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MECHANISMS OF CYTOKINE STORM DEVELOPMENT IN COVID-19 AND NEW POTENTIAL TARGETS OF PHARMACOTHERAPY

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The development of a "cytokine storm", characteristic of severe COVID-19 forms, can be defined as a state of uncontrolled release of a large number of inflammatory mediators.

The attachment of SARS-CoV-2 S-glycoprotein to angiotensin-converting enzyme 2 is considered a process that triggers complex molecular interactions that lead to hyperinflammation. In its turn, it is realized through several systems: renin-angiotensin-aldosterone, kallikrein-kinin and a complement system. Knowledge of these mechanisms suggests potential therapeutic interventions that can be targeted by existing therapeutic agents to counter the cytokine storm and treat the acute respiratory distress syndrome associated with COVID-19.

The aim of the review article is to summarize the currently known data on the molecular processes underlying the uncontrolled "cytokine storm" in the patients with severe COVID-19, and possible options for their pharmacological correction.

Materials and methods. The data base was represented by such systems as Medline, Cochrane Central Register of Controlled Trials, Scopus, Web of Science Core Collection, Cochrane Library, ClinicalTrials.gov, Elibrary, Google-Academy. A search was carried out for the following keywords and combinations: COVID-19, renin-angiotensin-aldosterone system, bradykinin, complement system, hyaluronic acid, pharmacotherapy.

Results. The development of a "cytokine storm" in COVID-19 is mediated by pathogenetic changes in the body in response to the penetration of SARS-CoV-2 into the cell. In the RAAS, suppression of ACE2 leads to a decrease in its ability to degrade ATII, which, on the one hand, leads to a decrease in the amount of AT1-7, and, on the other hand, to the effect of ATII on AT1R with the subsequent development of vasoconstriction and lung damage. The disturbances in the kallikrein-kinin system are associated, on the one hand, with the increased expression of kallikrein and an increase in the formation of bradykinin and its metabolite des-Arg 9-bradykinin. On the other hand, the disturbances are associated with the suppression of the expression of the C1-esterase inhibitor which prevents the formation of kallikrein, and impaired inactivation of des-Arg 9-bradykinin under the action of ACE 2. The nucleocapsid protein SARS-CoV-2 triggers the activation of the complement system through the lectin pathway. It leads to the production of anaphylatoxins C3a and C5a, which stimulate the synthesis of pro-inflammatory cytokines. Proinflammatory cytokines are potent inducers of the HAS 2 gene in the endothelium, which encodes the membrane enzymes of hyaluronate synthase. The sweating of the fluid into the alveoli caused by the "bradykinin storm" in combination with the overproduction of hyaluronic acid, which accumulates water 1000 times its own mass, can lead to the formation of a dense jelly-like substance that prevents gas exchange.

Conclusion. Promising areas of pharmacotherapy for "cytokine storm" are associated with its impact on the dysfunction of the listed above systems. However, the efficacy and safety of most drugs for the treatment of COVID-19, is to be studied through carefully designed clinical trials.

Keywords: SARS-CoV-2; "cytokine storm"; pharmacotherapy for COVID-19

Abbreviations: ACE – angiotensin converting enzyme; AT – angiotensin; GCS – glucocorticosteroids; CI – confidence interval; IL – interleukine; ARDS – acute respiratory distress syndrome; RAAS – renin-angiotensin-aldosterone system; RCS – randomised controlled study; TNF – tumour necrosis factor; AT1R – angiotensin receptor; BK – bradykinin; BKB 1R – type 1 Bradykinin Receptor; BKB 2R – type 2 Bradykinin Receptor 2; MASP-2 – Mannan-binding lectin-associated serine protease-2; MCP-1 – Monocyte chemotactic protein-1; MAPK – mitogen-activated protein kinase

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МЕХАНИЗМЫ РАЗВИТИЯ ЦИТОКИНОВОГО ШТОРМА ПРИ COVID-19 И НОВЫЕ ПОТЕНЦИАЛЬНЫЕ МИШЕНИ ФАРМАКОТЕРАПИИ

В.И. Петров, А.А. Амосов, А.С. Герасименко, О.В. Шаталова, А.В. Пономарева, А.Н. Акинчиц, И.С. Кулакова, В.С. Горбатенко

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Для тяжёлых форм COVID-19 характерно развитие «цитокинового шторма» – состояния неконтролируемого высвобождения большого количества медиаторов воспаления. Присоединение S-гликопротеина SARS-CoV-2 к ангиотензин-превращающему ферменту 2 рассматривается как процесс, запускающий сложные молекулярные взаимодействия, которые приводят к гипервоспалению, которое в свою очередь реализуется через несколько систем: ренин-ангиотензин-альдостероновую, калликреин-кининовую и систему комплемента. Знание данных механизмов позволяет предположить потенциальные точки терапевтического вмешательства, на которые можно воздействовать существующими терапевтическими средствами для противостояния «цитокиновому шторму» и лечения острого респираторного дистресс-синдрома, связанного с COVID-19.

