were more frequent in patients with LQTS compared with the control group (OR = 2.43; 95% CI of 1.23–4.79, p = 0.01), which indicates its role in the LQTS etiology [43]. In the publication by N.A. Bihlmeyer et al., there are already about 45 single nucleotide variants (SNVs) associated with ventricular repolarization, 10 of which were previously unknown [44].

CONCLUSION

A significant proportion of SCD cases in young people is associated with primary arrhythmia syndrome. Considering the electrical instability in the myocardium as one of the main reasons for the development of life-threatening arrhythmias (ventricular tachycardia / ventricular fibrillation) and SCD, one should be aware of such provoking factors as coronary heart disease, myocarditis, valvular heart disease, pharmacological influences, cardiomyopathy, and channelopathy. It is known that an increase or decrease in the duration of the QT interval, which reflects the work of ion channels and the processes of ventricular depolarization and repolarization, increases the risk of SCD.

Along with diagnosis and treatment strategies, one of the objectives of genetic testing for arrhythmia syndromes is to improve prediction of a risk of adverse events in each individual patient based on their own genotype. Therefore, the study of the relationship of molecular genetic markers with the QT interval duration contributes to better understanding of the pathophysiological mechanisms, correct choice of management strategy, and prevention and treatment of the patient, reducing the likelihood of SCD. The predictive value of genetic testing is the highest for LQTS, for which a gene-specific risk profile has been established, and to a lesser extent for other channel-

opathies. The large amount of genetic data may be a promising approach to quantifying the risk of SCD, especially at a young age, which is facilitated by further study of this problem.

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Pathogenetic mechanisms of postmenopausal osteoporosis formation and their relationship with cardiovascular pathology

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ABSTRACT

Involutional hormonal processes characteristic of the postmenopause are accompanied by disorders that deteriorate the quality of life in the female population and lead to an increased risk of developing metabolic diseases of the bones and cardiovascular system. In modern medicine, it is extremely important to understand the pathogenesis of postmenopausal osteoporosis (PMO) in association with cardiovascular diseases, which are the main causes of mortality in the population.

This review is devoted to determining the key aspects of the pathogenesis of PMO and identifying their relationships with cardiovascular pathology. Epidemiological data are assessed, the main mechanisms of PMO and vascular pathology development are considered, the fundamental role of hormone deficiency, immune dysregulation disorders, and disorders of macrophage polarization is described, and data on the association between the pathogenesis links of the studied pathological processes are analyzed.

The obtained data will form a unified approach to reducing the growing prevalence of cardiovascular diseases and complications of PMO and contribute to the development of new research areas in disease prevention.

Keywords: osteoporosis, postmenopause, cardiovascular diseases, atherosclerosis, estrogen, macrophages

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Патогенетические механизмы формирования постклимактерического остеопороза и их взаимосвязь с кардиоваскулярной патологией

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РЕЗЮМЕ

Инволюционные гормональные процессы, характерные для постклимактерического периода, сопровождаются расстройствами, ухудшающими качество жизни женской половины населения и приводящими к повышению риска развития метаболических заболеваний скелета и сердечно-сосудистой системы. С позиции современной медицины крайне важным является понимание патогенеза постклимактерического остеопороза (ПКО) в ассоциации с кардиоваскулярными заболеваниями, занимающими лидирующие позиции в структуре смертности населения.

Данный обзор посвящен определению ключевых аспектов патогенеза формирования ПКО и выявлению их взаимосвязей с кардиоваскулярной патологией. Проведена оценка эпидемиологических данных, с разных позиций рассмотрены основные механизмы развития ПКО и сосудистой патологии, описана основополагающая роль гормонального дефицита, дисрегуляторных расстройств иммунной системы, нарушений поляризации макрофагов, проанализированы данные, доказывающие сопряженность звеньев патогенеза изучаемых патологических процессов.

Собранные в обзоре сведения позволят сформировать единый подход к снижению растущей распространенности сердечно-сосудистых заболеваний и осложнений остеопороза среди женщин постклимактерического периода и будут способствовать разработке новых научных направлений в профилактике заболеваний.

Ключевые слова: остеопороз, постменопауза, сердечно-сосудистые заболевания, атеросклероз, эстроген, макрофаги

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

Currently, the observed trend toward increased life expectancy in developed countries leads to an increase in the number of postmenopausal women and a rise in the incidence of related diseases. Osteoporosis and cardiovascular diseases are among the most common postmenopausal complications [1, 2].

Postmenopausal osteoporosis (PMO) is understood as a widespread systemic metabolic bone disease, characterized by progressive loss of bone tissue following the onset of natural or induced menopause [3,4].

PMO belongs to type I primary osteoporosis and accounts for 85% of all forms of this group of diseases [1]. The social significance of this disease is determined by its consequences – fractures of the vertebrae and tubular bones, which is one of the main causes of disability in the elderly population [5]. While the majority of premenopausal women have normal bone mineral density (BMD) parameters, by the age of 70, 27–60% of patients are diagnosed with osteopenia, while 70% of women develop osteoporotic changes in hip and lumbar spine [6]. According to statistics, almost half of women are unable to work as a result of fractures, and about 20% of them become disabled [7].

The most commonly occurring hip fracture is a serious complication of osteoporosis, and the associated high mortality rates vary from 12 to 40% during the first year after the fracture. The risk of fatal outcomes also increases due to high probability of a vertebral compression fracture, since it is the trabecular bone tissue that is the most susceptible to damage [3, 8]. The analysis of fracture frequency dynamics revealed a statistically significant increase in rates in recent years. According to the prognosis, the number of osteoporotic femoral neck fractures is expected to double by 2050 [9].

The multifactorial nature of the disease, complex pathogenetic mechanisms with underlying hormonal deficiency, and the impact on other body systems allow to consider osteoporosis as a multidisciplinary problem. In addition to pronounced osteoporotic changes and related fractures, it is characteristic of postmenopausal women to have progression of cardiovascular diseases (CVDs). A number of authors [7, 10–12] have proven that the frequency of CVDs significantly increases in people suffering from osteoporosis. Globally, the prevalence of CVDs in postmenopausal women exceeds the prevalence of CVDs in premenopausal women and men of the same age [13].

One of the known causes of this phenomenon is osteolysis, which leads to an increase in the level of calcium ions in the vascular bed and their further deposition on the inner wall of the vessels. Calcification of the vascular wall ultimately leads to the development of ischemic heart disease, acute cerebrovascular accident, and other CVDs [12].

However, these risk factors do not fully reflect a more severe course of cardiovascular pathologies in this group [14]. Therefore, from the standpoint of modern medicine, comprehensive understanding of the pathophysiological mechanisms of the relationship between postmenopausal osteoporosis and CVDs is certainly an important task, since it might not only improve the diagnosis of serious pathologies and affect further progression of diseases with the formation of common risk groups, but also become an incentive to discover new directions in their treatment.

Pathogenetic role of immune dysregulation in the development of PMO

PMO is considered to be a multifactorial disease. At the same time, most of the studies indicate the fundamental role of hypoestrogenism in the process of bone loss in postmenopausal women. The effect of estrogens in this case is described either as direct, due to the influence on specific estrogen receptors by regulating the transcription of target genes, or as indirect, through a change in the production of stimulants and blockers of bone remodeling [15]. Moreover, in comparison with the reproductive period, when adequate estrogen secretion maintains mineral homeostasis and ensures the formation of a peak in bone mass and maintenance of BMD in the future, the onset of menopause is characterized by significant shifts in the hormonal regulation of bone metabolism [3, 15].

Of course, the mechanisms that underlie the loss of bone mass associated with estrogen deficiency are much more complex and are not limited to the model of direct regulatory effects of these hormones on bone tissue [15]. Recent studies of osteoimmunology have proven the role of immune dysregulation in the initiation of various inflammatory diseases of bone tissue, including osteoporosis. Cells of immune and bone systems have many common molecules, such as transcription factors, signaling molecules, cytokines or chemokines, and the osteoclast at the cellular level can be considered as a prototype of an osteoimmune cell [16]. Therefore, the so-called immunoporosis was clearly revealed from the standpoint of the effect of T helper 1 (Th1), T helper 2 (Th2), and T helper 17 (Th17) cells, as well as regulatory T cells and other cells of the immune system [17].

It was also found that postmenopausal women with osteoporosis have certain immune status disorders compared with healthy women of the same age, and their T cell activity parameters exceed normal values. Now this is associated with the absence of an estrogen blocking effect on the production of pro-osteoclastogenic cytokines (tumor necrosis factor alpha, interleukin-1 beta (IL-1β), interleukin-6 (IL-6) [18]. Thus, the presence of PMO in women is combined with an increase in the concentrations of IL-1β, IL-6, IL-8, IL-17A, and receptor activator of nuclear factor kappa-B ligand (RANKL) and a decrease in the levels of IL-4 and IL-10 [15]. Moreover, under normal conditions, suppression of pro-osteoclastogenic cytokine secretion by hormones is implemented both due to a direct effect on T cells and an indirect effect by inhibiting IL-7 and stimulating transforming growth factor-beta (TGF-β) [18]. Estrogens are able to directly induce apoptosis of bone-resorbing osteoclasts, while the pool size of preosteoclasts not involved in bone resorption is also reduced by estrogens [19].

In total, the processes of osteoclast differentiation in PMO are stimulated by two regulatory factors:

The cytokine ligand – receptor system RANKL / RANK / OPG. It is known that in a state characteristic of PMO, there is an increased production of RANKL, which is necessary for further connection with RANK and its activation. A subsequent increase in the expression of nuclear factor kappa-B (NF-κB) activates the nuclear factor of activated T cells 1 (NFATc1), which, in turn, is the protein initiator of bone resorption and contributes to progressive bone destruction [20, 21].

The macrophage colony-stimulating factor (M-CSF) produced by osteoblasts. It stimulates intracellular tyrosine kinase, resulting in the subsequent proliferation and differentiation of osteoclast progen-

itor cells – monocytes, macrophages, preosteoclasts [21].

Macrophages are of particular interest among progenitor cells, since it is a heterogeneous population of cells that exhibit unique plasticity under changing environmental conditions [20, 22, 23]. Over the past decades, a lot of research was dedicated to the functional features of various macrophage phenotypes, and nowadays there are reliable data proving the role of these cells in the pathogenesis of osteoporosis [4, 17].

Thus, it is known that for women who have reached the age of postmenopause and have a low level of estrogens, there is an imbalance between the proinflammatory M1 and anti-inflammatory M2 phenotype, leading to a change in the polarization of macrophages. The shift in the ratio of macrophage phenotypes and the resulting discrepancy between the number of osteoclasts and their precursors negatively affect the state of bone tissue and contribute to the development and progression of osteoporosis [4, 19, 23].

It is estrogens that coordinate the balance between macrophages and osteoclasts, determining the course of PMO [21], as evidenced by a number of studies. Thus, in a model of experimental osteoporosis after ovariectomy in mice, bone marrow macrophages were isolated from the femur, followed by induced polarization of M1 macrophages by lipopolysaccharide (LPS) / interferon gamma (IFNγ) and M2 macrophages – by IL-4 / IL-13, respectively. After stimulation of the M1 and M2 RANKL phenotypes, it was found that it was the M2 phenotype that mainly differentiated into a functional osteoclast, and not M1, which led to a change in the M1 / M2 ratio [19]. Conducting a similar study, but under conditions of a normalized hormonal background using 17β-estradiol (E2), it was showed that restoring the level of estrogens prevented osteoclastogenesis from M2 macrophages, and the ratio of phenotypes remained normal. The effect of the endocrine profile on the balance between macrophages and osteoclasts is shown in Figure 1.

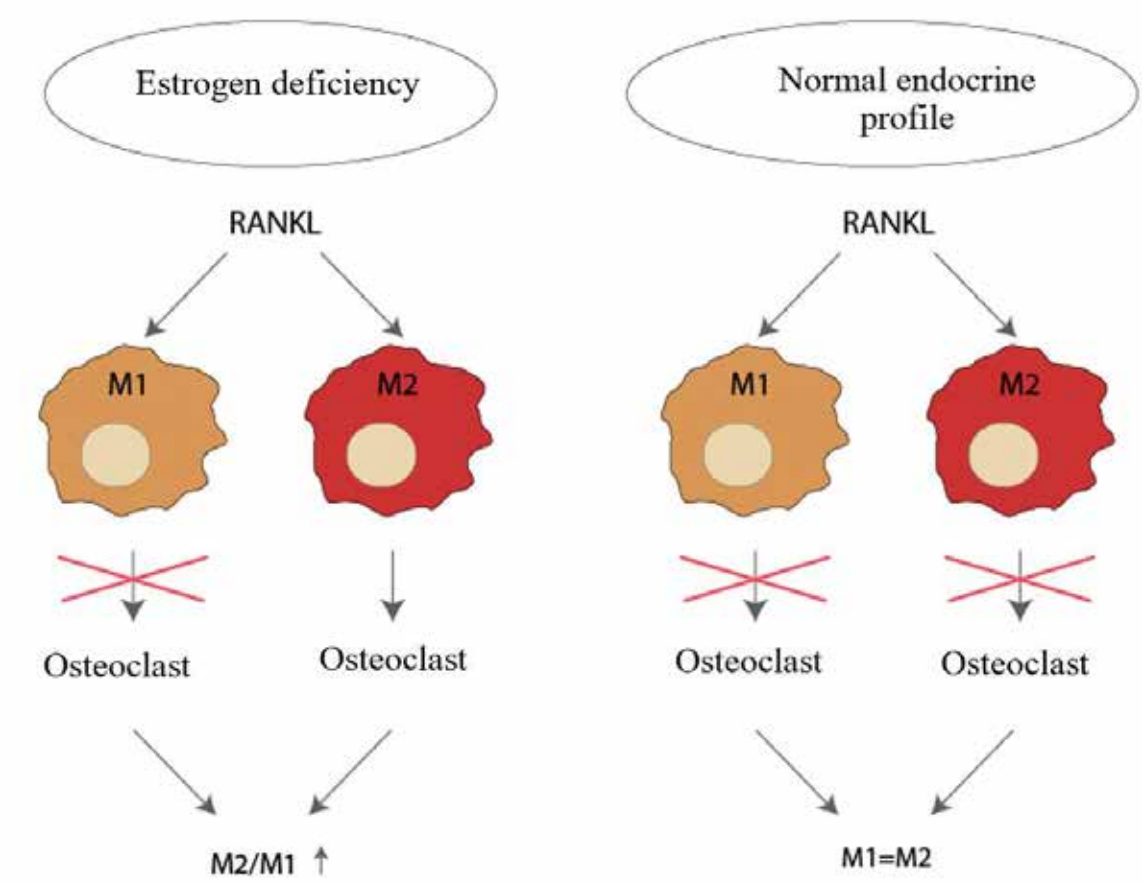

Fig. 1. Effect of endocrine profile on the balance between macrophages and osteoclasts: M1, M2 – macrophage phenotypes; M2 / M1 – a shift in the ratio of macrophages toward the M2 phenotype

These phenomena are explained by the fact that estrogen protects the M2 macrophage from RANKL stimulation due to selective action through the α-estrogen receptor (ERa) and subsequent blocking of the nuclear translocation of NF-kB p65 [19, 20]. A selective α-estrogen receptor agonist PPT (4, 4′, 4″ -(4-propyl-[1H]-pyrazole-1, 3, 5-triyl) trisphenol) can reproduce a similar therapeutic effect in treatment of osteoporosis in ovariectomized mice.

Therefore, against the background of estrogen deficiency, osteoclastogenesis of macrophages of the M2 phenotype leads to a shift in the M1 / M2 ratio and contributes to the progression of PMO.

MECHANISMS OF THE RELATIONSHIP BETWEEN PMO AND CARDIOVASCULAR DISEASES

The postmenopausal period is dangerous not only due to progressive loss of bone mass, but also due to an increased likelihood of CVD. Unfortunately, quite often, these processes mutually aggravate each other, worsening the quality of patients' lives and causing the development of serious complications [7, 10]. Currently, there are data from a number of authors confirming the relationship between vascular diseases and bone tissue, which can be traced both in the presence of similar pathogenesis links and in observations conducted during experimental and clinical studies [24, 25].

In particular, estrogen deficiency underlying PMO, is also a risk factor for CVDs in women after their reproductive function has declined. Moreover, the effect of sex hormones on CVD is diverse: they regulate the mechanisms of vasodilation, the relationship between hypoxia and angiogenesis, and the formation and development of left ventricular diastolic dysfunction. They are also crucial in the regulation of calcium homeostasis and participate in the coordination of the contraction and relaxation of cardiomyocytes [2, 13].

17β-estradiol (E2) mentioned earlier as a factor that prevents osteoclastogenesis from M2 macrophages and normalizes the ratio of phenotypes in osteoporosis is also involved in vasodilation processes. This occurs mainly due to the regulation of nitric oxide synthesis by binding to estrogen receptors that are present in endothelial cells [21, 26].

The cytokine system RANKL / RANK / OPG, which affects both the stages of bone remodeling and the development of arterial calcification, is one of the common factors involved in the regulation of bone tissue and vascular mineralization [27]. In this case, special attention should be paid to osteoprotegerin (OPG), an important secreted protein of the tumor necrosis factor family, which regulates bone density by inhibiting osteoclast differentiation and activation and affects the development of arterial calcification. By binding to its ligand (OPGL), it suppresses the interaction between RANK and OPGL on osteoclasts and their precursors, acting as a secreted inhibitor of the RANK signaling pathway [11]. Studies on mice involving targeted deletion of the osteoprotegerin gene showed a significant decrease in total bone density and high incidence of fractures, as well as a decrease in the incidence of calcification in the aorta and renal arteries. Statistical data confirm the relationship between a high degree of aortic calcification and a pronounced decrease in BMD. Besides, they confirm that the risk of carotid artery calcification for women with osteoporosis is approximately 4 times higher than that in women with a normal BMD index in the femoral neck [24, 27].

One of the significant pathogenetic factors affecting the development of CVD is disturbance of macrophage polarization. Currently, a lot of data have been obtained on the role of macrophage phenotypes in the development of atherosclerotic lesions [11, 13, 22]. As in the case of osteoclastogenesis regulation, the effect of M1 and M2 phenotypes is opposite, contributing to a shift in the overall ratio of macrophages and progression of the lesion or, conversely, restoration of the vascular tissue.

However, in this case, activation of each phenotype is closely related to the change in the metabolic processes occurring in atherosclerosis. Thus, proinflammatory M1 macrophages are characterized by an anabolic type of metabolism, so they mainly regulate glycolysis or the pentose phosphate pathway. The anti-inflammatory M2 phenotype, in turn, is associated with oxidative phosphorylation and fatty acid oxidation. Therefore, such factors as hyperlipidemia, hypoxia, and hyperglycemia can change polarization of macrophages toward the glycolytic M1 phenotype and shift the initial equilibrium, contributing to the progression of atherosclerosis [22].

The localization of phenotypes also confirms the dual role of macrophages. For example, staining of M1 phenotype markers is mainly limited to one of the most unstable areas inside the plaque, while M2 phenotype markers are more often present in the vascular adventitia or areas of stable plaques. M1 macrophages are also more common in the foci of infarction than

M2 macrophages [11]. These data confirm that the M1 phenotype, which, according to many authors, is proatherogenic, characterizes progressive lesions, while regressing plaques are enriched with the antiatherogenic M2 phenotype, which promotes restoration of vascular wall tissues (Fig. 2).

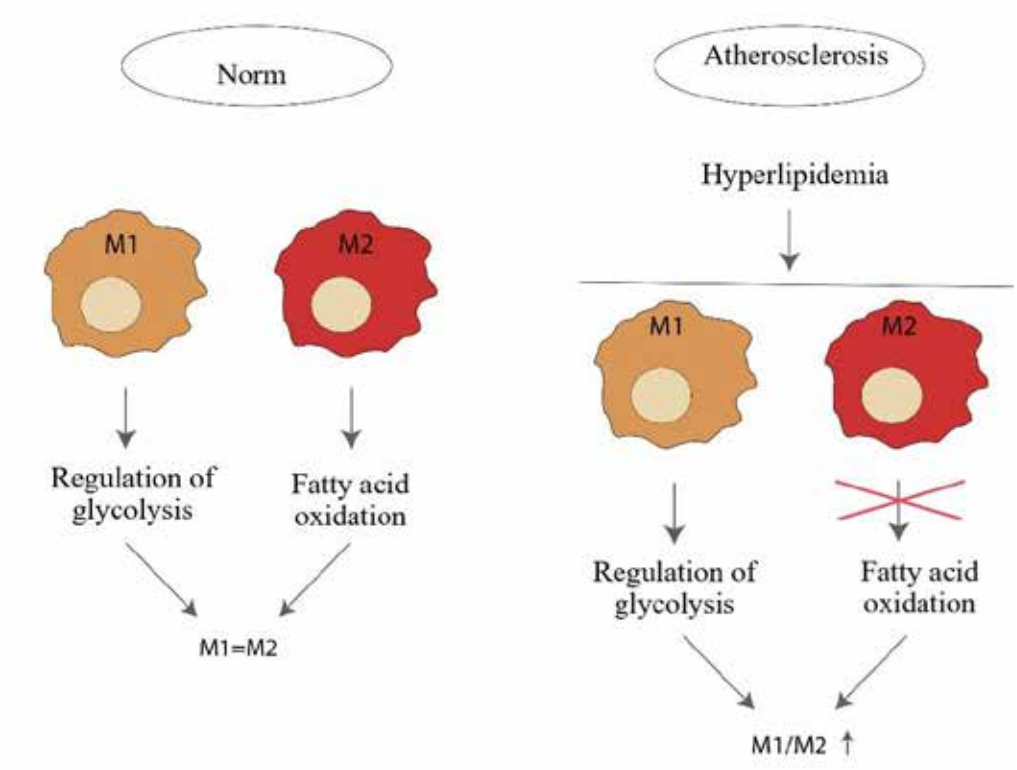

Fig. 2. The role of macrophage phenotypes in the development of atherosclerotic lesions. FA – fatty acids; M1 / M2 – shift in the ratio of macrophages toward the M1 phenotype

In addition to the common links in the pathogenesis of vascular lesion formation and bone tissue reduction, these pathological conditions are reciprocal in relation to corrective therapy. The results of some studies indicate that bisphosphonates, which are used in treatment of osteoporosis as bone resorption inhibitors, have a positive effect on the vascular wall and even reduce the degree of vascular calcification [11]. In order to prevent or reduce progression of subclinical atherosclerosis and vascular calcification, other groups of drugs used in the treatment of osteoporosis were also studied, which included antiresorptive agents (monoclonal antibodies targeting RANKL, selective estrogen receptor modulators) and anabolic bone therapy (teriparatide). However, currently, such studies are few, which is an incentive for the development and introduction into clinical practice of new pathogenetically substantiated methods for treatment of comorbid diseases under consideration [25].

CONCLUSION

The mechanisms of PMO formation following the impact of hormonal imbalance on bone tissue are extremely complex. Hypoestrogenism is the main trigger in the imbalance of bone remodeling which can directly or indirectly induce bone resorption processes. Metabolic disorders and dysregulated immune disorders perform a particular function in maintaining degenerative processes and disease progression. The change in the immune status is largely due to the lack of a blocking effect of estrogens on the regulatory factors of the RANKL / RANK / OPG system, which affects activation of NFATc1 and M-CSF and is responsible for proliferation and differentiation of osteoclast progenitors.

Impairment of macrophage polarization in conditions of estrogen deficiency is associated with the absence of a blocking effect of the hormone on estrogen receptors. In such an environment, activation of M2

phenotype osteoclastogenesis leads to increased bone resorption, which underlies osteoporotic changes. In addition, the analysis made it possible to establish the presence of a close correlation between PMO and cardiovascular pathology from the standpoint of the hormone factor, bone proteins, cytokines, and macrophage polarization. Thus, osteoprotegerin, acting as a secreted inhibitor of the RANKL signaling pathway, is responsible for changing the overall bone density, as well as for the development of large vessel calcification, and its effects on the relationship between a decrease in BMD and an increase in the risk of calcification have been confirmed by statistical data.

It is also shown that due to the disruption of polarization processes, macrophages undergo structural changes during the development of both PMO and cardiovascular pathologies. Only in atherosclerosis, macrophage phenotypes are described according to metabolic shifts, as pro-atherogenic M1 and anti-atherogenic M2, while in osteoporosis, they are classified as proinflammatory M1 and anti-inflammatory M2 phenotypes. At the same time, during the analysis of the collected data, it was found out that in case of PMO, the M1 / M2 ratio is characterized by a shift toward the M2 phenotype, and in case of atherosclerosis, the shift is mainly toward the M1 phenotype. However, the observed pattern in the relationship between cardiovascular pathology and osteoporosis has not been studied enough.

Therefore, it is necessary to further study these and other links in the pathogenesis of the considered diseases, both individually and in relation to each other. Clear understanding of the described and possible new mechanisms can not only make a great contribution to the development of strategies to reduce the growing prevalence of cardiovascular pathologies and complications of osteoporosis among postmenopausal women, but will also help to form a unified approach to the prevention of these diseases.

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Comorbidity of coronary artery disease and its significance in predicting the results of coronary artery bypass grafting

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ABSTRACT

The review presents an analysis of the scientific literature on comorbidity of coronary artery disease (CAD) and assessment of its impact on the results of coronary artery bypass grafting (CABG). Arterial hypertension (AH), chronic obstructive pulmonary disease (COPD), metabolic syndrome (MS), and diabetes mellitus (DM) have been shown to be the most common comorbidities in CAD patients. Clinical manifestations of cardiovascular comorbidities also include atrial fibrillation, acute cerebral ischemia, atherosclerosis of carotid and lower limb arteries, and chronic heart failure.

Concomitant COPD doubles the risk of postoperative complications after CABG and reduces the 10-year survival rate in patients to 30%. In CAD patients with MS, the risk of postoperative mortality increases by 1.4 times, and the 5-year survival rate decreases by 3 times. Diabetes significantly worsens the long-term survival of patients after CABG and is an independent predictor of acute cardiovascular events after revascularization in the long term. The presence of various comorbidities in CAD patients requires a personalized approach to managing the risks of adverse outcomes after CABG and introduction of modern artificial intelligence (AI) technologies into clinical practice, which significantly increase the accuracy of prognosis.

Keywords: comorbidity, coronary artery disease, coronary artery bypass grafting, prognosis

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Коморбидность ишемической болезни сердца и ее значение в прогнозировании результатов аортокоронарного шунтирования

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РЕЗЮМЕ

Представлен анализ научной литературы по проблеме коморбидности ишемической болезни сердца (ИБС) и оценке ее влияния на результаты аортокоронарного шунтирования (АКШ). Показано, что наиболее частыми вариантами коморбидной патологии у больных ИБС являются артериальная гипертензия (АГ), хроническая обструктивная болезнь легких (ХОБЛ), метаболический синдром (МС) и сахарный диабет (СД). Сердечно-сосудистая коморбидность помимо АГ проявляется также фибрилляцией предсердий, острой церебральной ишемией, атеросклерозом сонных артерий и артерий нижних конечностей, хронической сердечной недостаточностью.

Респираторная коморбидность, представленная ХОБЛ, увеличивает риск послеоперационных осложнений АКШ в 2 раза, а 10-летняя выживаемость этих больных снижается до 30%. У больных ИБС с МС риск послеоперационной летальности увеличивается в 1,4 раза, а 5-летняя выживаемость снижается в 3 раза. СД существенно ухудшает долгосрочную выживаемость больных после АКШ и является независимым предиктором острых сердечно-сосудистых событий в отдаленный период после реваскуляризации. Наличие различных вариантов коморбидности у больных ИБС требует персонифицированного подхода к управлению рисками неблагоприятных исходов АКШ и внедрения в клиническую практику современных технологий искусственного интеллекта, повышающих точность прогнозирования.

Ключевые слова: коморбидность, ишемическая болезнь сердца, аортокоронарное шунтирование, прогнозирование

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Currently, comorbidity is understood as coexistence of two or more diseases, regardless of their intensity, which are pathogenetically interconnected or coincide in time for one patient [1]. Comorbidity has been proven to be associated with adverse disease outcomes, an increased risk of adverse effects of drug therapy, and a significant increase in treatment costs. One of the main reasons for the growth of concomitant conditions is increased life expectancy in developed countries. Thus, more than 50% of people aged over 60 years have at least three concomitant diseases, and the proportion of patients from older age groups with five or more comorbid pathologies is constantly increasing [2]. Comorbidity of coronary artery disease (CAD) with other pathologies significantly aggravates its clinical course and reduces the effectiveness of

therapy and survival prognosis after myocardial revascularization. Therefore, the problem of comorbidity in such groups of patients is of special interest [3].

Today, coronary artery bypass grafting (CABG) is one of the most common types of surgical treatment for CAD, which restores coronary blood flow and increases the quality and expectancy of patients' life. Evaluation of its long-term results is the subject of numerous studies. Thus, the accumulated clinical experience indicates that CABG does not fully prevent progressive or recurrent myocardial ischemia (MI). It has been proven, in particular, that within 2–3 years after CABG, recurrent angina pectoris develops in 3.5–7.2% of patients, and by the fifth year, their number increases up to 17–36% [4].

The frequency and timing of angina pectoris recurrence are determined by the clinical features of CAD before myocardial revascularization, surgical risk factors, the presence or absence of postinfarction cardiosclerosis, the prevalence and localization of coronary artery (CA) stenosis, and the presence and intensity of concomitant pathology [5–9]. In the multicenter study conducted by the US Society of Thoracic Surgeons, factors that most commonly appear as the main causes of rehospitalization for patients after CABG were identified. These included: chronic obstructive pulmonary disease (COPD) (odds ratio (OR) 1.81), cerebrovascular disease (OR 1.56), diabetes mellitus (DM) (OR 1.44), and chronic heart failure (CHF) (OR 2.21).

It was shown that one year after CABG, in patients of older age groups with comorbidities, behavioral risk factors affecting their quality of life and disease outcome were more likely to persist (smoking, irregular intake of medications, non-compliance with diet, sedentary lifestyle, etc.) [10,11]. According to the results of the national study in Iceland, it was found that 5-year survival for patients after CABG was 89.9%, and the most significant predictors of mortality were concomitant conditions, such as COPD, chronic renal failure, and diabetes [12]. Another study found that significant predictors of long-term mortality (7 years after CABG) are cerebrovascular diseases, significant peripheral arterial disease, COPD, CHF, and ventricular arrhythmias [13].

According to the data from the Russian Cardiac Surgery Center registry, 10-year survival rate for patients after CABG was 76.4%, and comorbidities, such as DM, COPD, and ischemic stroke (IS) were among the factors that significantly influenced survival [14]. A number of studies have shown that negative CABG prognosis is also associated with the presence of left ventricular (LV) aneurysms, low ejection fraction (EF), small CA diameter, and the use of vein grafts [4, 8, 12, 14]. The quantity and intensity of concomitant pathology for CAD patients is associated with certain gender differences: women are more likely to have hypertension, diabetes, thyroid pathology, bronchial asthma (BA), and varicose veins, while men more commonly have hypertension, lower extremity artery stenosis, COPD, and urolithiasis.

It was noted that the presence of comorbidities in CAD patients planning to undergo CABG is generally a rule, not an exception, which requires an optimal strategy for personalized therapy for comorbidities in preoperative period, as well as adherence to recommendations on their examination and treatment in the postoperative period. In addition, understanding the clinical and pathophysiological features of various types of comorbidities, as well as common biological patterns of non-random co-occurrence of diseases (syntropic diseases) should provide higher efficiency of risk management for adverse outcomes upon surgical myocardial revascularization. In such cases, interdisciplinary interaction of specialists, including the information technology field, is particularly relevant, making it possible to evaluate a wider range of predictors characterizing the postoperative period and long-term consequences of CABG prognosis. However, the problem of CAD comorbidity with account of its typological features, including cardiovascular, respiratory, metabolic, and other clinical variants of the disease, has not been fully resolved and requires further study.

COMORBIDITIES OF CAD WITH OTHER CARDIOVASCULAR DISEASES

As a rule, pathologies of the coronary, cerebral, and peripheral arteries develop simultaneously due the same pathophysiological mechanisms of atherosclerotic lesion formation in the vascular pool. For example, a combination of carotid artery atherosclerosis and CA occlusion occurs in 74.7–92.4% of patients. The most common clinical variant of associated vascular comorbidity in CAD is arterial hypertension (AH), which, according to various sources, can be seen in 64–81.2% of CAD patients [15–17]. In younger groups of patients (up to 50 years of age), this combination is found in around 45% of cases, while in patients over 70 years, it is observed in 85% of cases. The combination of these pathologies leads to an increase in LV stiffness due to its hypertrophy, which worsens the clinical prospects in patients af-

ter myocardial revascularization [18]. According to 3-year follow-up, in the absence of AH treatment, the frequency of LV hypertrophy (LVH) in patients with CAD increased from 57 up to 77%, and the number of cases with diastolic and systolic dysfunction of both ventricles increased drastically [19].

In other studies, LVH was registered in 11.8–59% of patients with CAD. The clinical significance of LVH lies primarily in the fact that it is an independent and strong predictor of cardiovascular mortality: its presence doubles the risk of death for CAD patients [18]. In CAD with comorbid AH, increased LV myocardial mass has greater prognostic value in terms of mortality than a degree of blood pressure (BP) increase and other risk factors, except age, with the worst prognosis occurring for patients with concentric LVH [19]. That happens due reduction of the number of resistance microvessels per unit of myocardial tissue during LVH, which in turn accelerates coronary remodeling, reduces its vasodilation potential, and increases intracardiac vascular resistance. In addition, these changes contribute to the development of perivascular fibrosis, which also limits the coronary flow reserve and increases myocardial oxygen demand.

A combination of AH, LVH, and CAD significantly increases CA stiffness and worsens the prognosis in both diseases [20]. At the same time, it was shown that for CAD patients with LVH, a combination of CABG and surgical LV reconstruction to reduce its volume did not lead to a decrease in the severity of cardiac symptoms, exercise tolerance, hospitalization rates, and mortality [21]. In patients with CAD associated with AH, 3–5 years after CABG, relapses of angina pectoris, coronary atherosclerosis progression, and vein graft stenosis were noted significantly more often than in patients with effective control. These changes were closely related with the degree and time index of the average daily systolic and diastolic blood pressure, as well as with the insufficient degree of its nighttime drop.

A frequent combination of CAD, AH, and carotid artery atherosclerosis determines a high risk of IS in such patients. Thus, CAD was diagnosed in 74% of patients with IS [22]. In other studies, the combination of CAD and IS was recorded in 30–60% of cases [23]. According to various authors, hemodynamically significant carotid artery stenosis was found in 2.8–17% of CABG patients. It was also shown that around 28–40% of patients after carotid endarterectomy are diagnosed with concomitant CA lesion [24]. For patients with a history of transient ischemic attack or IS, the risk of recurrent acute ischemic stroke in the perioperative period increases up to 8.5%. At the same time, the risk of death for patients after simultaneous CABG and endarterectomy is around 10–12% [23, 24].

In the postoperative period, paroxysmal atrial fibrillation (PAF) develops in 30–50% of patients who underwent CABG with cardiopulmonary bypass (CPB) and less frequently – in off-pump CABG, which increases the risk of embolic complications by 4 times and the risk of sudden cardiac death by 3 times [25]. Higher incidence of atrial fibrillation (AF) is associated with older age, male sex, prolonged mechanical ventilation, multifocal atherosclerosis, COPD, left atrial enlargement, valvular lesions, episodes of AF before surgery, diabetes, and obesity.

Pathogenetic features of postoperative AF are determined by the presence of new triggers associated with cardiac surgery and CPB: impaired blood ionic composition, atrial edema, compliment activation and expression of proinflammatory cytokines, increased sympathetic activity, oxidative stress, and pericardial effusion. It was also established that the presence of even a single episode of PAF in the postoperative period acts as an independent risk factor for development of a persistent form of the disease (risk ratio (RR) 4.99). Long-term comorbidity of CAD and AF in the preoperative period exacerbates ischemic myocardial damage and aggravates patient's hemodynamic status. AF episodes before CABG dramatically increase the risk of sudden cardiac death in the postoperative period (9 vs. 2%), ischemic cerebrovascular events (7 vs. 2%), and development of end-stage CHF (18 vs. 7%) [26]. To prevent such complications, various options for simultaneous surgical treatment of paroxysmal and persistent AF during CABG have been developed, which contributes to restoration and stabilization of sinus rhythm in a significant number of patients and improves the structural, functional, and electrophysiological characteristics of the myocardium [27].

Chronic critical lower extremity ischemia combined with CAD is observed in 37–78% of patients [28]. Combined atherosclerotic damage to the lower extremity arteries and branches of the aortic arch and abdominal aorta occurs in almost half of CAD patients [24]. For such patients, the risk of IS and MI in the early postoperative period after CABG reaches 12% (against 3.8% in the general population) [22, 23]. Depending on the number of damaged arteries, the risk of postoperative vascular events increases by 1.64–10.5 times compared with isolated coronary artery disease. Independent predictors of overall and cardiovascu-

lar mortality after CABG were determined for patients with multifocal atherosclerosis, which include the presence of clinical manifestations of lower limb ischemia (OR 3.6) and a decrease in the ankle-brachial index without clinical manifestations (OR 2.4). The 3-year cardiovascular mortality after CABG in patients with multifocal atherosclerosis is 18.5% vs. 11.2% in the general population of CAD patients [29].

In the European epidemiological study of patients with CAD, functional class (FC) III–IV CHF was detected in 5 and 0.6% of CAD patients, respectively [30]. It was proven that chronic impairment of coronary circulation leads to structural changes in cardiomyocytes and a decrease in myocardial contractility due to ischemic cardiomyopathy formation. On the other hand, limitation of LV contractility contributes to development of arterial hypoxemia, increases myocardial oxygen demand, and aggravates the clinical course of CAD. It was established that in CAD patients with CHF, surgical myocardial revascularization is more preferable than percutaneous coronary intervention. According to meta-analysis data, patients with left ventricular ejection fraction (LVEF) ≤ 35% after CABG show operative mortality of around 50% and 5-year survival of up to 73% [13, 24]. In patients with normal ejection fraction (EF), the same parameters are 2 and 83.8%, respectively. Therefore, AH with LVH, CHF, AF, atherosclerotic stenoses of carotid and peripheral arteries, and episodes of acute ischemic stroke should be attributed to the most frequent and dangerous manifestations of cardiovascular comorbidity in CAD patients. The effectiveness of CABG in such cases depends on the effectiveness of risk stratification in the preoperative period, as well as on personalized programs for preparation for surgery and postoperative rehabilitation for such patients.