Цель. В обзоре обобщаются известные на сегодняшний день данные по молекулярным процессам, лежащим в основе неконтролируемого «цитокинового шторма» у пациентов с тяжёлой формой COVID-19 и возможные варианты их фармакологической коррекции.

Материалы и методы. В системах Medline, Cochrane Central Register of Controlled Trials, Scopus, Web of Science Core Collection, Cochrane Library, ClinicalTrials.gov, Elibrary, Google-академия был проведен поиск по ключевым словам и комбинации этих слов: COVID-19, ренин-ангиотензин-альдостероновая система, брадикинин, система комплемента, гиалуроновая кислота, фармакотерапия.

Результаты. Развитие «цитокинового шторма» при COVID-19 опосредованно патогенетическими изменениями, происходящими в организме в ответ на проникновения SARS-CoV-2 в клетку. В РААС подавление АПФ2 приводит к снижению его способности расщеплять АТII, что, с одной стороны, ведет к уменьшению количества АТ1-7, а с другой – к воздействию АТII на АТ1R с последующим развитием вазоконстрикции и повреждения лёгких. Нарушения в калликреин-кининовой системе связаны, с одной стороны, с повышенной экспрессией калликреина и усилением образования брадикинина и его метаболита des-Arg 9-брадикинина, с другой стороны, с подавлением экспрессии ингибитора С1-эстеразы, который препятствует образованию калликреина, и нарушением инактивации des-Arg 9-брадикинин под действием АПФ 2. Нуклеокапсидный белок SARS-CoV-2 запускает активацию системы комплемента по лектиновому пути, что приводит к продукции анафилатоксинов С3а и С5а, которые стимулируют синтез провоспалительных цитокинов. Провоспалительные цитокины являются сильными индукторами гена HAS 2 в эндотелии, который кодирует мембранные ферменты гиалуронатсинтазы. Вызванное «брадикининовым штормом» пропотевание жидкости в альвеолы в сочетании с гиперпродукцией гиалуроновой кислоты, которая накапливает воду в 1000 раз больше собственной массы, может приводить к образованию плотного желеобразного вещества, препятствующего газообмену.

Заключение. Перспективные направления фармакотерапии «цитокинового шторма» связаны с влиянием на дисфункцию перечисленных систем. Однако эффективность и безопасность применения большинства препаратов для лечения COVID-19 еще предстоит изучить с помощью тщательно спланированных клинических исследованиях.

Ключевые слова: SARS-CoV-2; «цитокиновый шторм»; фармакотерапия COVID-19

Список сокращений: АПФ – ангиотензин-превращающий фермент; АТ – ангиотензин; ГКС – глюкокортикостероиды; ДИ – доверительный интервал; Ил – интерлейкин; ОРДС – острый респираторный дистресс-синдром; РААС – ренин-ангиотензин-альдостероновая система; РКИ – рандомизированное контролируемое исследование; ФНО α – фактора некроза опухоли α; АТ1R – ангиотензиновый рецептор первого типа; БК – брадикинин; BKB 1R – рецептор брадикинина 1 типа; BKB 2R – рецептор брадикинина 2 типа; MASP-2 – маннан-связывающая лектин-ассоциированная сериновая протеаза 2

INTRODUCTION

The novel coronavirus infection (COVID-19), which has spread on a pandemic scale in 2020, was caused by SARS-CoV-2, the enveloped RNA virus, which belongs to the Coronaviridae family, a beta coronavirus genus. Structural and auxiliary proteins of the virus are involved in the penetration into the cell and affect the immune response of the infected [1].

The scientific evidence suggests that the immune response to viral infection contributes to the development of severe infections such as MERS-CoV, SARS-CoV and SARS-CoV-2 [2]. The immune responses in severe COVID-19 are known as cytokine storms. “Cytokine storm” is a massive and uncontrolled release of cytokines. This phenomenon is observed in some infectious and non-infectious diseases, leading to a hyperinflam-

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matory response of the body associated with a poor clinical prognosis [3]. This hyperimmune response correlates with a high rate of admissions to intensive care units and a high rate of deaths from COVID-19.

The initial phase of penetration by SARS-CoV-2 into the cell is mediated by the binding of the S-glycoprotein of the viral envelope to the membrane-bound form of the angiotensin converting enzyme (ACE2) on the target cell [1]. The attachment of the virus to ACE2 as its cellular receptor provides penetration into the target cell by viropexis, which leads to the penetration (internalization) of the enzyme into the cell and suppression of its function [4]. The internalization of ACE2 can potentially lead to an increase in the concentration of angiotensin II (AT II) and a decrease in the level of AT1-7, which subsequently leads to the triggering of inflammatory reactions associated with an imbalance in the functioning of the renin-angiotensin-aldosterone system (RAAS) [4].

There are other key mechanisms for the onset of the cytokine storm. Thus, the analysis of bronchoalveolar lavage showed that SARS-CoV-2 causes an increase in the level of bradykinin in the cells (the so-called "bradykinin storm"). It is known that bradykinin is involved in the formation of pain and dilates blood vessels, increasing their permeability, which leads to edema and inflammation. Besides, it was found out that in the patients with COVID-19, the production of hyaluronic acid increases, and the synthesis of hyaluronidase enzymes that could destroy it, decreases [5]. The sweating of fluid into the alveoli, caused by the "bradykinin storm," in combination with the overproduction of hyaluronic acid, leads to the formation of a dense jelly-like substance, preventing gas exchange. Thus, not only an imbalance in the RAAS system, directly leading to a "cytokine storm", but also a "bradykinin storm" with an overproduction of hyaluronic acid, can cause a severe course of COVID-19. However, the potential cellular and molecular mechanisms for the development of severe forms in COVID-19, have not been well studied yet.

THE AIM of the review article is to summarize the currently known data on the molecular processes underlying the uncontrolled "cytokine storm" in the patients with severe COVID-19. Understanding these processes will be critical to the selection of an effective therapeutic target in the pathogenesis of COVID-19. Understanding these processes will be crucial for the selection of an effective therapeutic target in the pathogenesis of COVID-19.

MATERIALS AND METHODS

The data base was represented by such systems as Medline, Cochrane Central Register of Controlled Trials, Scopus, Web of Science Core Collection, Cochrane Library, ClinicalTrials.gov, elibrary, Google-Academy. A search was carried out for the following keywords and combinations: COVID-19, renin-angiotensin-aldosterone system, bradykinin, complement system, hyaluronic acid, pharmacotherapy – both in Russian and English equivalents. There was no limitation by date. The references were checked in manual way, and electronic archives of the clinical trials were used to search for additional studies on the topic. The date of the application was 12/14/2020. The search was carried out by two researchers, and the disagreements were solved by reaching a consensus.

RESULTS

Renin-angiotensin-aldosterone system

RAAS is a system of vasoactive peptides that regulates blood pressure, the volume of circulating fluid, the balance of plasma ions, and also participates in the maintenance of inflammation. One of the key enzymes of this system, ACE2, is expressed in the heart, kidneys, testes, gastrointestinal tract, and lungs [6]. It cleaves ATI to form the AT1-9 peptide, which can be converted to the AT1-7 peptide using ACE or other peptidases. ACE 2 can also directly metabolize ATII with the formation of AT1-7 [7]. AT1-7 is a vasodilator peptide that has antiproliferative, antithrombotic and anti-inflammatory kinds of activity, weakens the effects of activation of type 1 angiotensin receptors (AT1R) [8], reduces the expression of inflammatory factors such as interleukin (IL)-6, tumor necrosis factor α (TNFα) and IL-8 [9].

The positive effects of AT1-7 listed above, occur under the influence of the MasR receptor [9]. MasRs are expressed in the epithelium and smooth muscles of the bronchi; therefore, AT1-7 can modulate acute and chronic inflammatory processes in the lungs by activating MasR [10]. AT1-7 also indirectly affects the production of IL-10, which, in its turn, induces the differentiation of T-helpers into T-helpers of type 2 [11]. Type 2 T-helpers regulate immune responses by producing anti-inflammatory cytokines: IL-4, IL-5, IL-9 and IL-13 [12]. In addition, IL-10 can be involved in the prevention of tissue alteration [13].

Thus, the RAAS can be considered as a hormonal system with two axes: the ACE/ATII/AT1R axis – pathological – and, opposite to it, the protective axis – ACE2/AT1-7/MasR (Fig. 1).

The penetration of SARS-CoV-2 into the cell and its subsequent binding to ACE2 leads to a deviation of the system towards the pathological axis, since the ability of ACE2 to break down ATII decreases. On the one hand, it leads to a decrease in the amount of AT1-7; and on the other hand, to the effect of ATII on AT1R with the subsequent development of vasoconstriction and damage to the lungs [14].

The obtained clinical data confirm the influence of the RAAS imbalance in the pathogenesis of the development of acute respiratory distress syndrome (ARDS). In laboratory studies, the patients with COVID-19 showed higher concentrations of ATII, which linearly correlate with the severity of lung damage [15]. In addition, the use of ACE inhibitors and angiotensin receptor blockers for the treatment of hypertension, is associated with a

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lower severity of the disease and a trend towards low IL-6 levels in the patients with COVID-19 [16]. The beneficial effects of RAAS inhibitors are confirmed in a meta-analysis of a total of 24,676 patients with COVID-19, which showed a reduction in the risk of deaths and/or development of critical illnesses by about 23% (the odds ratio of 0.768.95% confidence interval (CI): 0.651–0.907, p = 0.0018) [16].