COMORBIDITY OF CAD WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

According to numerous studies, COPD prevalence in the Russian Federation is around 15.3% in the general population and 21.8% among people with respiratory symptoms. At the same time, mortality from COPD ranks 4th around the world. COPD is classified as a multisystem disease, the most important extrapulmonary manifestation of which is damage to the cardiovascular system. Large epidemiological studies demonstrate that the leading cause of mortality for COPD patients is cardiovascular pathology, which is recorded in at least 50% of these patients [31]. It has been shown that every 10% decrease in forced expiratory volume in 1 second (FEV1) increases the risk of cardiovascular mortality by 28% and non-fatal coronary events by 20% [32].

The key mechanisms of cardiovascular comorbidity in COPD are chronic systemic inflammation, arterial hypoxemia and tissue hypoxia, oxidative stress and degradation of the extracellular matrix, which accelerate atherogenesis and are involved in the pathogenesis of intracardiac, central, and pulmonary hemodynamic disorders and myocardial contractile dysfunction. A number of authors point to certain difficulties in diagnosing CAD in COPD patients due the fact that in the clinical presentation of the concomitant pathology, signs of dyspnea due to respiratory failure prevail instead of the coronary syndrome [33, 34].

One of the factors contributing to the atypical course of CAD in patients with COPD is chronic arterial hypoxemia, which increases the pain threshold. This explains, in particular, that painless forms of myocardial ischemia are more common for patients with COPD, they are recorded in 66.7–84.4% of cases. According to results of various studies, atypical localization of chest pain is recorded much more often in patients with comorbidity, than in individuals with isolated CAD [35, 36].

In patients with CAD against the background of COPD, a more severe course of angina pectoris and CHF is also observed. In addition, in CAD patients with comorbidity, the severity of bronchial obstruction is significantly higher than in patients with isolated COPD. The main mechanisms of mutual aggravation of these diseases include augmented pulmonary shunt both due to ventilation – perfusion mismatch characteristic of COPD and alveolar shunt against the background of left ventricular failure and diffusion disorders associated with ischemic cardiomyopathy. It should be noted that progression of CAD and COPD during their comorbidity is characterized by commonality of some links in their pathogenesis. In particular, formation of secondary pulmonary hypertension increases the load on the right heart and left atrium, limiting the coronary flow reserve, which increases MI in both ventricles and contributes to progression of coronary and pulmonary heart disease [37].

It is believed that a severe course of COPD reduces traditional clinical and functional manifestations of CAD [33]. In these situations, more careful monitoring of physiological functions in the dynamics of the disease is required. In addition, according to various authors, the frequency of the established COPD diagnosis in patients with CAD referred for CABG ranges

from 6.7 up to 14% [36]. However, spirometry for all patients before CABG makes it possible to diagnose obstructive disorders in more than a third of all cases. Thus, in real clinical practice, the problem of cardiorespiratory comorbidity in patients with CAD is underestimated due COPD underdiagnosis. The SYNTAX II scoring scale has been proven to be a useful tool for optimizing the choice of surgical revascularization and predicting 4-year survival outcomes among patients with complex CAD forms, which include associated COPD. It was shown that risks of complications during the hospital stay in patients with cardiorespiratory comorbidity double [35]. Other studies identified an increase in the risk of mortality in patients with COPD after CABG by 1.4–1.8 times compared with the control group (27.2 vs.14.5%) [34]. According to the results of the Spanish Registry, the 10-year survival rate for patients with CAD and concomitant COPD after CABG is 30% [33].

A number of studies indicate a predictive role of individual parameters of lung ventilation function in assessing early and long-term CABG consequences in patients with COPD. In particular, FEV1 has been shown to be an informative predictor of 5-year survival in COPD patients who underwent CABG [33]. According to other data, FEV1 less than 60% of the expected value is an unfavorable prognostic factor for postoperative respiratory complications and long-term results of revascularization [36].

The predictive value of forced vital capacity in assessing the risk of developing hospital-acquired pneumonia, atelectasis, and postoperative wound infections and increasing the duration of mechanical ventilation after surgery and reintubation was also shown. One of the factors aggravating respiratory failure and increasing the risk of cardiovascular complications in COPD patients in the postoperative period is respiratory muscle dysfunction, which timely diagnosis and correction at the preoperative stage can reduce the risk of postoperative complications. It has been shown that prehabilitation of COPD patients with respiratory simulators and modern methods of bronchodilator therapy significantly improves postoperative period prognosis and respiratory function recovery rate [33].

COMORBIDITY OF CAD WITH METABOLIC SYNDROME

Metabolic syndrome (MS) is defined as a complex of endocrine and metabolic disorders associated with insulin resistance. A detailed study of its development mechanisms is of great clinical importance, since this syndrome is reversible and amenable to therapeutic control. In the “classic” variant of MS, it is characterized by abdominal obesity, dyslipidemia, impaired carbohydrate metabolism, and hypertension, which determines its high atherogenic potential. Thus, the incidence of CAD in people with MS increases by 2.9–4.2 times and mortality rate – by 2.6–3 times. After CABG, the presence of MS is recorded in 55.4% of women and 41.3% of men [38]. In patients with MS, the incidence of life-threatening cardiovascular diseases increases by almost 4 times [39].

The importance of individual MS components and their combinations in the development of coronary artery atherosclerosis, LVH, LV diastolic dysfunction, AF, and other variants of cardiovascular comorbidity has been proven [39, 40]. According to individual studies, a complete MS cluster is formed within at least 10 years, and its multicomponent variants are characterized by severe comorbidity [41, 42].

Development of acute cardiovascular events after CABG in patients with MS is significantly more frequent, and a number of authors indicate a significant increase in mortality in such groups (2.4 vs. 0.9%) [42]. According to the American Society of Thoracic Surgeons, the presence of MS increases the risk of postoperative mortality by 1.4 times [43]. In MS, the risk of developing respiratory complications and postoperative wound increases by 2.5 times, and thorough correction of carbohydrate metabolism in the perioperative period reduces it by 2 times. Another study confirmed a significant association of MS with wound infections and pulmonary complications (OR 6.64), cardiac arrhythmias (OR 5.47), prolonged mechanical ventilation (OR 5.47), and almost a 3-fold increase in the risk of postoperative mortality after CABG, compared with the control group (3.1 vs. 1.1%) [42, 43]. In CAD patients with MS, CA lesions are usually more diffuse and distal, which in some cases limits the use of endovascular revascularization. This explains the advantage of CABG with arterial bypasses compared with percutaneous coronary intervention for such patients. In CAD patients with MS over 65 years, surgical myocardial revascularization increases the 10-year survival rate by almost 50% [39].

Besides, it has been shown that with coronary artery occlusion in patients with MS, the degree of collateral circulation development is lower, and the clinical course of atherosclerosis is more aggressive with an increased risk of developing various complications. A high risk of adverse cardiac events, shunt obstruction or new coronary artery damage within 2–5 years

after CABG correlates with triglyceride and blood glucose levels. It has been shown that in MS patients with high triglyceride levels and elevated (>8.6%) levels of glycosylated hemoglobin in the preoperative period, mortality after CABG increases by 4 times [44]. In case of CAD with concomitant MS in the long-term period after revascularization, the incidence of CHF increases by 46%. According to the Scottish registry, after CABG, patients with a body mass index (BMI) of 27.5–30 kg / m^2 had a decrease in the 5-year mortality rate by 41% compared with patients whose BMI was more than 30 kg / m^2 [12]. These data support the hypothesis on atherogenic and inflammatory activity of visceral adipose tissue in patients with MS. Thus, according to the results of a five-year cohort study, patients with MS after CABG, distributed according to the presence or absence of obesity, significantly differed in terms of early postoperative complications (30.26 vs. 20.75%, OR 2.04) and five-year mortality (11.84 vs. 3.74%, OR 4.65) [45].

COMORBIDITY OF CAD WITH DIABETES MELLITUS

Based on the WHO data, the number of patients with diabetes mellitus (DM) is steadily growing and now accounts for about 2.1% of the population, while DM contribution to the overall mortality by 2030 will reach 3.3%. According to the DECODE study, DM prevalence increases with age, regardless of gender, and the lifetime risk of morbidity is 30–40%. DM prevalence among CAD patients planning to undergo surgical treatment is about 35% [46, 47].

DM is one of the leading pathogenetic factors for development and progression of circulatory disorders, and the risk of cardiovascular mortality in patients with CAD and associated DM increases by 2 times in males and by 4 times in females [48]. In particular, it has been shown that mortality in patients with acute coronary syndrome and DM is 2–3 times higher than in patients with normal carbohydrate metabolism [49]. In case of CAD and DM comorbidity, coronary artery atherosclerosis is more diffuse, with frequent involvement of the epicardial ST segment and an increased tendency to restenosis and shunt obstruction [44]. A number of studies have noted an increase in mortality during CABG in patients with DM (3 vs. 1%) [39, 42, 44]. Other studies have shown that immediate CABG outcomes in patients with DM may not differ compared with patients without DM [45]. Thus, according to a retrospective analysis of 4,508 bimammary CABG surgeries, the presence of DM did not affect the overall hospital mortality, but was associated with wound infection and postoperative bleeding. In a similar study, the presence of DM did not increase the risk of mortality and cardiovascular and other hospital-acquired complications, with the exception of renal dysfunction [50, 51]. However, the clinical prospects of long-term CABG results for patients with DM are not so optimistic. Thus, patient's 1-, 5-, 10-, and 20- year survival after CABG with and without DM was 94 vs. 94%, 80 vs. 84%, 56 vs. 66%, and 20 vs. 32%, respectively [52].

DM significantly impairs long-term survival after surgical revascularization, being an independent predictor of acute cardiovascular events [41,48]. Moreover, in patients with type 2 diabetes receiving insulin therapy, IS, MI or sudden cardiac death were significantly more likely to develop both during the short-term (30 days) and medium-term follow-up (1 year) – OR 1.66 and 1.50, respectively [53]. For patients with type 1 diabetes, according to the SWEDEHEART registry, the risk of mortality after CABG increased by more than 2 times compared with the general population (OR 2.04) [54].

The 5-year mortality after CABG in patients with DM was 6 times higher than in the control group (12.1 and 2.1%, respectively). In the structure of 5-year mortality in patients with DM after CABG, acute heart failure prevails, usually associated with MI [52]. The long-term prognosis is also affected by the presence of prediabetes detected in the preoperative stage. Thus, after CABG, the risk of acute cardiovascular events for such patients is significantly higher (OR 1.40), which proves the importance of an active approach to DM detection [50]. The results of 6-year survival after off-pump CABG in patients with DM differ significantly from the results after CABG with CPB and reach 69.9 and 54.7%, respectively [54].

CONCLUSION

Thus, literature data indicate that the presence of certain types of comorbidities in patients with CAD is the most important prognostic factor characterizing the expectancy and quality of life after CABG. The most frequent and unfavorable concomitant pathologies include cardiovascular, respiratory, and metabolic comorbidities, the clinical manifestations of which have a significant impact on the effectiveness of surgical myocardial revascularization. The presence of concomitant pathology in CAD patients requires personalized approaches to peri- and postoperative management, as well as development of automated programs for predicting complications and

long-term consequences of surgical treatment, taking into account individual risk factors. In addition, introduction of modern artificial intelligence technologies into clinical practice will significantly improve the accuracy of CABG outcome prediction and optimize treatment costs.

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The problem of multimorbidity in a modern therapeutic clinic

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ABSTRACT

A review of modern studies on the problem of multimorbidity, its definition, frequency of occurrence, prevalence, medical and social consequences, factors predisposing to its formation, and approaches to diagnosis, therapy, and rehabilitation is presented. According to modern understanding, multimorbidity is the presence of two and / or more chronic diseases that are pathogenetically interconnected and / or coincide in time in one patient.

Currently, multimorbidity is becoming an epidemic, affecting people of different ages and gender and with various diseases. The literature describes multiple adverse medical and social consequences of multimorbidity, such as increased rates of hospitalization, disability, and mortality, decreased functional capabilities and quality of life in patients, as well as increased volume, timing, and cost of medical care. Today, issues of the unified terminology, identification of multimorbidity, and the structure and clinical manifestations of associated pathology are being studied. There are single works on the study of possible factors contributing to the formation of multimorbidity. Approaches to management of patients in conditions of multimorbidity are being developed. A more detailed study of the mechanisms of multimorbidity formation and common pathogenetic links of associated diseases will make it possible to develop more effective strategies for the diagnosis, treatment and rehabilitation of multimorbid patients.

Keywords: multimorbidity, comorbidity, associated pathology

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Проблема полиморбидности в современной терапевтической клинике

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РЕЗЮМЕ

Представлен обзор современных исследований, посвященных проблеме полиморбидности, ее определению, частоте встречаемости, распространенности, медико-социальным последствиям, факторам, предрасполагающим к ее формированию и подходам к диагностике, терапии и реабилитации. Согласно современным представлениям, полиморбидность представляет собой наличие двух и (или) более хронических заболеваний, патогенетически взаимосвязанных между собой и (или) совпадающих по времени у одного пациента.

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В настоящее время полиморбидность приобретает масштабы эпидемии, затрагивая лиц различного возраста и пола, с различными нозологиями. В литературе описаны множественные неблагоприятные медицинские и социальные последствия полиморбидности: повышение показателей госпитализации, инвалидизации и смертности, снижение функциональных возможностей и качества жизни пациентов, увеличение объема, сроков и стоимости оказания медицинской помощи. В настоящее время активно исследуются вопросы единства терминологии, идентификации полиморбидности, проводится изучение структуры и клинических проявлений сочетанной патологии. Появляются отдельные работы по изучению возможных факторов, способствующих формированию полиморбидности, разрабатываются подходы к ведению больных в условиях множественной сочетанной патологии. Более детальное изучение механизмов формирования полиморбидности, исследование общих патогенетических звеньев сочетанных заболеваний позволит разработать более эффективные стратегии диагностики, лечения и реабилитации полиморбидных пациентов.

Ключевые слова: полиморбидность, коморбидность, мультиморбидность, сочетанная патология

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

In recent decades, wide spread and growth of associated pathology, which is considered within the concept of multimorbidity (MM), has become a serious medical, social, organizational, and economic problem [1–5].

A combination of diseases should be taken into account for targeted prevention, adequate treatment, and prediction of complications. At the same time, it should be noted that to date, most studies are predominantly mononosological in nature, and most guidelines have been developed for individual diseases. This presents the greatest difficulties in managing patients with multimorbid pathology, as it does not allow to fully analyze risk factors and prognosis for the development of associated diseases and effectively solve the problems related to their prevention and treatment. A lack of knowledge in this area leads to defects in the organization of medical care. Thus, an analysis of 10 studies conducted in seven countries (Belgium, England, Germany, Ireland, Scotland, the Netherlands, and the USA) showed that most difficulties in the treatment of patients with multimorbidity occurred following disorganization and fragmentation of medical care and inadequacy of guidelines and evidence-based medicine [6].

To solve this problem, a thorough comprehensive study of multimorbidity, its structure, clinical manifestations, risk factors, and features of its formation is required. This review presents current information on the definition, identification, frequency of occurrence, and medical and socioeconomic consequences of multimorbidity. Scientific works on the study of possible mechanisms and factors contributing to the formation of multimorbidity, as well as currently available approaches to the management and treatment of multimorbid patients are also considered.

CONCEPT AND DEFINITIONS OF MULTIMORBIDITY

Currently, multimorbidity is defined as the presence of two and / or more chronic diseases, pathogenetically interrelated and / or coinciding in time in one patient, regardless of the intensity of each of them [3, 7–11]. The term “multimorbidity” has various synonyms: comorbidity, polymorbidity, multifactorial, combined, and associated diseases, polypathia, etc. There is still no mainstay of the definition [9, 10]. Most often, in the Russian-language literature, the terms “polymorbidity” and “comorbidity” are used, in foreign literature – “multimorbidity” and ‘comorbidity”.

Comorbidity is most often defined as the coexistence of 2 and / or more diseases in one patient, pathogenetically and genetically interrelated [12, 13]. Comorbidity can proceed as syntropy – organ damage under the influence of common pathogenetic factors or interference – the occurrence of one disease under the influence of another [14]. Diseases or disorders

that are comorbid with a particular disease are understood as disorders that occur in this disease most often and have some common etiological or pathogenetic mechanisms with it [3].

According to some authors, the term "comorbidity" usually implies interaction of several diseases, one of which being the underlying one [15, 16]. At the same time, multimorbidity is also considered as the presence of two or more diseases that may not be in a cause-and-effect relationship. Consequently, multimorbidity is a broader concept focused on the entire complex of patient's diseases, and not on one underlying disease [15, 16].

It is believed that the relationship of several diseases can be represented by various variants. Thus, one disease may be the cause of another disease. In another variant, associated diseases may have common risk factors and common pathogenetic mechanisms. One more variant suggests that multiple diseases may not have a cause-and-effect relationship or may have only a weak association [17]. Moreover, one disease may be caused by iatrogenic factors, occurring in connection with another disease [18].

Transsystemic, transnosological, and chronological multimorbidity are identified. The first two variants represent the coexistence in one patient of two and / or more syndromes or diseases, pathogenetically interrelated with each other, and the last variant requires their temporal coincidence [19].

In recent years, the concept of "associative multimorbidity" has also emerged, which implies non-random occurrence of a certain complex of diseases that develop together much more often than one would expect from a pure chance [20]. M. van den Akker (1998) [21] and A. Prados-Torres (2014) [20] initiated the cluster theory of multimorbidity, according to which a number of diseases tend to appear in clusters [21].

METHODOLOGICAL APPROACHES TO THE DETERMINATION OF MULTIMORBIDITY PARAMETERS

There are two main groups of multimorbidity parameters. The first group consists of simple counts from various lists of chronic diseases. The second group of measurements introduces weighting for included chronic diseases, thus creating a weighted index of multimorbidity [22].

According to a review article by V. de Groot et al. (2003), there are 13 methods for scoring multimorbidity [23]. Data from a systematic review by A.L. Huntley et al. (2012) [24] cover 17 methods for assessing multimorbidity, of which the estimations of Chronic Disease Score (CDS), Charlson Index, Cumulative Illness Rating Scale (CIRS), and Duke Severity of Illness (DUSOI) indices are the most commonly used methods. The Charlson Index is based on an elaborate list of specific diagnoses. The CIRS system summarizes the state of each system and makes it possible to assess the "ranked" effect of comorbid states on specific organs and systems of the body. The DUSOI scale assesses the severity of the patient's condition according to four scales (symptoms of the disease, complications, prognosis without treatment, and possibility of cure) [25].

PREVALENCE AND FREQUENCY OF MULTIMORBIDITY

Epidemiological studies of recent years convincingly show high prevalence of multimorbidity around the world, associated, among other factors, with population aging and an increase in life expectancy [1, 2, 5, 26]. The global estimates of the multimorbidity prevalence among different age groups vary from 14 to 90% [26]. Such a large difference in magnitude can hardly be associated only with the real difference between populations, but rather with different methodological criteria for its determination. Thus, according to C. Violan et al. [1], the prevalence of multimorbidity varies from 13 to 95% depending on the studied population and the method of collecting and recording incidence data.