The accumulated clinical and epidemiological data on COVID-19 show that in the patients with an initially reduced level of ACE2, the expression or an impaired RAAS function (elderly patients, males and suffering from diabetes mellitus, hypertension, obesity), ACE2 depletion associated with the action of SARS-CoV-2, leads to a more severe clinical course [17].

Kallikrein-kinin system

The kallikrein-kinin system includes an inactive precursor kininogen. Bradykinin is formed from it under the action of the serine protease of kallikrein, the active metabolite of which is des-Arg 9-bradykinin. These peptides can act on two types of receptors associated with the G-protein: the type 1 bradykinin receptor (BKB 1R), the main agonist of which is des-Arg 9-bradykinin, and the type 2 bradykinin receptor (BKB 2R), which is activated by bradykinin [18].

Fundamentally different effects are associated with the stimulation of these receptors, as the activation of the BKB 2R receptor induces vasodilation, increases sodium excretion and lowers blood pressure, while the exposure to BKB 1R leads to the release of pro-inflammatory chemokines and enhances the neutrophil migration to tissues (Fig. 2).

The kallikrein-kinin system is closely related to the RAAS, since signaling of the bradykinin receptor is enhanced by the exposure to AT1-9, and ACE2 inactivates bradykinin [19]. Experimental studies have shown that des-Arg 9-bradykinin is a substrate of pulmonary ACE2, and weakening of its activity leads to impaired inactivation of des-Arg 9-bradykinin and, thus, to an increase in signaling through BKB 1R [18].

Studying the expression of separate genes in the samples of bronchoalveolar lavage from the patients with COVID-19 showed a pronounced expression of kininogen and kallikreins, which was not found in controls [5].

The expression of ACE, which cleaves bradykinin, is suppressed by a factor of 8, and the expression of BKB 2R is increased by a factor of 207, BKB 1R – by a factor of 2945. It should be notified that BKB 1R is usually expressed in a very low amount in almost all the tissues, but in this case, the expression of this receptor is pronounced, while in the controls it is practically not detected.

Circulating plasma kallikrein is activated by factor XIIa (Hageman factor) of the intrinsic coagulation pathway, which is suppressed by the C1 esterase inhibitor encoded by the SERPING1 gene [20]. In bronchoalveolar lavage samples from the patients with COVID-19, the expression of Hageman factor was not altered, while the SERPING 1 gene was suppressed by a factor of 33, which led to a decrease in the concentration of the C1 esterase inhibitor and, as a consequence, insufficient suppression of Hageman factor. This promoted the synthesis of bradykinin from kininogen and an even greater increase in the concentration of this neurotransmitter in the patients with COVID-19 [20]. The resulting "bradykinin storm" is potentially responsible for most of the symptoms seen in COVID-19: dry cough, fatigue, headaches, myalgia, dyspeptic disorders, cognitive decline, arrhythmias, and sudden cardiac deaths [5].

Complement system

The complement system includes many proteins and their cleavage products that can coordinate the inflammatory response in the sites of infection. The activation of the complement system occurs through several mechanisms, which include three main pathways: classical, lectin and alternative. The lectin pathway and its effector enzyme, mannan-binding lectin-associated serine protease 2 (MASP-2), are directly related to the lung damage in the coronavirus infection. In particular, it was found out that the nucleocapsid protein SARS-CoV-2, as well as the SARS and MERS proteins activate MASP-2, and the traces of MASP-2 are observed in the vasculature of the lung tissue of the patients with COVID-19 [21]. Activated MASP-2 initiates a series of enzymatic reactions that lead to the production of anaphylatoxins C3a and C5a, and to the formation of a membrane attack complex C5b-9 [22].

Factor C5a is the strongest inflammatory peptide in the complement system, which triggers the release of a number of pro-inflammatory cytokines [22] and TNF-α [23]. The membrane attack complex C5b-9 induces the release of IL-6 by activating the nuclear transcription factor, activating protein-1 [24] and chemotactic protein-1 of monocytes [25]. The increased concentration of C3a leads to the overproduction of IL-1, IL-6 and TNF-α [26]. In addition, the lectin pathway of the complement activation is associated with endothelial damage and thrombosis [27].