According to C. Buffel du Vaure et al. [27], 55% patients with chronic diseases are multimorbid. According to A.L. Vertkina et al. [19], the frequency of comorbidity in patients with decompensation of chronic diseases (average age of 67.8 ± 11.6 years) is 94.2%. Most frequently, in clinical practice, combinations of two and three diseases are observed, however, in 2.7% of cases, up to 6–8 diseases proceed in one patient simultaneously.

In recent decades, there has been a significant increase in the incidence of multimorbidity in a therapeutic clinic [3, 4, 28, 29]. Thus, the analysis of data on the occurrence of multimorbid pathology in patients of a therapeutic clinic in Novosibirsk, carried out by the authors of this review, showed an increase in the degree of transnosological multimorbidity (average number of nosological forms) from 4–6 disorders in one patient in 2003–2005 to 11–14 pathologies in 2015 [30]. The dynamics of transsystemic multimor-

bidity (average number of affected systems) showed an increase in the average number of body systems involved in the pathological process in one patient from 2–4 in 2003–2005 up to 5–6 in the current time period [30].

MEDICAL, SOCIAL, AND ECONOMIC CONSEQUENCES OF MULTIMORBIDITY

The issues of effective prevention, treatment, and long-term follow-up of patients with multiple chronic comorbidities are a major problem facing public health and society [31, 32]. Multimorbidity leads to adverse medical and socioeconomic consequences: an increase in hospitalization, disability, and mortality rates, worsening of disease prognosis, a decrease in functionality and quality of life in patients, and an increase in the volume, timing, and cost of medical care [3, 7, 8, 12, 33].

In patients with associated diseases, diagnosis and choice of treatment methods and strategy become more complicated, or when following the standards of compulsory medical services, the target results are not achieved in patients of this category [34]. All this leads to a decrease in the treatment effectiveness and patient's adherence to therapy, as well as polypharmacy [32, 33, 35, 36]. The interaction of comorbidities changes the classical clinical presentation and the course of diseases, increases the number and severity of complications, and worsens the quality-of-life prognosis [33, 36–38]. Patients with multimorbid pathology are characterized by higher rates of hospitalization and complications, significant deterioration in the prognosis of morbidity, reduced functionality and quality of life, and the highest mortality rates [35–37, 39–44].

Multimorbidity is associated with an increased risk of mortality [36, 39, 41–43]. A systematic review and meta-analysis of 26 articles showed a significant association between multimorbidity and mortality. The number of diseases is also positively associated with the risk of death [36]. Thus, the risk of death in patients with two diseases is 5–10%, while with five or more diseases it increases to 70–80% [21, 45].

Multimorbid pathology is a heavy burden on the country's economy. In the presence of multimorbidity, the costs for diagnosing and treating diseases significantly increase [39, 46, 47]. Thus, 75% of the US budget for medicine is spent on patients with multiple comorbidities. Medical insurance payment for patients with one disease is 211 USD, with four or more diseases – hundreds of times more (13,973 USD) [33]. The Russian National Clinical Guidelines "Comorbid Pathology in Clinical Practice" [46] emphasize the impact of an increase in the prevalence of multimorbidity on the impoverishment of individual families due to catastrophic medical care costs and a high share of costs from family funds.

RISK FACTORS FOR THE FORMATION OF MULTIMORBIDITY

The common risk factors and pathogenetic mechanisms of multimorbidity formation should be identified to develop effective approaches to disease prevention, treatment, and rehabilitation in multimorbid patients. Chronic somatic symptom disorders form the majority of multimorbid pathologies in the therapeutic clinic. The common etiological factors and risk factors are identified, which determine the commonality of some links in the pathogenesis of chronic somatic symptom disorder [48]. The formation and frequency of associated chronic diseases are influenced by many risk factors, including gender, age, social conditions, place of residence, etc. [8, 17, 28, 35, 49, 50].

Many studies have shown the relationship of multimorbidity with age; researchers focus on a significant increase in multimorbidity with increasing age [1, 8, 28, 32, 50–53]. According to M. van den Akker et al. [54], multimorbidity increases from 10% in patients under the age of 19 to 78% in people that are 80 years and older. According to M. Fortin et al. [45], multimorbidity occurs in up to 69% of young patients, in up to 93% of cases among people of middle age, and in up to 98% of patients of older age. At the same time, the average number of chronic diseases varies from 2.8 in young patients to 6.4 in elderly and senile patients.

L.B. Lazebnik et al. [48] analyzed the number of diseases in patients of therapeutic departments of a geriatric hospital depending on age. The authors obtained the following data: the number of diseases per one patient aged 60–65 years was 5.2 ± 1.7; aged 66–70 years – 5.4 ± 1.4; aged 71–75 years – 7.6 ± 1.7; aged 76–80 years – 5.8 ± 1.6; aged 81–85 years – 5.8 ± 1.8; aged 86–90 years – 4.4 ± 1.6; in centenarians aged 91–95 years – 3.2 ± 0.5. According to A. Marengoni et al. [7], the prevalence of multimorbidity in older people varies from 55 to 98%. According to C. Violan et al. [1], in patients aged 18 years and older, the prevalence of multimorbidity is 12.9%, and in patients older than 65 years it reaches 95.1%.

It has been noted that the onset of diseases forming multimorbidity and their chronicity occur mainly in middle age, but the result of their accumulation, that

is, a period of vivid manifestation, begins precisely in old age [51]. Some authors put forward a hypothesis according to which the formation of multimorbidity and aging are based on the same mechanisms, and multimorbidity can be considered as a marker of accelerated aging [55]. There is growing evidence on gender differences in the prevalence of multimorbidity. Most studies have shown that a greater frequency and severity of multimorbidity is associated with the female gender [1, 2, 7, 8, 28, 56, 57].

The formation of multimorbidity is significantly influenced by the socioeconomic status of a person [28]. In this regard, in recent years, the so-called social gradient or socioeconomic risk factors has begun to attract special attention. It includes the level of real income, the structure and standards of consumption, provision with housing and the comfort of life, the degree of cultural development, the social status of a person, the level of education, professional affiliation, etc. [58]. One of the significant social factors is the professional status, which largely forms the individual health of a population [3].

According to a meta-analysis of studies conducted using Medline and Embase databases, low level of education was associated with a 64% increase in the likelihood of multimorbidity, compared with high level of education. An increase in deprivation was consistently associated with an increased risk of multimorbidity, while data on income were ambiguous [59]. Other studies have also shown that the prevalence and severity of multimorbidity are associated with lower socioeconomic status, socioeconomic deprivation [1, 2, 60–63], and lower level of education [2, 7]. L. Mounce et al. [64] noted that individual factors of lifestyle, including obesity, sedentary lifestyle, smoking and excessive alcohol consumption; psychosocial factors (negative life events, external locus of control, and limited social contacts); mental health problems (depression and long-term treatment with certain drugs), also contribute to the formation of multimorbidity.

Modifiable hemodynamic and metabolic risk factors for chronic noncommunicable diseases are of great interest for practical medicine. These factors include high blood pressure, obesity, dyslipidemia, hyperglycemia, hyperuricemia, and others. However, their role in the formation of multimorbidity is much less studied. In recent years, there have been works indicating a possible relationship of multimorbidity formation with an imbalance in metabolic parameters [30, 46, 65] and with behavioral risk factors (smoking, alcohol abuse, low physical activity, overweightness) [1, 60].

Our previous studies have shown a direct relationship between the formation and development of multimorbidity and some of the main risk factors for chronic noncommunicable diseases, such as arterial hypertension, hyperglycemia, dyslipidemia, hyperuricemia, and obesity [30]. It is known that these metabolic and hemodynamic risk factors for chronic noncommunicable diseases, which are also risk factors for cardiovascular diseases, are closely related within the metabolic syndrome [66]. Literature data indicate that metabolic syndrome significantly increases the risk of developing multiple diseases, primarily diseases of cardiovascular and endocrine systems, digestive system, as well as musculoskeletal system, which can serve as a pathogenetic basis for the formation of multimorbidity [46, 67, 68].

APPROACHES TO TREATMENT OF MULTIMORBIDITY

The existing problems and unfavorable trends associated with multimorbidity have necessitated creation of guidelines and national recommendations for management of multimorbid patients.

The American Geriatrics Society developed a concept of individual approach to an elderly patient with multimorbidity. The basic principles of the concept included defining the key role of the patient's values and priorities; building a relationship between the doctor and the patient, ensuring shared decision-making; and a coordinated approach to interdisciplinary care [69]. In this document, particular attention is given to optimization of treatment regimens. It is recommended to avoid polypharmacy in patients with multimorbidity, reduce the number of drugs, especially high-risk drugs, systematically review the list of drugs to eliminate drugs that people no longer need, and select drugs with minimal drug interactions [69].

In 2016, the National Institute for Health and Clinical Excellence (NICE) guidelines "Multimorbidity: Clinical Assessment and Management" were issued [70]. The NICE Guidelines for the management of patients with multimorbidity are based on other guidelines created to provide optimal management of patients with isolated diseases or conditions (that is, for patients without multimorbidity) that require regular medication. The main principle of these guidelines is a personalized assessment and development of an individual management plan; the purpose is to improve the patient's quality of life by reducing the treatment load and unplanned or uncoordinated care.

In accordance with these guidelines, when prescribing research and treatment to multimorbid patients, it is recommended to carefully consider all the risks and advantages of the methods recommended for the diagnosis and treatment of certain diseases and conditions on an individual basis and discuss the risks and advantages with the patient, taking into account their preferences. When analyzing the expected positive and negative effects of treatment methods (including drugs), it is recommended to use databases of treatment method effectiveness. The need to analyze all drugs and non-pharmacological methods of treatment that the patient is already receiving and assess which of them can be canceled and which new treatments are reasonable for prescription is noted. Separately, it is recommended to consider the likelihood of a slight potential benefit of some treatment methods aimed at improving the prognosis in the long term in patients with a limited expected life expectancy or fragility.

In the subsequent Recommendations on the tactics of managing patients with multimorbidity (JA-CHRODIS consensus, 2017), on the basis of expert discussions, 16 principles of providing medical care to patients with multimorbidity were formulated [71]. In this document, tactical and organizational issues of managing multimorbid patients were worked out in detail. Particular attention is paid to the need for a regular comprehensive assessment of the patient's condition, as well as interdisciplinary, coordinated work of a team of specialists.

On the initiative of Academician of the Russian Academy of Sciences, Professor R.G. Oganov, the Working Group developed Russian Clinical Recommendations "Comorbid Pathology in Clinical Practice" (2017) [46], and "Comorbid pathology in clinical practice. Algorithms for diagnosis and treatment" (2019) [13]. The approaches to diagnosis and treatment of multimorbid patients are set out in these Recommendations. Due to the fact that in modern clinical practice multimorbidity is based on chronic diseases, the influence on common risk factors for chronic noncommunicable diseases has been proposed as an effective therapeutic approach in multimorbidity. Among the modifiable risk factors, the largest number of associations with polypathies is noted for behavioral risk factors and obesity. In addition, by the examples of the most common combinations of diseases, it is recommended to conduct a thorough analysis of drug therapy for the underlying disease, taking into account the concomitant disease (the choice of drugs that do not adversely affect the course of the comorbid disease) [13, 46].

In connection with the need to minimize and optimize therapy for multimorbidity (by the example of the gastrointestinal tract disorders), the concept of multitarget therapy of comorbid disorders was developed. In accordance with this concept, in case of comorbidities, it is advisable to use drugs affecting the common links in the pathogenesis of associated diseases and leading to positive changes in the course of all components of comorbid pathology [13].

CONCLUSION

In the modern therapeutic clinic, there is a significant increase in multimorbidity, which significantly affects the course and outcome of many diseases. It is of great medical and social importance and determines the need for a detailed study of risk factors and features of its formation. Due to the prevalence and socioeconomic consequences of multimorbidity, this problem poses a challenge to healthcare in the XXI century. Scientists and specialists are focusing on the need to develop effective strategies for managing multimorbid patients, set treatment priorities in order to improve the quality of life, reduce unwanted interventions and consequences of polypharmacy, and create a network of health professionals.

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Dynamic changes in the tumor microenvironment under the effect of estradiol as a diagnostic tool and target for targeted cancer therapy

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ABSTRACT

Activation of the estrogen receptor-α (ER-α) signaling pathway is a significant factor in the initiation of carcinogenesis in various types of tumors due to the genomic and non-genomic effects of estradiol in cancer cells. However, data on the expression of ER-α and aromatase on stromal and immune cells in the tumor microenvironment (TME) point to an additional mechanism by which estrogens increase tumor malignancy. There is growing evidence that TME can affect tumor immunity by increasing the immune response or reducing immunoreactivity.

The important role of estrogen and the estrogen receptor signaling pathway in the response of the tumor microenvironment in cancer of various localizations, not only classical hormone-dependent cancers, has been proven. However, the clinical effectiveness of blocking the effect of estrogen on tumor growth has been primarily shown in cancer of the female reproductive system. At the same time, data on the significant role of TME in the development of endocrinotherapy resistance in breast cancer treatment are of great interest.

Despite the possibilities of standard therapy, a more in-depth study on the role of various TME components in cancer evolution, creation of a micrometastatic niche, as well as in the response to therapy may result in development of new strategies for cancer treatment. It is also necessary to study the possibilities of overcoming the immunosuppressive effect of the estrogen receptor signaling pathway on TME in order to increase the survival rates in patients with hormone-dependent cancers, particularly, breast cancer.

Keywords: estrogen receptor expression, tumor microenvironment, review, tumor-associated fibroblasts, T-lymphocytes, tumor-associated macrophages

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Динамические изменения опухолевого микроокружения под влиянием эстрадиола как диагностический критерий и мишень лекарственной терапии рака

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РЕЗЮМЕ

Активация сигнального пути эстрогенового рецептора-альфа (ЭР-α) является значимым фактором в инициации канцерогенеза при различных типах опухолей ввиду геномных и негеномных эффектов эстрадиола в опухолевых клетках. Тем не менее данные об экспрессии ЭР-α и ароматазы на стромальных и иммунных клетках в микроокружении опухоли (МО) говорят о дополнительном механизме, с помощью которого эстрогены повышают злокачественность опухоли. Появляется все больше доказательств того, что МО способно влиять на опухолевый иммунитет, повышая иммунный ответ или снижая иммунореактивность.

Доказано немаловажное значение роли эстрогена и ЭР-сигнального пути в реакции микроокружения опухоли при раке различных локализаций, не только классических гормонально-зависимых опухолей. Однако клиническая эффективность блокирования влияния эстрогена на рост опухолевых клеток доказана в основном при раке женской репродуктивной системы. При этом весьма интересны данные о значимой роли микроокружения опухоли в развитии резистентности к эндокринотерапии рака молочной железы.

Несмотря на возможности стандартной терапии, более углубленное изучение роли различных компонентов МО в эволюции опухоли, создании ниши микрометастазов, а также в ответе на терапию может привести к появлению новых стратегий лечения рака. Также необходимо изучить возможности преодоления иммуносупрессивного влияния сигнального пути рецептора эстрогена на МО с целью увеличения показателей выживаемости больных гормонально-зависимыми опухолями, в частности раком молочной железы.

Ключевые слова: экспрессия рецептора эстрогена, микроокружение опухоли, опухоль-ассоциированные фибробласты, Т-лимфоциты, опухоль-ассоциированные макрофаги

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Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

Estrogen receptor-α (ER-α) is a transcription factor regulating cell proliferation, migration, and survival. In the mammary gland, ER-α plays a key role in tumor growth, being activated by 17β-estradiol (E2). The estrogen receptor signaling pathway is based on dimerization of ER located in the nucleus in response to steroid hormone binding. Dimerized nuclear ERs bind to estrogen-sensitive elements (ESE) in the promoter regions of target genes, regulating their transcription [1].

In classical hormone-dependent tumors, ER expression as a prognostic and predictive marker is currently evaluated only on tumor cells. Therefore, the efficacy of blocking the ER-dependent signaling mechanism is analyzed from the standpoint of reducing the proliferative activity of malignant cells. So, endocrine therapy is the most effective treatment method for ER-positive breast cancer (BC). However, its effectiveness is limited by primary and acquired tumor resistance. Undoubtedly, resistance may be associated with ER expression loss, which is observed in 15–20% of patients with disease progression. However, ER mutations and amplifications are considered to be rare in the primary tumor and are detected only in 0.5% and 2.6% of luminal breast cancers, respectively. However, in metastatic foci, ER mutations in the ligand-binding domain of the receptor were noted in about 20% of cases [2].

It was found that endocrine therapy resistance can also provoke dynamic changes in the tumor microenvironment (TME). A lot of data have been accumulated on the significant role of TME components in treatment response, resistance, and tumor progression. Interactions between cancer-associated fibroblasts (CAFs), adipocytes, immune cells, endothelial cells, pericytes, extracellular matrix (ECM), and soluble factors lead to tumor evolution and disease progression. Therefore, the role of TME in cancer progression, as well as in a response of the tumor stroma to drug therapy in various tumors is intensively studied [3–6].

Resistance mechanisms independent of tumor cells have been studied in murine models of breast cancer. It was found that estradiol promotes the growth of tumor cells without ER-α expression due to the activation of stromal estrogen receptors [7].

TUMOR STROMAL COMPONENTS AS DIRECT PARTICIPANTS OF THE ER-SIGNALING MECHANISM MODULATION

Cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs) are the most numerous cells in the tumor stroma. They act as a paracrine source of chemokines, soluble factors for cell activation of signaling pathways involved in cancer cell survival, invasiveness, and metastasis [8]. In breast cancer, ER expression was detected on CAFs, and in response to estradiol, they secrete growth and angiogenesis factors, as well as immunoregulatory and proinvasive soluble factors. It was found that soluble factors from CAF conditioned medium induce tamoxifen resistance in murine models of ER-positive cancers. This may be due to CAF secretion of growth factors, proteases, and the b1 integrin signaling pathway activation in response to endocrine therapy [9].

Despite similar ER expression level on normal fibroblasts and CAF, E2-sensitive genes and LRH-1 (liver receptor homolog-1) are hyperactive in CAF [8]. *LRH-1* is a target gene for E2 and a transcriptional regulator of the aromatase gene (*CYP19A1*) [10]. Aromatase is co-expressed on breast cancer cells with LRH-1, which indicates the paracrine mechanism of E2 synthesis and the role of TME in estrogen-mediated carcinogenesis in breast cancer. Endometrial CAFs also express ER and can promote tumor proliferation when co-cultured with human endometrial cancer cells. They induce *in vitro* tumor cell proliferation partly by activating PI3K and MAPK signaling pathways, which are regulated by an ER-dependent signal in breast cancer and lung cancer [1].

When studying human CAF co-cultured with MCF-7 breast cancer cell lines, two CAF subpopulations were identified based on different CD146 expression in breast cancers. CAFs without CD146 expression inhibit the ER expression on MCF-7 cells, reduce their sensitivity to estrogen, and increase tamoxifen resistance. The presence of CD146+ CAF stimulated ER expression and supported estrogen-dependent proliferation and sensitivity to tamoxifen. Conditioned medium of

CD146+ CAF restored sensitivity to tamoxifen in breast cancer cells which were resistant to it. Gene expression profile of breast cancer patients with CD146− CAF correlated with a decrease in the frequency of a clinical response and a worse disease prognosis [11].

M.M. Morgan et al. showed an increase in ER signaling activation in the presence of 17β-estradiol, while co-culturing MCF-7 cells with human breast fibroblasts in a 3-dimensional model. The addition of fibroblasts increased proliferation rate and caused estrogen-induced hyperplasia, which was explained by the inhibition of apoptosis during co-culture [12]. According to other data, dermal fibroblasts and bone marrow mesenchymal stem cells also affected ER-signaling pathway regulation in MCF-7 and T47D cells [10, 13]. In the first case, the authors showed that CAF induce resistance to tamoxifen by increased mitochondrial activity in breast cancer cells. In the second case, the paracrine stromal signaling mechanism led to a decrease in the activity of the ER signaling pathway in MCF-7 and T47D cells. The transfer of exosomes from stromal cells to breast cancer cells is the mechanism of resistance to endocrine therapy. The transfer of microvesicles containing OncomiR-221 microRNA from CAF to a breast cancer cell induces expansion of cancer stem cells (CSCs) with an increased self-renewal ability and resistance to endocrine therapy. It was previously shown that tamoxifen led to an increase in the number of CSCs in breast cancer in murine and human models of breast cancer and in the patient's primary tumor.

The question of different origin of the luminal and basal-like types of breast cancer remains important. A high degree of plasticity between these types of tumors is assumed. The transformation of luminal or basal-like cancers into each other under the influence of the microenvironment was demonstrated *in vitro*, which indicates the relationship with the progenitor cell [14]. PDGF receptors and their ligands are important factors that modulate the molecular type of tumor and the response to antiestrogenic therapy. It was established that platelet-derived growth factor (PDGF-CC) is an independent prognostic marker of poor survival in breast cancer.

Using xenografts of triple-negative breast cancer of MDA-MB-231 cell lines orthotopically inoculated into an immunodeficient mouse, an increase in ER-α expression was shown with a decrease in activity or inhibition with a response to tamoxifen. CAFs of all analyzed tumors expressed PDGFR-α and PDGFR-β, which indicates the paracrine type of PDGF-CC signaling mechanism from the epithelium to the stroma. In response to the activation of the PDGF-CC signaling cascade, CAFs secrete HGF, IGFBP3, and STC1 molecules that induce the formation of the luminal breast cancer phenotype [15, 16]. Thus, CAFs act as determinants of the molecular subtype of breast cancer and represent a promising target for therapy that modulates the tumor epithelial component [15].

Myeloid-derived stromal cells

Myeloid-derived stromal cells (MDSCs) are an important component of TME, affecting immune tolerance and promoting tumor development [17]. ER-α expression was also detected on tumor MDSCs, as well as in the bone marrow and peripheral blood in ovarian cancer. In a murine model of E2-dependent ovarian cancer, ovariectomy led to an increase in overall survival. At the same time, E2 caused tumor progression and reduced the effect of ovariectomy. This effect was observed only with normal immune status. However, the immunodeficient mouse did not benefit from ovariectomy due to the absence of T cell infiltration of the tumor.

Thus, it was found that the antitumor effect eliminating the influence of E2 is realized by modulating acquired immunity. When E2 was prescribed, T helper cells and cytotoxic T lymphocytes decreased, but the number of MDSCs in the spleen and tumor niche increased. At the same time, the immunosuppressive activity of granulocytic MDSCs was increased. In a murine model of ovarian cancer, exposure to E2 in the peritoneal cavity increased activation of signal transducer and activator of transcription 3 (STAT3), which is a signaling mechanism that regulates myeloid cell differentiation and development, by transcriptional regulation of JAK2 and SRC. Similar data were obtained in murine models of lung cancer and breast cancer. Under E2 stimulation, tumor growth stopped when MDSCs were affected by antibodies to Gr1 [18].

Extracellular matrix

Extracellular matrix (ECM) is an important component of the tumor tissue, which plays an essential role in tumor progression and treatment resistance in various cancers, including breast cancer. It was established that the tumor is a non-healing wound, since the host microenvironment receives signals for tissue repair by formation of fibrous tissue. Breast cancer progression is accompanied by stromal changes and the appearance of stiffness. The basement membrane of a normal mammary gland clearly separates the epithelial component from the stroma. Laminin, type IV collagen, fibronectin, and entactin are the main components of the basement membrane which are produced by the epithelium, endothelium, and stromal cells. Interstitial ECM consists of collagen fibrils, fibronectin, glycoproteins, and proteoglycans [19].

The biochemical characteristics of ECM make it possible to modulate the cellular response to various soluble factors, such as hormones, polypeptide growth factors, and chemokines. Malignant breast tissue becomes stiff, which is due to a change in the biochemical properties of ECM. ECM remodeling involves continuous synthesis of matrix proteins, their connection, interaction, and cleavage by proteases. This leads to an increase in ECM stiffness, which is a consequence of increased collagen deposition following lysyl oxidase (LOX) expression and parallel orientation of collagen fibers [1]. The reorganization of collagen into thin, linearly oriented fibers correlates with tumor progression and the clinical outcome, and increased ECM stiffness may also be the cause of certain cancer types [20].

M.P. Jansen et al. showed an association of the ECM gene cluster with ER-positive breast cancer progression in patients taking tamoxifen. The authors analyzed 112 ER-positive locally advanced tumors in patients with breast cancer and identified types of responses to tamoxifen therapy. Differences in the expression of 91 genes between tumors sensitive and resistant to tamoxifen were noted. Overexpression of the ECM genes *TIMP3, FN1* (fibronectin 1), *LOX, COL1A1* (collagen type 1 alpha 1 chain), *SPARC,* and *TNC* (tenascin C) was associated with progression of the disease in all cases [21].

Another study focused on these 6 genes and examined 1,286 tumor samples. The level of mRNA expression was associated with disease progression. The results showed that high expression of FN1, LOX, and SPARC was associated with low metastasis-free survival rates in patients who received adjuvant systemic therapy [22]. Studies have shown that fibronectin is associated with breast cancer progression. In breast cancer cell cultures, fibronectin led to endocrine therapy resistance via binding to beta-1 integrin. It was found that upon contact with fibronectin, ER-α activity does not decrease after 1 hour of exposure to estradiol. Estradiol induces endocytosis in breast cancer cells, and the ER-α of the cell membrane in the form of endosomes is directed to the nucleus. Pharmacological or biological endocytosis inhibition led to ER transcriptional activity inhibition.

Therefore, in the presence of fibronectin, ER-α undergoes endocytosis and is brought back to the cell surface by beta-1 integrin. So, ER-α is not co-localized with the lysosomal component, so it is obvious that the interaction of beta-1 integrin with fibronectin determines the fate of ER and the response to tamoxifen. Thus, ECM directly regulates the effect of the estrogen signaling mechanism on breast cancer cells [23].

INFLUENCE OF THE ER SIGNALING MECHANISM ON THE INFLAMMATORY RESPONSE AND TUMOR IMMUNE MICROENVIRONMENT

Numerous studies have confirmed the significant role of chronic inflammation in tumor progression. TME produces cytokines that activate protumorigenic proliferation pathways, leading to immune evasion and metastasis. The proinflammatory cytokine interleukin (IL)-6 increases growth and invasiveness of ER-positive breast cancer. Local CAFs act as paracrine sources of IL-6, activating STAT3 and ER-positive tumor cell proliferation *in vitro* and *in vivo* [24].

Tumor necrosis factor (TNF) regulates the expression of genes associated with the metastatic phenotype of ER-positive breast cancer and increases aromatase expression in cultured human adipose-derived stromal cells. An association between aromatase transcription and TNF and IL-6

cytokines in breast cancer was also found. A similar correlation was observed between aromatase and cyclooxygenase-2 (COX-2). COX-2 affects prostaglandin E2 (PGE2) synthesis, which causes an increase in aromatase transcription by elevating the concentration of cyclic adenosine monophosphate (cAMP) in breast cancer [25].

Significant correlations were found between the expression of ER, TNF, and NF-kB in breast cancer. The NF-kB signaling pathway is involved in the initiation of tumor growth and inflammation. Activation of NF-kB is observed in some types of cancer and is associated with the profile of IL-6 and TNF cytokines. Binding of DNA to NF-kB and activator protein-1 is associated with resistance to antiestrogens in ER-positive breast cancer cell lines and tissue samples [26]. Moreover, exposure to E2 in a murine model of tobacco-induced lung cancer increased the inflammatory response through increased activation of the NF-kB signaling pathway and expression of VEGF and IL-17A. A combination of aromatase inhibitor and NSAIDs prevented lung carcinogenesis in mice, reducing the activity of STAT3 and MAPK signaling pathways, the level of circulating IL-6, and expression of IL-17A. Thus, a relationship between the E2 signaling pathway and regulators of tumorigenic inflammation is obvious. This opens up promising strategies for targeted cancer therapy through additional E2 signal inhibition [27].

Involution of breast tissue after pregnancy and obesity are serious risk factors associated with inflammatory TME and breast carcinogenesis. After pregnancy and subsequent involution of the mammary glands after lactation suppression, the risk of developing cancer increases within 10 years. A high risk of breast cancer and a poor prognosis of this cancer type are associated with inflammatory mediators involved in the involution process. Nevertheless, ER expression status is not discussed at this point. Some studies have found low incidence of ER-positive tumors in this cancer type. Other data indicate a decrease in the estrogen and progesterone receptors due to a high estrogen level [28].

Postmenopausal period and obesity are associated with an increased risk of developing ER-positive breast cancer, endometrial cancer, and tamoxifen resistance. As a result, the risk of relapse increases during endocrine therapy [29]. This can be explained by the fact that a characteristic feature of inflammation is recruitment of macrophages to the adipose tissue. Adipocytes and macrophages trigger activation of the proinflammatory transcription factor NF-kB. The degree of macrophage infiltration is also associated with the development of resistance to tamoxifen. Research data suggest that tumor-associated macrophages (TAMs) protect cancer cells from an antitumor immune response [30].

Tumor-associated macrophages

Macrophages isolated from humans and mice are able to suppress the T cell response *in vitro*, and removal of macrophages leads to an increase in the number of CD8+ T cells in a breast cancer model when exposed to chemotherapy. It was shown that circulating M2-like monocytes were elevated in this population in comparison with healthy volunteers and patients with benign lesions. Another study revealed an association between CD204 expression on TAM and clinical and pathohistological characteristics in patients with invasive breast cancer [1, 31]. In a study involving 108 patients with luminal breast cancer subtypes, high CD204 expression was associated with a decrease in relapse-free survival and long-term event-free survival [32].

The immune response involving macrophages is tissue-specific and depends on local TME polarization by various cytokines. Polarized M1 macrophages produce proinflammatory cytokines, including interferon (IFN), IL-12, and TNF to trigger the tumor immune response and antigen presentation. M2-macrophages produce type 2 cytokines – IL-4, IL-5, IL-6, and IL-10, which promote tumor growth and cause immune evasion. TAMs are represented by the M2 phenotype, being a promising target for drug therapy. TAM infiltration is observed in a large number of various cancer types and is associated with a poor prognosis [1, 33]. In ER-positive cancer, premenopausal patients show an increase in the number of TAMs compared with postmenopausal women. While TAM infiltration was associated with a poor prognosis in ER-positive and ER-negative breast cancer, an increase in TAMs with their proliferation is more often detected in hormone receptor-negative tumors [34]. However, M1 polarization was not observed in these studies, as opposed to M2 polarization. In addition, the immunohistochemical analysis of

breast cancer samples showed aromatase expression in TAM, indicating local production of E2 in TME and increased proliferation of cancer cells in ER-positive tumors.

There is evidence that E2 is able to induce M2 polarization and TAM infiltration. In a murine model of ER-positive cancer, E2 increased infiltration of TAMs with the M1 phenotype, and in the control group, infiltration of CAFs with the M1 phenotype was shown. E2 also increased secretion of VEGF by M2-polarized TAMs, VEGFR expression, and the content of macrophages in murine lungs during tobacco-associated carcinogenesis [35–37].

A study of the tumor growth in high-grade serous ovarian carcinoma induced by E in a murine model found that E2 not only enhanced tumor growth, but also increased M2 CAF infiltration compared with an untreated ovariectomized mouse [33]. It is known that endometrial M2-polarized TAMs affect ER activation through epigenetic regulation and IL-17A secretion, increasing E2-associated proliferation of endometrial cancer cells [38].

Thus, a potential positive feedback between the ER-signaling pathway and M2 CAF infiltration has been identified in certain cancer types. These connections may become a therapeutic target. Recent studies on lung cancer xerographs confirmed an effect of the phytoestrogen resveratrol similar to that of selective estrogen receptor modulators (SERMs). Resveratrol reduces tumor growth by inhibiting M2 TAM polarization and decreases STAT3 signaling pathway activation [39].

Tumor-infiltrating CD4+/CD8+ T lymphocytes and natural killer cells

The composition of TME lymphocytes varies significantly depending on the type of cancer. At the same time, they promote tumor progression or activate antitumor immunity, depending on the primary tumor. The CD4+ T cell polarization is one of the evading immune mechanisms. Th1-mediated cellular response is associated with tumor suppression and hyperactivation of INF and IL-12. The Th2-associated cellular response is based on IL-4 production and implements a protumorigenic effect. Studies have noted that increased E2 induces Th2-type response and increases IL-4 production. An increase in the infiltration of ER-negative breast cancer by immune Th1 cells, B cells, and cytotoxic T lymphocytes was revealed in comparison with ER-positive tumors.

Thus, a negative relationship was noted between the ER-α signaling pathway and immune infiltration. An increased number of tumor-infiltrating lymphocytes, in particular CD8+ T cells, significantly increases overall survival of patients with ER-negative tumors [40]. Analysis of gene expression in ER-positive cancers showed that treatment with the aromatase inhibitor letrozole increased tumor infiltration with B cells and T helper cells both at early and late stages of treatment [41].

Granule exocytosis is one of the ways to initiate apoptosis by cytotoxic T lymphocytes and natural killer (NK) cells to fight pathogens and tumor cells [25]. CD8+ cytotoxic T cells play an important role as effectors in acquired immunity. Cells expressing a foreign antigen in association with the main histocompatibility complex (MCH I) are recognized by cytotoxic T lymphocytes through a specific interaction between the T cell receptor and the presented antigen. This interaction causes the activated T cells to release the proteins perforin and granzyme B, resulting in lysis of the cell membrane and cell death. These mechanisms may affect malignant cells due to atypical antigen presentation.

X. Jiang et al. co-cultured ER-positive hepatocellular carcinoma cells with E2, and the expression of granzyme B inhibitor, proteinase-9 inhibitor (PI-9), increased. This mechanism protected cells against NK-related and cytotoxic apoptosis [42]. E2-induced expression of PI-9 was also detected in the culture of ER-positive MCF7 cells, which decreased the influence of NK-cells. Elimination of PI-9 blocking reduced the protective effect of E2 from NK-mediated apoptosis. Thus, E2 enhanced immunosuppression through inhibition of NK and cell death mediated by cytotoxic T lymphocytes [43].

Analysis of 12,439 tumor samples, of which 8,775 were ER-positive, showed that intratumoral CD8+ T cells were associated with a 27% decrease in the risk of death from breast cancer [44]. The analysis of PD-L1 expression revealed that 20% of ER-positive tumors show positive marker expression in comparison with 58% of patients with triple-negative breast cancer phenotype [45, 46].

Regulatory T lymphocytes

Activation of T cells and their differentiation are mandatory in acquired immunity. Regulatory (T-reg) T cells expressing FoxP3 are involved in suppressing the antitumor immune response by secreting immunosuppressive cytokines and inhibiting T cell expansion [47]. Physiological doses of E2 increased the amount of CD4+CD25+ T-regs and Foxp3-expression in many tissues of immunodeficient mice after ovariectomy. At the same time, ER-positive CD4+CD25-negative cells acquired the ability to express CD25 after exposure to E2. The CD4+CD25+ T cells transformed by estradiol acquired the immunosuppressive T regulatory lymphocyte phenotype and inhibited proliferation of T cells *in vitro*. It was also found that Foxp3 expression by murine estradiol-stimulated T-regs was critically important for their functioning. And an increase in the number of FoxP3+ T-regs in patients was a predictor of a poor prognosis in various types of cancer [25, 48].

The results of a meta-analysis indicate that FoxP3+ T-reg infiltration significantly correlates not only with poor overall survival in ER-positive breast cancer, but also with higher survival rates in ER-negative breast cancer patients [49]. When prescribing letrozole to patients with ER-positive breast cancer, a significant decrease in FoxP3+ T-regs was shown after therapy [1]. E2 also stimulates *in vitro* expression of the programmed death ligand (PD-L1) on ER-positive endometrial cells and breast cancer cells through PI3K signaling pathway activation. The interaction between cells with PD-L1 expression and PD-1positive T cells inhibits recruitment of cytotoxic T cells, which leads to immune evasion. Data on E2 hyperactivation of both PD-L1 and PD-1 suggest a critical impact of the E2 signaling pathway on the PD-1 / PD-L1 signaling mechanism [50, 51].

Targeting the ER-signaling pathway in tumor microenvironment as a way to increase tumor immunoreactivity

Despite the fact that immunotherapy is an effective treatment strategy for cancer, often the immunosuppressive microenvironment reduces its possibilities. Immune checkpoint inhibitors (CTLA4) and PD-1 / PD-L1 are currently the most discussed drugs. However, the response rate to treatment remains about 20–35% with varying response duration depending on the stage, tumor type, and PD-L1 expression [25, 52]. In addition, during treatment, resistance to therapy and disease progression may occur [31, 32]. It was found that impaired DNA repair mechanisms and an increase in somatic mutation load and neoantigen presentation correlate with tumor heterogeneity and better clinical outcomes [1, 53]. Mechanisms leading to immune evasion include impaired ability to antigen presentation and decreased neoantigen presentation by MHC-1 [54, 55].

It should be noted that the described mechanisms can serve as potential predictors of a response to treatment with immune checkpoint inhibitors and a target for increasing the effectiveness of breast cancer therapy. Despite the data on the clinical effectiveness of anti-PD-1 / PD-L1 therapy for metastatic triple-negative breast cancer, it was found that the objective response frequency does not exceed 12%, and the clinical response duration of more than 24 weeks is observed in 20% of cases [56, 57]. The immune-suppressive TME may be the biological explanation for such disappointing results.

When analyzing 61 ER-positive cancer samples of primary breast cancer, C.A. Egelston et al. found that tumor-infiltrating CD8+T lymphocytes have a weakened ability to produce effector cytokines and degranulation capacity, despite PD-1 expression. In addition, the ability of CD8+ T lymphocytes treated with CD3:CD19 bispecific antibodies to influence breast cancer cells as effectively as peripheral blood mononuclear cells was shown [58].

In studies on cell lines, ER-α acts as a negative transcription regulator of the *PD-L1* gene. Moreover, TCGA data in the breast cancer sample analysis showed that the PD-L1 mRNA level in ER-positive tumors was significantly lower than in ER-negative tumors [59]. There are also data indicating a positive effect of blocking the ER-signaling mechanism on the increase in TME immunoreactivity. For example, in lung cancer, the antiestrogen fulvestrant increases tumor sensitivity to immune-mediated lysis [60]. Fulvestrant is an ideal candidate for combined use with anti-PD-1 / PD-L1 agents due to its proven safety and a lack of cross-toxicity. This strategy may improve the immediate and long-term results of cancer immunotherapy [61].