The hypothesis for the role of the components of the complement system in the pathogenesis of ARDS in the coronavirus infection has been confirmed in experimental and clinical studies. In particular, in the study by Gralinski et al, the course of SARS-CoV-1 in the mice with and without C3 deficiency (the control group) was investigated. It was established that the mice with C3 deficiency showed significantly less weight loss and less respiratory dysfunction compared to the control group, despite an equivalent viral load in the lungs [28]. And in the autopsy studies of the patients who had died from COVID-19, there were deposits of complement components (C3, C3a and C5b-9) in the lungs, and increased levels of C5a in plasma [27].

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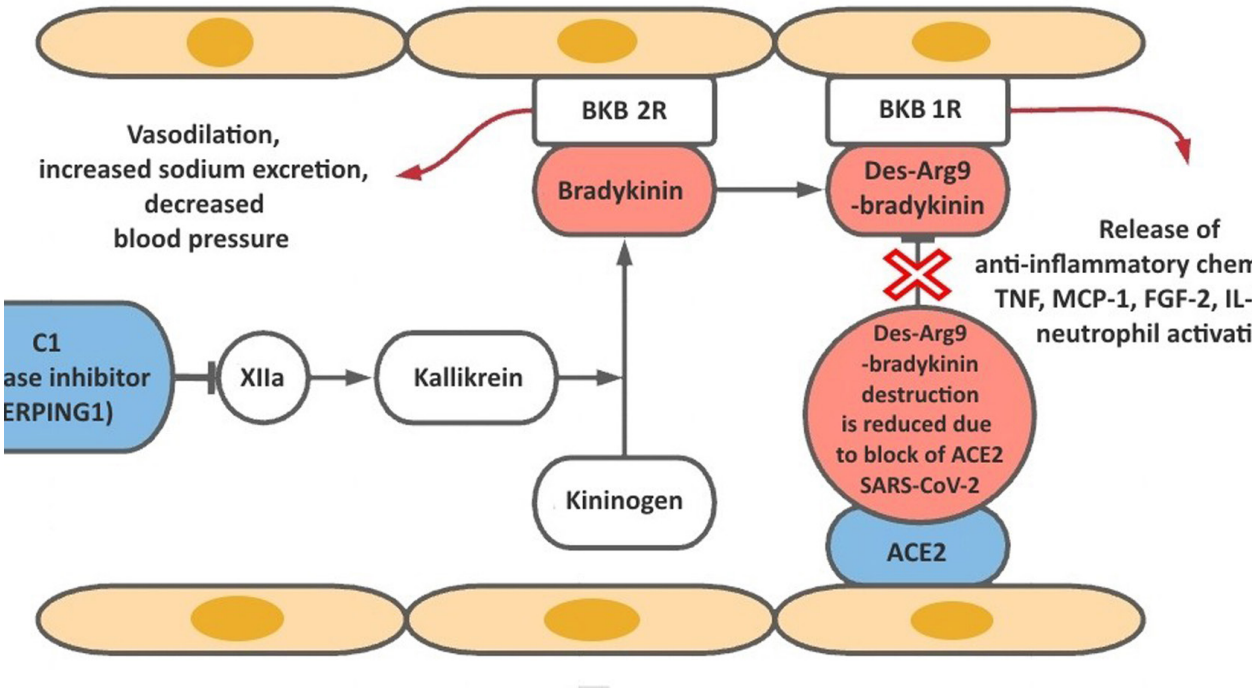

Figure 1 – RAAS is a system with two axes: the ACE/ATII/AT1R axis – pathological – and, opposite to it, the anti-inflammatory axis – ACE2/AT1-7/MasR. The penetration of SARS-CoV-2 into the cell and its subsequent suppression of ACE2, shifts the balance towards the pathological axis and, as a result, an increased total ratio of ATII to AT1-7 leads to a deterioration of lung function and lung damage

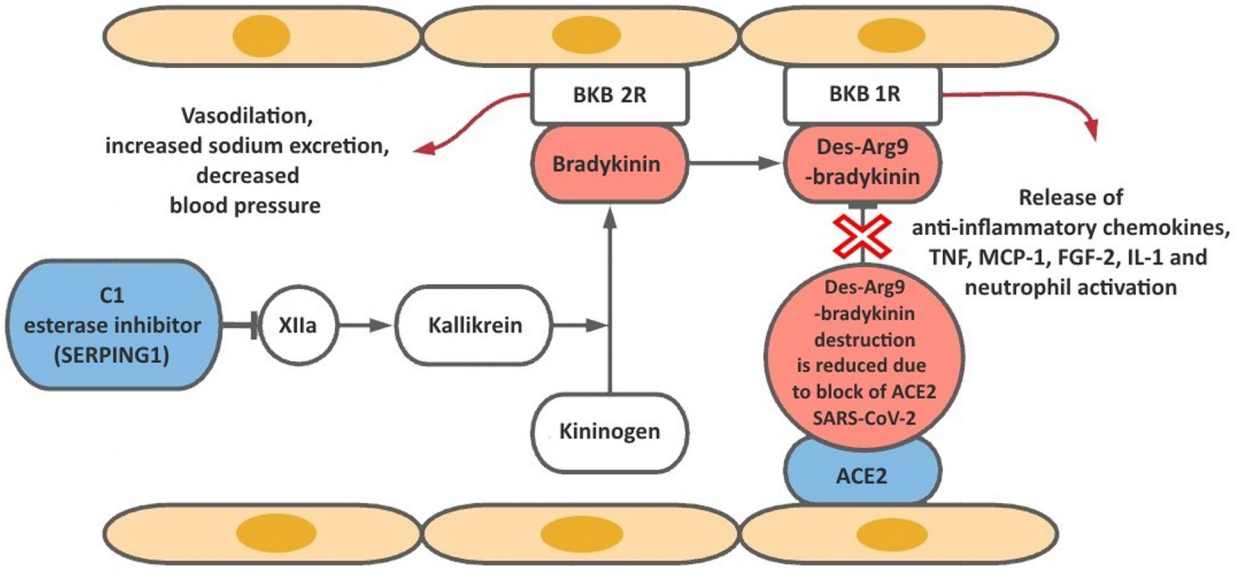

Figure 2 – Disturbances in the kallikrein-kinin system are associated, on the one hand, with increased expression of kallikrein and an increase in the formation of bradykinin and its metabolite des-Arg 9-bradykinin; on the other hand, with the suppression of the expression of the C1-esterase inhibitor, which prevents the formation of kallikrein, and violation of des-Arg 9-bradykinin inactivation by ACE2

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Table 1 – Potential targets of "cytokine storm" pharmacotherapy in COVID-19

Impact target	Therapeutic solution
	Plasmapheresis is an elimination of the components of the complement system, immune complexes and cytokines, circulating in the plasma
Renin-angiotensin-aldosterone system	Human recombinant soluble ACE2
	Administration of exogenous AT1-7
	Administration of Vitamin D
Kallikrein-kinin system plasmic	Selective BKB 1R blockers
	Conestat alfa is a recombinant form of a C1 inhibitor
	Lanadelumab is a human monoclonal antibody that binds plasmic kallikrein
	Icatibant is a selective BKB 2R antagonist
Complement system	Narsoplimab is a high affinity human monoclonal IgG4 antibody that binds MASP-2 and blocks the lectin pathway
	Eculizumab is high affinity monoclonal antibody to C5 protein
	Compstatin AMY-101 inhibits the cleavage of C3 to C3a and C3b by C3 convertases
Hyaluronic acid	Intranasal administration of exogenous hyaluronidase
	Gimecromone (4-methylumbelliferone) is an inhibitor of the expression of hyaluronate synthases (HAS2 and HAS3)
Other components of pathogenesis	Siltuximab is a preparation of monoclonal antibodies against IL-6 and tocilizumab, sarilumab preparations against IL-6 receptor Systemic glucocorticosteroids

Hyaluronic acid

Hyaluronic acid is a polysaccharide that can hold 1000 times as much as its own weight in water, to form a dense hydrogel. The HAS1, HAS2 and HAS3 genes encode membrane enzymes of hyaluronate synthase. The destruction of hyaluronic acid occurs under the action of hyaluronidase enzymes encoded by the HYAL1 genes (lysosomal hyaluronidase) and HYAL2 genes (membrane-bound hyaluronidase) [29]. The synthesis and decomposition of hyaluronic acid is regulated according to the principle of the negative feedback: hyaluronic acid stimulates CD44 (hyaluronic acid receptor), which, in its turn, induces the synthesis of hyaluronidases [30].

The hypothesis about the role of hyaluronic acid in the pathogenesis of severe forms of COVID-19 was made after the discovery of a transparent jelly-like exudate, which filled the lung tissue, at the autopsy of the patients who had died from this infection [31]. Although the nature of the detected changes had not yet been determined at that time, the assumption about hyaluronic acid was associated, first, with the fact that its accumulation in ARDS had been notified in the earlier study [32], and second, there was a violation of the regulation of hyaluronic acid production in the SARS infection [33]. Pro-inflammatory cytokines (IL-1, TNF-α), an increase in the amount of which, is observed in the lungs with ARDS, have witnessed being strong inducers of the HAS2 enzyme in the endothelium, alveolocytes and fibroblasts. The analysis of bronchoalveolar lavage of the patients with COVID-19, revealed a significant increase in the activity of the genes involved in the synthesis of hyaluronic acid: HAS1 (9113 times as active), HAS2 (493 times as active) and HAS3 (32 times as active) [5].