CONCLUSION

Literature data indicate the important role of estradiol and activation of the ER-signaling mechanism in TME, which provokes immunosuppression and tumor progression. These features have been identified in various cancers and are not limited to tumors of the female reproductive system. Nevertheless, in clinical practice, the study of estrogen and antiestrogenic effects on TME is of the greatest value in classical hormone-dependent tumors.

The results of studies showing that antiestrogen therapy has the potential for a reversible effect on immunosuppressive TME due to a pronounced response in hormone-dependent tumors are promising. However, a significant role of TME in resistance to endocrine therapy, particularly in luminal breast cancer, was revealed. These data will further lead to expansion of the panel of predictive and prognostic molecular markers of malignant diseases with mandatory identification of potential drug therapy targets in TME.

The relationship between the tumor immune response and the ER-signaling mechanism of TME also opens up prospects for improving the effectiveness of immune checkpoint inhibitors and overcoming resistance to hormones. There is a need to standardize the method for detecting the expression of ER and aromatase in TME, taking into account the possibilities of activating the immune response through combined use of antiestrogens and immune checkpoint inhibitors. In addition, studies should focus on gender differences and demographic data, including menopausal status and body mass index with information on the degree of obesity, to clarify the degree of E2 involvement in tumor immunity.

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Implantable cardioverter-defibrillators in sudden cardiac death prevention: guidelines and clinical practice (literature review)

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ABSTRACT

Implantable cardioverter-defibrillators (ICDs) are considered to be the most beneficial in preventing sudden cardiac death (SCD), especially in patients with reduced left ventricular ejection fraction (LVEF). However, major large-scale randomized clinical trials on ICD effectiveness were conducted 20 years ago and do not reflect current realities. Modern ICDs and methods for treating heart failure have drastically improved. New clinical reality requires reconsideration of approaches to determining the risk of SCD and indications for ICD, personalization of device selection and programming, and identification of barriers that prevent ubiquitous use of the method in real clinical practice.

The article reviews the available evidence base on the use of ICDs, current clinical guidelines, complications following the device implantation, and any difficulties associated with ICD application in routine clinical practice.

Keywords: implantable cardioverter-defibrillators, sudden cardiac death, prevention, clinical practice

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Имплантируемые кардиовертеры-дефибрилляторы в профилактике внезапной сердечной смерти: современные рекомендации по применению и реальная клиническая практика (обзор литературы)

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РЕЗЮМЕ

После завершения основных крупномасштабных рандомизированных клинических исследований около 20 лет назад, имплантируемые кардиовертеры-дефибрилляторы (ИКД) являются основой профилактики внезапной сердечной смерти (ВСС), особенно у пациентов с низкой фракцией выброса левого желудочка. За прошедшее время эволюционировали как сами устройства, так и методы лечения сердечной недостаточности. Новые медицинские реалии требуют пересмотра существующих подходов к определению риска ВСС, показаний для ее профилактики с помощью ИКД, индивидуализации выбора и программирования устройства, а также объективизации проблем, ограничивающих широкое применение метода в реальной клинической практике.

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В обзоре рассматриваются существующая доказательная база использования ИКД и позиции современных клинических рекомендаций, проблемы, возникающие после установки ИКД и пути их решения, а также вопросы применения ИКД в реальной клинической практике.

Ключевые слова: имплантируемые кардиовертеры-дефибрилляторы, внезапная сердечная смерть, профилактика, клиническая практика

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

Sudden cardiac death (SCD) is one of the most common causes of death worldwide, including young and able-bodied individuals. According to recent data, SCD accounts for 15–20% of all deaths worldwide [1]. Coronary artery disease (CAD) is known to be the most common pathology underlying SCD, followed by cardiomyopathies, inherited arrhythmia syndromes, and valvular heart diseases [2, 3].

During the past 3 decades, declines in SCD rates have not been as steep as for other causes of CAD deaths, with a growing fraction of non-ischemic SCD particularly among young population [4]. Although the effectiveness of prehospital resuscitation methods is improving throughout the world, the majority of individuals with sudden cardiac arrest will not survive, which makes prevention of SCD a highly relevant issue [1]. The mainstay of primary and secondary prevention of SCD is implantable cardioverter – defibrillator (ICD), since in 80% of cases the causes of sudden cardiac arrest are ventricular arrhythmias (VA), such as ventricular tachycardia (VT) or ventricular fibrillation (VF) [4].

EVIDENCE BASE FOR USING IMPLANTABLE CARDIOVERTER – DEFIBRILLATORS AND MODERN CLINICAL GUIDELINES

First implanted in individuals who experienced cardiac arrest due to VF, ICDs have been in use since 1980 [4]. Current guidelines for ICDs are based on the research data from clinical trials, such as Cardiac Arrest Study Hamburg (CASH); Canadian Implantable Defibrillator Study (CIDS), and Antiarrhythmics Versus Implantable Defibrillators (AVID) study, which have shown the benefits of ICDs compared with antiarrhythmic drug therapy, including amiodarone [5–7]. The total number of patients included in these randomized controlled trials (RCTs) was 1,963, the average follow-up was 3 years. However, all three trials were completed before 2005, therefore, they do not reflect the clinical realities of improved CAD and heart failure (HF) treatment.

According to the meta-analysis performed by S.J. Connolly et al., the use of ICDs for secondary prevention demonstrated a 50% decrease in the risk of SCD and a 28% decrease in the overall mortality [7]. Although secondary prevention ICDs proved to be more effective in patients with severe left ventricular (LV) dysfunction, all current guidelines recommend secondary prevention of SCD in case of VF / hemodynamically unstable VT in the medical history, irrespective of the left ventricular ejection fraction (LVEF).

The Russian Scientific Society of Clinical Electrophysiology, Arrhythmology, and Cardiac Pacing (2017) guidelines on the use of pacemakers, ICDs, cardiac resynchronization therapy devices, and implantable cardiac monitors have six indications for secondary prevention ICDs:

1) diagnosed VF or VF with adverse hemodynamic effects;

2) syncope of unknown origin, clinically similar to hemodynamically unstable VT or VF induced during an electrophysiology study (EPS);

3) unstable VT due to prior myocardial infarction (MI) with LVEF < 40% and sustained VT or VF induced during EPS;

4) sustained VT with LVEF < 45%, irrespective of a possibility to perform catheter ablation and its results;

5) recurrent sustained postinfarction VT with normal LVEF;

6) recurrent sustained non-coronarogenic VT, in case its eradication is unavailable [8].

To perform the implantation, the following conditions must be met: no transient causes of VA; 48 hours passed after MI; the patient receives optimal drug therapy (ODT); the predicted life expectancy of the patient exceeds 1 year [8]. The first recommendation is based on the findings of the three RCTs – CASH, CIDS, AVID [5–7] and corresponds to class IA indications. The second recommendation is based on the results of CIDS [6], in which one of the inclusion criteria was sustained VT with syncope. The third recommendation is based on the findings of the study performed by A.E. Buxton et al. on prevention of SCD in patients with CAD [9].

The study included 704 patients with MI, LVEF < 40%, and induced asymptomatic VT. The ICD therapy resulted in significant reduction of the SCD risk compared with standard therapy (odds ratio (OR) 0.24; 95% confidence interval (CI) 0.13–0.45; $p < 0.001$). The remaining recommendations mentioned are based on the expert consensus. The European Society of Cardiology (ESC) and the American Heart Association (AHA) take a more rigorous and reasonable approach to determining indications for secondary prevention ICDs in their guidelines [10, 11].

The ESC guidelines (2015) contain only two indications for secondary prevention ICDs: diagnosed VF or hemodynamically unstable VT in the absence of reversible causes of VT / VF (excluding the first 48 hours after MI, class IA) and recurrent sustained VT (excluding the first 48 hours after MI). In all these cases, patients should be on long-term optimal drug therapy and their life expectancy should exceed 1 year [10].

Thus, for secondary prevention ICDs, there is only one clearly defined class IA indication, based on the RCTs performed 10–15 years ago, while all other recommendations are based mainly on the expert consensus, confirming once again the existing gaps in the evidence base [10–12]. Subsequent RCTs focused on the effectiveness of ICD in patients at high risk of SCD as a primary prevention method, provided that there are no other diseases limiting the life expectancy to 1–2 years.

The guidelines for primary prevention are based on data of three relatively recent studies (Multicenter Autonomic Defibrillator Implantation Trial II (MADIT II), Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT), and Multicenter Unsustained Tachycardia Trial (MUSTT)). They demonstrated an increase in the life expectancy by an average of 2–6 years in ICD patients with reduced ejection fraction (rEF) and symptomatic HF compared with amiodarone-treated patients (amiodarone did not improve the prognosis) [13–15]. However, patients with reduced LVEF are still included in the primary prevention ICD group despite a lack of data on the impact of ICDs on the heart failure (HF) and non-sudden cardiac death [10–12].

Patients with CAD have an increased risk of SCD due to VA, especially with rEF. Two RCTs included patients with stable CAD prior to MI and reduced LVEF. The first study, MADIT II, included patients with NYHA class I–III HF and LVEF ≤ 30%. The follow-up period was 20 months, all-cause mortality was 14.2% in the ICD group, as opposed to 19.8% in the control group, with relative risk reduction of 31% [13]. The second study, SCD-HeFT, included patients with NYHA class II–III chronic HF and LVEF ≤ 35%. After 5-year follow-up, the absolute risk of death in the group with ICD was 7%, with relative risk reduction of 23% [14].

In the MUSTT study, all patients underwent an EPS for the induction of sustained VT, and 353 patients with induced VT were randomized into two groups – an antiarrhythmic therapy group and a placebo group. According to the results of 5-year follow-up, a significant decrease in SCD and all-cause mortality in the antiarrhythmic therapy group compared to the placebo group was revealed. However, a detailed analysis identified that the statistically significant reduction in mortality concerned only the ICD patients [15].

The clinical trials showed that ICDs had no positive impact on mortality in case the device was implanted in the early postinfarction period or during cardiac surgery [16, 17]. The results of a meta-analysis of RCTs in CAD patients (excluding studies in which the device was implanted during surgery or cardiac surgery) indicate a statistically significant

reduction of the risk of all-cause mortality with ICD by 24% compared with non-ICD therapy in this group [18]. Thus, cardiologists in Europe, the United States, and the Russian Federation have identical guidelines for primary prevention ICDs in patients with CAD, NYHA class II – III HF, and LVEF ≤ 35% after at least 3 months of optimal drug therapy and not earlier than 40 days after MI, if life expectancy exceeds 1 year [10–12].

The evidence base underlying guidelines for ICD therapy for non-ischemic HF is not as considerable as that for ischemic HF. Guidelines are based on the findings of Defibrillators in Non-Ischemic Cardiomyopathy Treatment Evaluation (DEFINITE) [19], SCD-HeFT [14], Cardiomyopathy Trial (CAT) [20], and Amiodarone Versus Implantable Cardioverter – Defibrillator: Randomized Trial in Patients With Non-ischemic Dilated Cardiomyopathy and Asymptomatic Nonsustained Ventricular Tachycardia (AMIOVIRT) [21] and a large meta-analysis performed by A.S. Desai et al. with a total of 1,854 patients [22]. Data from the DEFINITE study, which included 458 patients with non-ischemic HF, LVEF ≤ 35%, and Holter monitoring showing unstable VT, showed a statistically significant decrease in the SCD prevalence after 29-month follow-up, but no decrease in the all-cause mortality [19].

In the SCD-HeFT study mentioned above, nearly half of the patients had a non-ischemic HF [114]. The CAT and AMIOVIRT studies showed no statistically significant difference in survival of the patients from the ICD therapy and control groups [20, 21]. However, the meta-analysis of performed by M.J. Shun-Shin et al., which incorporated all the studies mentioned above, showed decreased all-cause mortality rate in the ICD group compared with the control group (OR 0.69; 95% CI 0.55–0.87; $p = 0.002$) [18].

The results of the Defibrillator Implantation in Patients with Non-Ischemic Systolic Heart Failure (DANISH) study (which included 1,116 patients with non-ischemic HF, LVEF less than 35%, and NYHA class II-IV HF, the average follow-up was 67.6-months) showed that ICD therapy had no positive impact on the all-cause mortality (the ICD group had 4.4 cases per 100 person – years versus 5.0 per 100 person – years in the control group, the differences were not significant). However, the frequency of SCD in the ICD group was 2 times lower. The main cause of HF was idiopathic cardiomyopathy (76% of cases). Moreover, this RCT showed that the benefits of ICD lessen with age and become minimal in elderly patients (68 years and older) [23]. In contrast to DEFINITE and SCD-HeFT, 96% of patients in the DANISH study were receiving renin – angiotensin – aldosterone system blockers (RAAS), 92% – beta-blockers, more than half – aldosterone antagonists (doses titrated sufficiently to achieve target levels), and 58% of patients in both groups received cardiac resynchronization therapy (CRT) for HF, which could have affected the results [14, 19, 23].

Despite the results of the DANISH study, a meta-analysis performed by L. Shen et al. showed that primary prevention ICDs in patients with non-ischemic HF are associated with statistically significant benefits in terms of survival, which are identical to those observed in patients with ischemic HF [24]. The current ESC, AHA, and Russian Clinical Guidelines indicate primary prevention ICDs for patients with non-ischemic HF, in case the following conditions are met: LVEF ≤ 35%, NYHA class II–III HF, patients after 3 months of optimal drug therapy, and a predicted life expectancy of more than 1 year [10–12].

There are a number of rare, genetically determined disorders associated with a high risk of SCD, such as hypertrophic cardiomyopathy (HCM), arrhythmogenic right ventricular dysplasia (ARVP), Brugada syndrome, long QT syndrome (LQTS), etc. RCTs on the effectiveness of ICD therapy in the prevention of SCD in these diseases have not been conducted. Currently, HCM is the main cause of SCD among young population [25].

According to the Russian Scientific Society guidelines, ICD therapy in HCM is indicated in patients with an estimated 5-year risk of SCD > 6% (IIA, B) or with the predicted benefit of ICD in the long term (IIB, B) [8]. These recommendations are based on the findings of two retrospective, cohort, observational studies that showed higher mortality, frequent inappropriate shocks, and complications in patients with HCM [26, 27]. The HCM Risk – SCD risk prediction model is used for 5-year SCD risk estimation, it establishes a non-linear relationship between the risk of SCD and the maximum left ventricular wall thickness. The effectiveness of this predictive model is being constantly improved [27].

In the latest Russian guidelines for sudden cardiac death risk assessment and prevention (2018), ICD placement due to HCM is indicated for patients with a predicted 5-year SCD risk of ≥ 4%, calculated using the HCM Risk – SCD model (class IB), as well as for patients with at least one major risk factor (IIA, B) [25].

Arrhythmogenic right ventricular dysplasia is an indication for secondary prevention ICDs in case of severe dysfunction of one or both ventricles (class IB) and in the presence of risk factors (syncope, moderate ventricular dysfunction, episodes of unstable VT, (IIA, B)) [8]. The basis for these indications was the result of the meta-analysis performed by A.F. Schinkel, which included 24 small studies and a total of 610 patients (average age 40.4 years; 42% of patients were women) with ARVP and primary / secondary prevention ICDs. The author noted a reduced risk of overall and cardiac mortality in the ICD group [28]. However, it should be noted that the ICD group had higher frequency of myocardial perforation [25].

The number of patients with LQTS is increasing worldwide [29]. P.J. Schwartz et al. in a prospective analysis of 233 patients with ICD and LQTS (41% of cases – secondary prevention ICDs) showed that within 4.6 years, 28% of patients received appropriate shocks, and 25% had complications associated with the device [29]. Predictors of appropriate ICD shocks included: age younger than 20 years, prior cardiac arrest, and a prolonged QTc (greater than 500 milliseconds). Appropriate shocks were not observed in the absence of these factors. The authors concluded that it is necessary to specify the criteria for ICD placement and consider other existing treatment options [29].

According to the recommendations of the Russian Scientific Society, in patients with LQTS, ICD placement is indicated after cardiac arrest (class IB), in case of syncope or unstable VT with prescribed beta-blockers (IIA, B), and with history of SCD in the family (IIC, C) [8]. The study by C. Jons et al. confirms high risk of SCD and the need for ICD placement in the presence of syncope with prescribed beta-blockers, especially in women and children [30]. Taking into account the fact that the development of the syndrome is associated with mutations in 13 genes, each associated with a different risk of SCD, the latest Russian guidelines for sudden cardiac death risk assessment and prevention clearly indicate genetic testing [25]. Primary prevention ICDs are recommended for LQT3, and secondary prevention ICDs (IB) – in case of LQT1, LQT2, LQT5, and LQT6 and a prior cardiac arrest [25].

In the Russian Federation, the prevalence of Brugada syndrome is estimated at 1 to 3 cases per 10 thousand population [31]. This disorder requires ICD therapy in case the patient has the following adverse outcome predictors: male, syncope or SCD in the family history, spontaneous ST segment elevation in leads V1–V3 with syncope, spontaneous ST segment changes, and Brugada type 1 ECG pattern (ST segment elevation of 2 mm or more, ending in a negative T wave) [8, 25]. There are no available data concerning the routine use of genotyping to assess the risk of SCD in patients with Brugada syndrome.

LVEF is still the only parameter strongly associated with SCD in patients with cardiovascular diseases [1, 2]. Thus, LVEF and the NYHA functional classification of HF have been used for more than a decade to determine the indications for primary prevention ICD. However, recent advances in prevention of HF with rEF (HFrEF) have allowed specialists to use complete neurohormonal blockade with renin – angiotensin – aldosterone system (RAAS) blockers and beta-blockers, revealed a new group of drugs – angiotensin-receptor-neprilysin-inhibitor (ARNI), and helped to improve CRT and coronary revascularization. Due to this fact, AHA guidelines (2017) included an additional criterion for revaluating parameters after 90 days, if revascularization is to be performed [11].

Currently, the prognosis in patients with HFrEF has significantly improved due to higher survival rates and lower risk of SCD, compared with 10–20 years ago. Analyzing the outcome of the last 12 RCTs that are not related to ICD, a significant decrease (44%) in the rate of SCD was observed in more than 40,000 patients with HFrEF, which was comparable to ICD therapy [24]. This decrease occurred simultaneously with a rise in prescription of ODT. Additionally, an analysis of 4,000 MADIT patients showed significant reduction of VT contribution to the overall mortality in ICD therapy over the past two decades. VT involvement in the overall mortality decreased from 21% in MADIT-II

(conducted in 1997–2001) to 14% in Multicenter Automatic Defibrillator Implantation Trial: Reduce Inappropriate Therapy (MADIT-RIT, conducted in 2009–2011). Presumably, ODT for CHF reduces cases of VT and SCD and increases survival rates [32]. This is validated by the results of a recent DANISH RCT on the effectiveness of primary prevention ICD in patients with non-ischemic HF [23].

Therefore, the above mentioned data determine the need for new large-scale RCTs to assess the effectiveness of ICD therapy, define the indications for it, and search for new biomarkers and predictors of SCD.