The CD44 gene, encoding the CD44 receptor, which is necessary for the degradation of hyaluronic acid, and the gene encoding the extracellular hyaluronidase HYAL2, were suppressed (by a factor of 11 and 5 times, respectively). As a result of the disruption of the synthesis and decay of hyaluronic acid induced by SARS-CoV-2, its accumulation occurs in the alveoli, followed by their blockage, which was later shown in the histochemical study of the lung tissue of the patients who had died from COVID-19 [34]. It is with the accumulation of hyaluronic acid in the lungs that those changes in the form of the "ground glass" on computed tomograms are established. They are found out in the pneumonia associated with SARS-CoV-2 [34].

New Potential Targets for COVID-19 Therapy

Reducing the negative effect of the excessive production of inflammatory mediators, can be achieved by conducting plasmapheresis. The effectiveness of plasmapheresis in the treatment for severe COVID-19 has been demonstrated [35]. Currently, the safety of this method in the patients with COVID-19 has not been completed. It is advisable to consider the possibility of a therapeutic effect on individual systems involved in the pathogenesis of the "cytokine storm" development in COVID-19 (Table 1).

Renin-angiotensin-aldosterone system. For over a decade, work on the first human recombinant soluble ACE2 (GSK2586881) for the treatment of ARDS has been underway. Nowadays, two phases of clinical trials have been completed. In the first phase it was demonstrated that the drug is well tolerated by healthy volunteers and does not cause cardiovascular side effects [36]. In the second phase it was proven that this drug (GSK2586881)

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does not cause hypotension, but significantly more often causes hypernatremia, dysphagia and rash [37]. At the same time, its effectiveness in reducing IL-6 levels and improving clinical and instrumental parameters in a small number of sample patients with ARDS, has not been proven [37]. The second phase re-study (ClinicalTrials.gov ID: NCT04335136), including the patients with COVID-19 and ARDS, was completed at the end of 2020. In addition, it has been recently proven that human recombinant soluble ACE2 can prevent the penetration of SARS-CoV-2 into blood vessels and kidney cells [38].

It was proposed to administer AT1-7 in COVID-19 taking into consideration the role of AT1-7 in counteracting the inflammatory effects of ATII that protected endothelial cells and prevented lung damage with the subsequent development of ARDS [39]. However, the effectiveness of the use of AT1-7 in ARDS has been demonstrated only in experimental models. No studies involving patients with COVID-19, have been carried out yet [14].

Two reviews showing the potential benefit of vitamin D in the treatment of severe forms of coronavirus infection have also been published [40]. This effect was presumably associated with its ability to reduce the level of renin and consequently reduce the production of ATII, which has been displayed in a few studies [41]. The relationship between the production of renin and the concentration of vitamin D is not clear, but some studies demonstrate the relationship of its deficiency with the development of severe forms of COVID-19 [42]. However, the positive effect of vitamin D therapy on the course of coronavirus infection was recommended only in the review studies, while in randomized controlled trials (RCTs), its effect on any pathology other than skeletal, was not notified [43]. In another meta-analysis, a regular intake of vitamin D at the doses up to 2000 IU/per day is safe and protects against acute respiratory tract infection, especially in the patients with vitamin D deficiency [44]. Thus, it seems possible that prophylactic vitamin D therapy can reduce the severity of the disease caused by SARS-CoV-2, especially in the conditions where vitamin D deficiency is common [40].

Kallikrein-kinin system. Since many studies have shown a significant role for the "bradykinin storm" in the pathogenesis of COVID-19, the exposure by inhibiting the BKB 1R could potentially have positive therapeutic effects. In the period from 2004 to 2012, several large pharmaceutical companies simultaneously filed patent applications for the registration of chemical compounds with BKB 1R selective blockade properties and showed promising results in preclinical trials [45]. Sanofi, Merck and Boehringer-Ingelheim drugs even reached Phases I and II in the clinical trials, but further studies were abruptly stopped without any explanation [45]. Such factors as limited predictability of animal models, toxicological problems, and a non-optimal pharmacokinetic profile were probably the reasons for the termination of further development of this drugs group. Anyway, there is currently no registered blocker BKB 1R drug.

A decrease in the production of bradykinin (BK) in the body can be considered another potentially promising therapeutic area.One of the ways to achieve this is influencing the activity of a C1-esterase inhibitor, which suppresses the production of Hageman factor and, as a result, reduces the amount of bradykinin. The drugs with a similar mechanism of action are used to treat hereditary angioedema, a rare genetic disease associated with a decrease in the amount or activity of a C1 esterase inhibitor. Since a number of studies have demonstrated the suppression of the SERPING1 gene and a decrease in the concentration or the activity of the C1 esterase inhibitor in the patients with COVID-19, recombinant forms of the inhibitor (Berinert – registered in the Russian Federation, Cinryze, Haegarda, Ruconest) can be used to reduce the activity of bradykinin [46]. Currently, there are no randomised controlled studies that have studied the use of this group of drugs in the patients with COVID-19. In a small uncontrolled trial five patients with the signs of hyperactivation of the kallikrein-kinin system, were prescribed conestat alfa (Ruconest), and all the patients showed positive clinical and laboratory dynamics [47].