ICD IMPLANTATION-RELATED COMPLICATIONS AND THEIR PREVENTION

ICD-related complications can occur in the early and late postoperative period. Inappropriate shocks, infectious complications, and ICD malfunction are of particular interest due to their frequency.

According to the results of a recent study with a sample of more than 3,000 patients, the cumulative incidence of adverse events over 12 years of follow-up was: 20% – inappropriate shocks, 6% – infectious complications associated with an implanted device, and 17% – electrode failure. A population-based survey on the frequency of ICD-related infections in the United States (2016) showed that out of 191,610 placed ICDs, 8,060 caused infections (4.2%), hospital mortality was 4.7%, and the majority of patients (68.9%) with ICD-related infections had three or more comorbidities [33]. A much lower incidence of infectious complications (0.5–2.5%) was found by Russian observational studies, indicating relative safeness of the method [34, 35].

The problem of inappropriate ICD shocks is given a lot of attention in modern arrhythmology [4, 12, 35, 36]. There is growing evidence that ICD shocks lead to myocardial damage, contribute to the progression of left ventricular dysfunction (LVD), and multiply a risk of death by 1.9–5.6 times [24, 35, 36]. Moreover, social maladaptation, poor quality of life, anxiety, and depression can develop as the result of frequent shocks, worsening the course of the underlying disease. According to the data, 22–66% of patients complain about symptoms of depression, 31–83% of patients are concerned about anxiety within a year after ICD placement [37, 38], and the development of these mental health disorders is closely related to the frequency of ICD shocks [39]. Recent studies have shown that anxiety and depression in ICD patients have a bidirectional relationship with endpoints, such as hospitalization and death [40]. Currently, the cohort of patients with CHF and ICD is considered to have the most severe psychosocial distress and social maladaptation.

Data analysis suggests that compliance with the current recommendations on device programming, elaborated by the HRS / European Heart Rhythm Association (EHRA) / Asia Pacific Heart Rhythm Society (APHRS) / Latin American Heart Rhythm Society (LAHRS) in 2019, can help prevent inappropriate shocks and thereby reduce their frequency [41]. Optimal programming prolongs the time of arrhythmia detection, increasing the probability of triggering the antitachycardia pacing (ATP), rather than shock. Three RCTs (MADIT-RIT, Avoid Delivering Therapies for Non-Sustained Arrhythmias in ICD Patients III (ADVANCE III), and Programming Implantable Cardioverter – Defibrillators in Patients with Primary Prevention Indication to Prolong Time to First Shock (PROVIDE)) analyzed strategies of prolonging the tachycardia detection interval compared with conventional short detection intervals [42–44].

All three studies demonstrated that longer detection intervals were associated with a decrease in the frequency of inappropriate shocks. Moreover, improved survival rates in the groups randomized for prolonged detection were noted in MADIT-RIT and PROVIDE studies [42, 44]. T. Ananwattanasuk et al. compared two groups of patients with ICD: with programming according to recommendations and with random programming according to the doctor`s choice. The results showed that the first group experienced lower frequency of inappropriate shocks and had lower incidence of ICD therapy [45]. The results also demonstrated that only in 1/3 of patients in clinical practice ICDs were programmed in accordance with the existing recommendations [45].

Inappropriate ICD shocks might occur due to oversensing of the T-waves, atrial arrhythmia, R-waves, myopotential, electromagnetic noise, and sensing lead malfunction [46, 47]. Another common cause of inappropriate shocks is impaired detection and recognition of supraventricular tachycardia as

ventricular tachycardia, or a shock discharge instead of ATP [46, 47].

To solve this problem, modern ICDs use automatic algorithms for differentiation between arrhythmias, significantly reducing the frequency of inappropriate ICD shocks and recognizing supraventricular and ventricular arrhythmias, T-waves, noise, and interference. Non-compliance with the manufacturer's recommendations during programming can lead to an increase in the frequency of inappropriate shocks [48]. Medtronic Inc. have recently implemented algorithms to deliver the programmed number of ATP sequences during the charge after detection of arrythmia in the VF zone. The safety and effectiveness of this algorithm have been confirmed in a number of studies, moreover, they showed that the majority of episodes recognized as VF turned out to be VT that was treated with ATP therapy [49, 50].

Other similar algorithms for differentiating between types of arrhythmias are used in all modern devices, and subsequent studies have shown that their implementation can significantly reduce the number of inappropriate shocks [42, 51]. Devices made by different manufacturers differ significantly in their programming approaches, meaning there could be no standardized programming protocol. This particularity is reflected in the updates of the HRS/ EHRA / APHRS / LAHRS Expert Consensus [41]. According to the PainFree SmartShock Technology (PainFree SST) study, recently developed SmartShock technology (Medtronic Inc.) is comprised of six unique algorithms that effectively reduce the number of inappropriate shocks [52].

The development of new approaches to ICD placement based on the results of myocardial perfusion scintigraphy in patients with CAD is another way to reduce the stimulation threshold and the amplitude of the ventricular signal, allowing to prolong the longevity of ICD while minimizing the oversensing [53]. The compliance with current guidelines on the device programming and the use of modern ICD models proved to be an effective way to reduce the number of inappropriate shocks. Identifying the predictors of high-risk groups for frequent inappropriate shocks, specialists should take a more balanced approach to outlining the indications for ICD placement, its programming, and subsequent monitoring.

A significant contribution to the improvement of the follow-up efficiency after ICD placement is made by remote health monitoring (RHM) and telemetry technologies. Being a part of all modern devices, they can reduce the number of inappropriate shocks by appropriate programming. Data from numerous studies confirmed the effectiveness of RHM and telemetry technologies ((ALTITUDE (Long-term outcome after ICD and CRT implantation and influence of remote device follow-up), TRUST (Lumos-T Safely Reduces Routine Office Device Follow-Up), ECOST (Effectiveness and Cost of ICD follow-up Schedule with Telecardiology)) [54–56].

The large-scale ALTITUDE study (2006–2009) was devoted to the analysis of the advantages of RHM (69,556 patients) over conventional monitoring (116,222 patients) after the ICD placement. The results showed that implantation of the devices was associated with significant survival benefits during the first year – 92 and 88%, respectively, and it was RHM that ensured high efficiency of ICD in both groups ($p < 0.0001$) [54].A combination of telemetry and RHM allows for almost seamless process, providing daily self-monitoring of the implanted device and notifying the specialist in cases of abnormality, which could not be done using telemetry alone. The results of the TRUST study confirmed that RHM and telemetry are more effective than conventional follow-up visits, because patients were always under medical supervision [55]. In the ECOST study, cases of ICD therapy were identified as a secondary endpoint [56]. The results in the RHM group are explained by preemptive actions of the doctor that were taken after receiving an early warning via the RHM system. In the RHM group, 14.5% of device shocks were inappropriate, while in the control group the number was significantly higher, reaching almost 43% ($p < 0.001$) [56].

Another problem related to ICDs is the fact that, despite the 80% success rate of ICD therapy, the mortality rate in ICD patients continues to be high, which motivates researchers to further study the patterns and mechanisms leading to it. Thus, according to postmortem telemetry of 90 SCD cases in patients with ICD, 26% of patients died from uncorrected VT or VF, 29% – from post-shock electromechanical dissociation, 16% – from primary electromechanical dissociation, 13% – from inces-

sant VT or VF, and 7% – from VT or VF after ICD deactivation [57].

According to E. Cronin et al., only 33% of patients with primary prevention and 47% with secondary prevention ICDs receive appropriate shocks. Therefore, an upgrade in programming algorithms is required to differentiate life-threatening arrhythmias from other types of rhythm, cardiac, extracardiac, and external interferences. Monitoring ICD patients at high risk of SCD should be considered a priority [58]. According to the current guidelines, any inappropriate shock or non-response in the ICD patient with malignant arrhythmia is a reason for further studying SCD prevention methods, improving the device response, and verifying the population at risk [10–12].

For this purpose, several risk stratification systems were developed for ICD patients. However, most of them were focused on determining the risk of all-cause mortality in patients with reduced and preserved LVEF and were not widely used in clinical practice. The long-term follow-up withing the Leiden out-of-hospital cardiac arrest (LOHCAT) study (456 CAD patients with secondary prevention ICDs) added an adverse prognostic value to the QRS width [59]. D.B. Kramer et al. revealed that 2,717 patients with ICD had creatinine levels of more than 200 mg / l, LVEF < 20%, atherosclerosis, and an increased risk of mortality [60].

G.A. Gromyko et al. proposed a Russian system of risk stratification based on data of postinfarction patients, which included the following determinants: atherosclerosis, complete right bundle branch block, LV dilatation, stenosis of the anterior interventricular artery, and the value for the percentage of LV scar tissue. An important feature of this scoring system was the assessment of the relationship between the prognosis and the severity of the underlying and concomitant diseases [61]. Therefore, further validation and improvement of methods for ICD inefficiency assessment is another way to reduce medical and social losses for ICD patients.

DIFFICULTIES IN ICD APPLICATION IN CLINICAL PRACTICE

ICD therapy in real clinical practice is a complex issue. Firstly, there is an obvious gap between guideline recommendations and their clinical application in many countries, including the Russian Federation. Out-of-hospital all-cause mortality due to SCD reaches 39.4% worldwide [62]. There are no statistics available on the SCD-related death rate in the Russian Federation, but according to the latest estimates, 200,000–250,000 people die annually from cardiovascular diseases in the Russian Federation. The Sudden Cardiac Death in Patients with Coronary Heart Disease: Results of the Russian Multi-Center Epidemiological Study of Mortality, Morbidity, and Diagnostics and Treatment Quality in Acute CHD (RESONANCE) study revealed that the incidence of SCD is 156 (for men) and 72 (for women) per 100,000 population per year, although the real frequency of SCD might be higher [63].

For comparison, in the United States, the annual rate of SCD is 100 to 200 per 100,000 population [64]. This means that the number of ICD patients is too low, and there should have been more cases of ICD placement. The analysis showed that supply for surgical and interventional cardiology procedures in several regions of the Russian Federation is below average, while other regions are the most undersupplied in the world [65].

Only 66 clinics in the Russian Federation had cases of implanted ICDs in 2013. The total number of ICDs was 1,926 per year and the average was 0.05 ICDs per 100,000 population. The highest index was 0.06 per 100,000 in the Central Federal District (FD), and the lowest one was 0.01 per 100,000 in the North Caucasus FD [65]. According to recent data, the vast majority of ICD patients in the world belong to the primary prevention group, but even in countries where the ICD therapy is widespread, the implanted ICDs satisfy only 40–60% of the overall need [44]. There are several reasons for this situation: a high cost of the ICD device; ignorance or distrust of ICDs; lack of standards for patient selection and follow-up monitoring. Ultimately, doctors in the outpatient setting do not have necessary knowledge about the specifics of ICD patient management and experience of working with such patients due to their small number.

This leads to the second problem related to ICD patient management. Standard outpatient follow-up after implantation of the device implies complex cardiac care: echocardiography and ECG, compliance with medication treatment (including antiarrhythmic therapy), scheduled follow-up appointments with the arrhythmologist, and specialized

arrhythmological optimization of the device by the programmer (scheduled and in case of inappropriate shocks) [8].

Due to the occurrence of inappropriate shocks in 25% of patients, leading to premature depletion of the device battery, the need for unscheduled ICD follow-ups continues to persist. Other reasons for inappropriate shocks include device or lead malfunctions, excessive or insufficient sensing, and incorrect stimulation threshold [66]. However, many patients with ICDs do not receive such medical care, which was recognized, in particular, by HRS [67]. This problem has been especially relevant for the Russian Federation. The development of automated wireless RHM systems was a much-needed change in outpatient monitoring that helped to form the basis for new guidelines. Now all patients with ICD should be provided with RHM, which in turn reduces the number of follow-ups and justifies hospitalization in case of multiple malfunctions and is reasonable for evaluating the device performance and the battery life [67–69].

However, data from real clinical practice show that RHM data were never analyzed during the first year of ICD placement in 25% of patients [69]. Combined with the fact that the RHM system does not allow for reprogramming of ICD remotely, even more issues start to arise. They are primarily associated with a lack of standards and clinical guidelines for the use of RHM in the Russian Federation [68]. In many countries, neither clinics nor doctors receive monetary compensation for RHM / telemetry monitoring, despite the fact that these methods proved to be cost-effective and allow to increase the number of patients under medical supervision. Furthermore, this type of medical service is not funded by healthcare systems, which only complicates the work of specialists [57, 68, 69].

The difference in the ICD effectiveness according to foreign and Russian RCTs presents another significant problem. Data on the frequency of appropriate shocks in ICD patients in the Russian Federation indicate a lower number of effective responses compared with other RCTs, indirectly revealing the shortcomings of the selection process [35, 37]. According to A.S. Revishvili, 49% of patients did not receive single justified ICD therapy for 5 years, even though the frequency of inappropriate shocks was 39% [35]. Literature data indicate higher mortality rate among patients with both primary and secondary prevention ICDs in the Russian Federation compared with international statistics – 18.8 and 16% versus 12.7 and 14%, respectively [35, 70]. According to the study by M.A. Kamaliev et al., the survival rate in this category of patients during the year was 83.3%, which is a lower value than in international data on the annual survival of patients after ICD placement (92–98%) [71].

Therefore, studies on ICD therapy conducted in the Russian Federation indicate insufficient survival rate compared with international studies both in terms of the survival rate 1 year after ICD placement and in the long-term follow-up. It can be explained objectively by the level of healthcare system development and availability of medical care in certain regions; or subjectively by poor patient selection, non-compliance with recommendations on device programming or RHM, difficulties of outpatient management, and low adherence to ODT. However, these assumptions need to be confirmed by analyzing the cohort of patients with ICD in real clinical practice in every region of the Russian Federation.

Psychosocial rehabilitation is another problem of patient management in the Russian Federation. Depression and deterioration of the patient's quality of life after ICD placement can lead to a loss of contact with the specialist, which negatively affects survival [40]. The main sources of stress, anxiety, and depression can be both excessive information about the device, especially from other patients, as well as a lack of information [41]. Given these data, it may be useful to organize training sessions for ICD candidates – individual and group psychological counseling sessions, explanatory therapy, etc. The guidelines highly recommend assessment and treatment of psychosocial distress in ICD patients [10–12, 25]. Self-help groups and individual and group therapy have already proven to be effective in this cohort of patients [72].

However, such treatment options, including psychotherapy, are not available for patients with reduced mobility or financial difficulties [39, 72]. Research results have shown that online video and individual phone consultations can help in this situation [73, 74]. Given the availability and low cost, Internet-based consultation is a promising solution, even for elderly patients [40]. Supposedly, online consultations can be as effective as conventional

therapy for patients with ICD, and there is growing evidence in support of that [40, 73, 74].

Therefore, there are several ways to improve ICD patient management in the Russian Federation: active monitoring of the devices via implementation of the RHM system, optimization of drug therapy, promotion of drug compliance, application of modern ICD programming methods, raise of patient awareness (sessions, phone calls, monitoring diary), and psychological consultations and counseling, also via Internet-based technologies.

CONCLUSION

Significant progress has been made in the field of ICD therapy over the past two decades. As a result, there are currently multiple kinds of ICD devices to choose from [75]. Future efforts should be focused on improving methods of patient selection, which in turn requires large-scale RCTs against the background of ODT. It is also necessary to develop new comprehensive approaches to SCD risk stratification, based on the combined assessment of clinical risk factors on the basis of ECG, findings of medical imaging techniques, biomarkers, and genetic determinants, including patients with intermediate and preserved LVEF.

Evidently, ICD therapy has high relevance in healthcare. However, there are limiting factors for the Russian Federation, such as high cost of the device, distrust of the method due to ignorance of ICD benefits, lack of practical tools for risk assessment in SCD, and insufficient experience in managing patients with ICDs in the outpatient setting [75, 76].

Outlining problems associated with ICDs can assist in finding solutions among medical experts and device developers. One way to optimize ICD therapy is to create a registry of ICD patients, which can be crucial for developing cost-effective prevention strategies and bridging the gap between scientific data and limited healthcare resources.

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A clinical case of X-linked adrenoleukodystrophy in a 9-year-old boy

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ABSTRACT

X-linked adrenoleukodystrophy belongs to peroxisomal disorders characterized by combined damage to the nervous system and adrenal glands and often leading to death. This hereditary disease results in mutations in the *ABCD1* gene, leading to ineffective β-oxidation of fatty acids following a decrease in the activity of acetyl-CoA synthetase of their long chains. Accumulation of acyl-CoA derivatives of fatty acids takes place, which affect the physicochemical properties of cell membranes.

We have described a clinical case of X-linked adrenoleukodystrophy in a 9-year-old boy with the primary manifestation of the disease at the age of 7 years and 10 months in form of enterovirus encephalitis.

Early diagnosis, prenatal screening of adrenoleukodystrophy for performing gene-specific therapy, slowing the progression of the disease, and prolonging the life of the patient with the diagnosis of a rare hereditary disease are required.

Keywords: X-linked adrenoleukodystrophy, adrenal insufficiency, glucocorticoids, mineralocorticoids, leukoencephalomalacia

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Клинический случай Х-сцепленной адренолейкодистрофии у мальчика 9 лет

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РЕЗЮМЕ

Х-сцепленная адренолейкодистрофия относится к пероксисомным болезням, характеризуется сочетанным поражением нервной системы и надпочечников, часто приводящим к летальному исходу. Это наследственное заболевание вызывает мутации гена *ABCD1*, определяющим неэффективность β-окисления жирных кислот, в результате снижения активности ацетил-КоА синтетазы их длинных цепей. Происходит накопле-

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ние ацил-КоА-производных жирных кислот с нарушением физико-химических свойств клеточных мембран.

Нами описан клинический случай Х-сцепленной адренолейкодистрофии у мальчика 9 лет с первичной манифестацией заболевания в возрасте 7 лет 10 мес по типу энтеровирусного энцефалита.

Необходима ранняя диагностика, пренатальный скрининг для проведения специфической терапии, замедления прогрессирования заболевания, продления жизни больному с редким наследственным диагнозом.

Ключевые слова: Х-сцепленная адренолейкодистрофия, надпочечниковая недостаточность, глюкококортикоиды, минералокортикоиды, лейкоэнцефаломаляция

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. У родителей ребенка получено разрешение на публикацию анонимных данных.

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INTRODUCTION

X-linked adrenoleukodystrophy (X-ALD) belongs to the group of hereditary metabolic diseases associated with impaired peroxisomal function. Adrenoleukodystrophy (synonyms: Zimmerling – Creutzfeldt's disease, Schilder – Addison's disease) was first described by German neuropathologists Ernst Zimmerling and Hans Gerhard Creutzfeldt in 1923. This disease is not as rare as it was previously thought to be, it occurs everywhere, and its incidence rates prevail over other peroxisomal diseases [1].

The onset of the disease is associated with mutations in the *ABCD1* gene in the terminal region of the long arm of the X chromosome (locus Xq28) [2]. This gene encodes synthesis of a transmembrane carrier protein called adrenoleukodystrophy protein (ALDP), which is located on specific cellular organelles involved in oxidation reactions, peroxisomes, and is responsible for transport and further degradation of very-long-chain fatty acids (VLCFAs) [1]. With a structural defect in the peroxisomal transport protein, it becomes functionally incapable, which leads to accumulation of toxic compounds in the tissues.

Currently, more than 2,600 *ABCD1* mutations associated with the replacement of DNA nucleotides and loss of loci have been identified [3]. Many of them cause structural changes in ALDP. Adrenoleukodystrophy develops when there is a recessive gene in the genotype (in hemizygous men) or two of its variants (in heterozygous women). X-ALD is more common in boys; in girls, the disease is asymptomatic. The incidence of adrenoleukodystrophy is 1: 17,000 live-born boys [1, 3]. The disease is manifested by two groups of clinical symptoms: primary adrenal insufficiency and demyelinating disease. These disorders are caused by mutations in the *ABCD1* gene encoding the transmembrane protein ALDP, leading to ineffective β-oxidation of fatty acids following a decrease in the activity of acetyl-CoA synthetase of their long chains. Primary adrenal insufficiency is clinically manifested through hyperpigmentation of skin folds and mucous membranes, cicatricial changes, fatigue, weight loss, dizziness, increased anxiety, chronic nausea, and vomiting. Damage to the nervous system is clinically manifested through autistic behavior, learning problems, memory loss, attention deficit, convulsive syndrome, blindness, and deafness [4].

CLINICAL CASE

Patient S., born in 2011, was admitted to the Department of Pediatric Neurology of the City Clinical Hospital for the first time in March 2020 with complaints of weakness, lethargy, drowsiness, and disorientation.

Anamnesis vitae and anamnesis morbi. A boy from a second pregnancy that ended in childbirth by caesarean section at 39 weeks due to breech presentation. The first pregnancy ended in spontaneous abortion. The child was born with a weight of 3,200 g and a height of 52 cm, the Apgar score was 8 / 9.

The patient's health in early childhood was normal. The patient is vaccinated according to the calendar. The patient had rare respiratory infections and chicken pox. Until the age of 7, the parents did not report any complaints about the development of the boy. At the age of 7, mother began to notice periodic urinary incontinence in her son. According to his parents, the boy did not go to the toilet for a long time, and then he would not manage to get there in time. Therapy with oxybutynin was prescribed with appropriate water intake schedule. A positive effect was achieved.

At the age of 7 years 10 months, while the child was in a camp in Anapa, he complained of nasal congestion, single vomiting, weakness, and body temperature increase to febrile values. He was examined by a doctor, then he was isolated, an ambulance was called. Before the arrival of the ambulance, there was repeated vomiting, paroxysm developed in the form of a short-term loss of consciousness, eyes rolling back, and twitching of arms and legs. The boy was hospitalized in Krasnodar with the diagnosis of enterovirus encephalitis. He was administered carbamazepine (200 mg 3 times a day), and a positive effect was achieved.

During the autumn, the child did not feel well, he had continued weakness, lethargy, and rapid fatigability. The body temperature occasionally rose up to 37.2°C. It was recommended to consult a neurologist. Magnetic resonance imaging (MRI) of the brain was performed (December 16, 2019). According to MRI, for the first time, signs of leukoencephalomalacia were noted with symmetrical lesions in both parietal and temporal lobes, the posterior third and splenium of the corpus callosum, posterior parts of the basal ganglia, cerebral peduncles in midbrain, and pons. The boy was observed by a neurologist and an epileptologist in a local clinic. Diagnosis: "epilepsy"? Given the absence of epileptic seizures, therapy with carbamazepine was canceled. Aminophenyl-butyric acid was prescribed, which the patient took until the end of January 2020, therapy was then canceled according to the recommendation of a neurologist.

On March 28, 2020, immediately after waking up, the boy felt unwell. A single vomiting, weakness, and lethargy were noted, the child became distracted, inattentive, and disoriented in space. Paroxysm was noted in the form of fading (the patient did not respond to the mother) and eye movements. An ambulance was called. Before the arrival of the ambulance, a paroxysm developed in the form of eyes rolling back, lip twitching on the right, extended right arm and right leg. The patient did not respond to the people around him. Diazepam was administered. The child was admitted to the hospital for a comprehensive examination.

Objective clinical examination. An examination of the patient in the emergency room showed the following: a state of moderate severity, impaired consciousness, poor orientation in space and time, difficulties in make contact with the patient, he answered questions slowly and not always. His skin was dark and dry. The patient's skin on the elbows, in the armpits, and in the area of the buttocks was much darker (up to a chocolate brown color). In the right frontal region, a light brown spot of an irregular shape was noted. On the upper and lower extremities, 4 small (1–5 mm) light brown spots of an irregular shape were detected. A neurological examination did not reveal any changes, except for bad mood. The boy has phenotypic features: curly hair, protruding ears, deep set eyes, wide interdental spaces, dolichostenomyelia.

When the child was in the hospital, the following was revealed: hearing impairment, impaired understanding of addressed speech, and deterioration of handwriting. In the dynamics, child's space orientation became worse, and his gait became unsteady.

Laboratory tests and instrumental diagnosis were conducted. Blood test: ESR – 13 mm / h, other parameters were within the reference range. In the blood biochemistry test, the following parameters were increased: creatine phosphokinase 377 U / l (0–247), lactate dehydrogenase 426 U / l (0–378), glucose level was within the reference range: 4.36 mmol / l (3.3–5.9). The cortisol level was low: 01.04.2020 at 8 am – 25 nmol / l, at 10 pm – 31.5 nmol/ l; 08.04.2020 at 8 am – 12.6 nmol / l, at 10 pm – 3.2 nmol / l (reference range is 83–580 nmol / l). The level of adrenocorticotropic hormone was elevated: 127 pg / ml (with the reference range of 0–46 pg / ml).

Ultrasound examination of the thyroid gland: right lobe 24*9*9 mm, V – 0.9 cm ^{3}, left lobe 28*8.5*9 mm, V – 1.0 cm ^{3}. Contours were clear and even. No lesions were detected. The volume of the thyroid gland was 1.9 cm^3. Conclusion: thyroid hypoplasia. Electroencephalography (EEG) revealed no clear signs of impaired bioelectrical activity in the brain. Focal, paroxysmal, and epileptic activities were not registered in the study. Moderate cerebral changes with irritative zones were detected. On the basis of objective clinical examination data and the results of laboratory tests and instrumental diagnosis, the following diagnosis was made: demyelinating disease of the central nervous system, X-linked adrenoleukodystrophy.

The therapy was prescribed: valproic acid (Depakene) 180 mg 3 times a day, methylprednisolone 250 mg, thiamine 1 ml intramuscularly once every two days. The treatment had a positive effect, improving the patient's conditions, stopping psychotic states and seizures, and improving the child's mood. To carry out substitutive therapy, hydrocortisone (Cortef) was prescribed orally 10 mg per day in 3 divided doses (5 mg in the morning, 2.5 mg at lunch, 2.5 mg in the evening). The patient's discharge record was sent to the Department of Medical Genetics of the Russian Children's Clinical Hospital of Pirogov Russian National Research Medical University, to which the child was admitted in April 2020. At the time of hospitalization, the patient complained of visual and hearing impairment, lack of voluntary coordination of muscle movements, falling, dark skin, and periodic involuntary urination; the patient could not write in a straight line.

Objective clinical examination. Upon admission, he was in a state of moderate severity. Phenotypic features of the patient included dark skin, high forehead, wide nose bridge, wide set eyes, transverse palmar crease, and claw sign. The skin was dark without rash. The elbows and knees were hyperpigmented. There was a hyperpigmented area on the forehead on the right. Neurological status: convergence insufficiency on the right. Exotropia, more on the right. The face was slightly asymmetrical: the nasolabial fold on the right was somewhat smoothed. The patient had hearing impairment. The pharyngeal reflexes were reduced, without a clear difference in sides of the body. The patient had positive pyramidal signs. The gait was wide-based and atactic with polyneuropathy. Muscle tone – dystonia, more in the arms, D > S. Ankle jerk reflexes were reduced. The plantar reflex or Babinski sign was positive on both sides. Coordination tests: the patient tried to reach the hammer with intention tremor and slight asymmetry. Sensitivity was difficult to assess (due to hearing impairment). The patient almost did not execute commands. Speech was with dysarthria, slow and phrasal. It looked like speech and mental retardation.

The results of the conducted tests. Endocrine profile (29.04.2020): cortisol 82.92 nmol / l (reference value 83–580 nmol / l), adrenocorticotropic hormone (ACTH) 476 pg / ml (reference value 0–46 pg / ml). With continuous substitutive hormone therapy for a month, the level of cortisol increased, while the ACTH level remained high. The changes in the levels of blood electrolytes were the following: the level of sodium ion increased from 118 mmol / l to 138 mmol / l (reference value 135–148 mmol / l); and the level of potassium ion decreased from 4.5 mmol / l to 3.3 mmol / l (reference value 3.5–5.3 mmol / l).

Electrocardiogram (ECG) showed dysmetabolic syndrome in the myocardium (inverted T wave in V3, AVF), myocardial dystrophy. Electroneurography (27.04.2020) revealed several disorders in the muscle tone regulation: suprasegmental nature, signs of demyelination of peripheral nerves *n. Peroneus, n. Tibialis, nn. Suralis, nn. Medianus*, S > D. At that moment, no pathology of motor neuron disorders was observed at the level of cervical and lumbosacral enlargement. According to a video sleep EEG (28.04.2020), there were no data on continued regional, diffuse, and generalized epileptic activity (Table 1).

Table 1

Dynamics in the instrumental diagnosis of the brain

CT (August 2019)	MRI (December 2019)	MRI (March 2020)
Signs of reduced density areas in the splenium of corpus callosum and the area of the posterior horns of the lateral ventricles	Leukoencephalomalacia with symmetrical lesions in both parietal, both temporal lobes, posterior third and splenium of corpus callosum, posterior basal ganglia, cerebral peduncles of the midbrain, and pons (corticospinal tracts)	Leukoencephalopathy of unknown origin. No dynamics from 12.2019

The disease progressed rapidly, which was manifested through an aggravation in neurological symptoms: unsteadiness when walking, visual and hearing impairment, epileptic seizures, and intellectual disorders. Widespread demyelinating process on MRI of the brain (Lewis scale 11 out of 32 points) and adrenal insufficiency were also noted.

The primary diagnosis: a degenerative disease of the nervous system from the group of peroxisomal diseases (G31.8). X-linked adrenoleukodystrophy, cerebral form (E71.3). Symptomatic epilepsy. Atactic syndrome. Cortical visual and hearing impairments. Chronic primary adrenal insufficiency. Speech and mental retardation. Secondary diagnosis: myocardial dystrophy.

The following treatment was prescribed: low-fat diet, Lorenzo's oil in the 4 : 1 ratio of oleic acid and erucic acid triglycerides. Medications included substitutive therapy with glucocorticoids and mineralocorticoids: Cortef 5 mg in the morning, 2.5 mg at lunch, 2.5 mg in the evening; Cortineff ¼ tablet twice a day in the morning and in the evening; valproic acid 3 ml three times a day. Allogeneic stem cell transplantation was not advised when the boy

was hospitalized to the Russian Children's Clinical Hospital of Pirogov Russian National Research Medical University, given the rapid progression of the disease in the patient.

After hospitalization to the Russian Children's Clinical Hospital, the child was observed by a pediatric endocrinologist and a neurologist at the place of residence. The prescribed hormonal therapy with hydrocortisone upon discharge was increased for the summer period to 30 mg / day, taking into account a progressive decrease in cortisol levels: 09.2020 – 0.7 nmol / l; 10.2020 – 3.65 nmol / l (reference value 8.7–22.4 nmol / l). The level of adrenocorticotropic hormone also remained high: 09.2020 – 458 pg / ml, 10.2020 – 390.8 pg / ml (reference value 8–57 pg / ml).

In September 2020, the child's condition worsened significantly, which required a further increase in the dose of hydrocortisone to 40 mg / day. In dynamics, the ACTH level decreased to 390.8 pg / ml, and the cortisol level increased to 3.65 nmol / l. The blood biochemistry test showed moderately pronounced changes in all parameters (Table 2).

Table 2

Blood biochemistry test

Parameter	October 2020	Reference value
AST, U / l	77.8	0–50
ALT, U / l	54.8	0–50
Iron, μmol / l	5.8	9–21.5
Chlorine, mmol / l	90.58	98–107
Calcium, mmol / l	2.04	2.2–2.7
Potassium, mmol / l	2.88	3.5–5.3
Sodium, mmol / l	132.7	135–148
Total cholesterol, mmol / l	6.79	3.3–5.2
Alkaline phosphatase, U / l	49.75	86–315
Gamma-glutamyl transferase, U / l	23.86	3–22

DISCUSSION

Adrenoleukodystrophy is characterized by a pronounced phenotypic polymorphism, which is associated with differences in the penetrance and expressivity of the abnormal gene. There are several forms of the disease, the development of which is determined by the time of onset, main manifestations, and the rate of aggravation. In this case, the patient had a cerebral form of the disease, the main symptoms of which are neurological disorders. According to the time of the disease onset, this is a childhood form, which occurs more often (almost in 50% of cases) [5–7]. This form generally occurs between the ages of five and ten [8].

In the present clinical case, the first moderately pronounced symptoms (enuresis) appeared at the age of 7 years, and after 10 months, more manifestations of the disease appeared. Cerebral adrenoleukodystrophy is characterized by rapid progression [9–11], which is clearly shown in the present clinical case – 8 months passed from the onset of manifestations to a severe condition.

In most patients, neuropsychiatric disorders precede signs of adrenal insufficiency. At the onset of the disease, the child was diagnosed with convulsive disorder, which required differential diagnosis with epilepsy. The child was admitted to the neurological department with complaints of impaired hearing and understanding of addressed speech, worsening of handwriting, worsened space orientation, and unsteady gait. Changes in the skin color in the form of increased pigmentation and brown spots were first detected in the hospital, earlier, parents and doctors did not pay attention to these changes. Manifestations of adrenal insufficiency were confirmed by the endocrine test. The prescription of substitutive therapy with hydrocortisone led to an improvement in hormone levels without affecting the severity of clinical manifestations. In the dynamics, there were manifestations of behavioral disorders, motor functions, thinking, and unmotivated aggression appeared.

Presymptomatic therapy is particularly important in the treatment of adrenoleukodystrophy. At this stage, diet was effective [12]. In this clinical case, diet therapy is not justified due to the already developed clinical presentation of the disease. The use of substitutive therapy with glucocorticoids and mineralocorticoids is indicated for symptoms of hypocorticism [13]. However, these drugs do not have a pathogenetic effect on the pathological process in the central nervous system [14]. Symptomatic treatment of myelopathy is conducted with neurometabolic agents, muscle relaxants, and vitamins.

The main treatment method at early stages of adrenoleukodystrophy in childhood and adolescence is allogeneic stem cell transplantation, which can stop the progression of demyelination [15, 16]. Additional methods that do not affect the mechanisms of pathology development include acupuncture, transvertebral micropolarization, massage, and exercise therapy, which are also used at initial stages of the disease to relieve spastic symptoms and muscle stiffness. Patient S., 9 years old, was diagnosed at the stage of severe clinical symptoms, so the effectiveness of the

prescribed treatment is extremely low. The prognosis is poor.

CONCLUSION

Currently, there are no specific methods for prevention and treatment of adrenoleukodystrophy. Recommendations aimed at preventing the occurrence of this genetic pathology have been developed. Prenatal diagnosis of the disease is conducted. Treatment that is recommended at early stages of the disease involves bone marrow transplantation from a donor to a patient. Early diagnosis of adrenoleukodystrophy and comprehensive therapy significantly slow down the development and progression of the disease, prolonging the patient's life.

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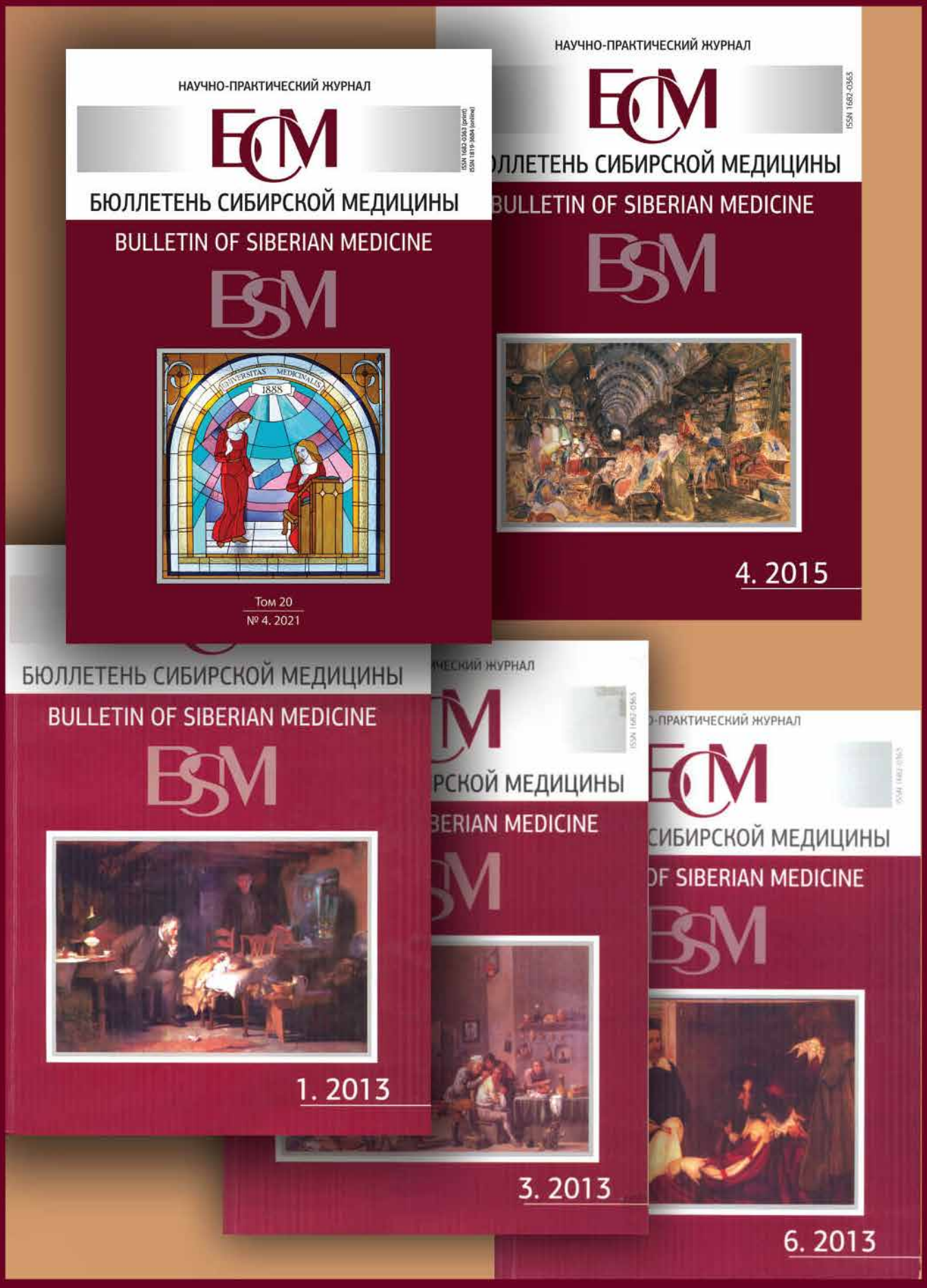

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