Another drug for the treatment of hereditary angioedema is lanadelumab. Lanadelumab is a human monoclonal antibody that binds plasmic kallikrein and prevents the *cleavage* of circulating high molecular weight kininogen to bradykinin. Lanadelumab has no effect on either the C1 inhibitor or the SERPING1 gene encoding the activity of the C1 inhibitor. Icatibant blocking the binding of bradykinin to BKB 2R prevents the development of vasodilation, increases vascular permeability, the contraction of smooth muscles of visceral organs and the development of edema. The efficacy of the drug in the treatment for COVID-19 was demonstrated in a case-control study of 27 patients with less than 90% saturation. They had been prescribed icatibant or standard therapy [48].

The patients taking icatibant, showed a decrease in the need for the oxygen therapy and the improvement in oxygen saturation values within 24 hours of starting the treatment. However, there have been no full-scale randomised controlled studies to research the effectiveness of this drug in COVID-19.

Complement system. The main therapeutic ways of influencing the complement system, are to block the lectin pathway of its activation and suppress the proteins that provoke the development of inflammatory reactions – C5a and C3.

Narsoplimab is a high-affinity, fully human anti-immunoglobulin G4 monoclonal antibody that binds MASP-2 and blocks the lectin pathway of the complement activation. This drug has shown efficacy in the treatment of patients with thrombotic microangiopathy associated with hematopoietic stem cell transplantation

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and also undergoing the third phase of clinical trials in the treatment for immunoglobulin A nephropathy and atypical hemolytic uremic syndrome [27]. In August 2020, the results of this drug study in the treatment of the patients with severe COVID-19 infection and ARDS were published [27]. The study involved 6 patients who had received narsoplimab at the dose of 4 mg/kg intravenously twice a week for 2–4 weeks, they had also received standard therapy as recommended, and a respiratory support. All participants in the trial showed clinical and laboratory improvement, the development of adverse reactions associated with taking the drug, was not notified. Narsoplimab does not interfere with the activation of the complement system through the classical pathway and does not interfere with the adaptive immune response, and these are the positive aspects of its use.

Eculizumab is a high affinity monoclonal C5 protein antibody. The drug has demonstrated encouraging results in the treatment for severe COVID-19 complicated by ARDS. In several small studies, an improvement was reported in all of them [49].

Nowadays, the drugs for the treatment of various diseases associated with pathology of the complement system, which are protein C3 inhibitors, are under clinical research. One of the representatives of a new generation of highly selective and potent C3 inhibitors called compstatins, AMY-101 is undergoing phase II clinical trials, demonstrating good safety and tolerability in volunteers during phase I study [50]. This drug has been proposed to be used in the treatment of critically ill patients with a novel coronavirus infection and ARDS. The results of several studies indicating its effectiveness have already been published [51]. In one of the studies, eculizumab was compared with AMY-101. The advantage of the latter in the rate of the onset of the effect and evidence of clinical and laboratory improvement in ARDS associated with COVID-19, has been demonstrated [51]. The authors associate the obtained results with the fact that the activation of C3 is the convergence point of all complement pathways, and inhibition at the C3 level ensures the simultaneous blocking of the formation of all downstream pro-inflammatory mediators involved in SARS-CoV-2-induced ARDS.

The limitations of these studies are a small population of patients, lack of control groups, and a concomitant use of other anti-COVID-19 drugs. SOLID-C19 (ClinicalTrials.gov identifier: NCT04288713), CORIMUNO19-ECU (ClinicalTrials.gov identifier: NCT04346797) and SAVE (ClinicalTrials.gov identifier: NCT04395456) are among the few ongoing studies investigating the therapeutic effect and tolerance of complement inhibitors in the patients with COVID-19 [52].

Hyaluronic acid. Scientists from China suggested that inhalation of hyaluronidase would degrade and decrease the amount of hyaluronic acid in the respiratory tract [53]. It has been shown experimentally that intranasal administration of exogenous hyaluronidase can reduce the quantity of hyaluronic acid in the lungs and restore the lung function after the influenza infection [33]. However, most likely, this method can be effective only in the early stages of the disease [34]. Another method of therapeutic influence on the synthesis of hyaluronic acid is the use of the drug 4-methylumbelliferone (gimecromone) which can inhibit the production of hyaluronic acid by inhibiting the expression of genes of two hyaluronate synthases (HAS2 and HAS3) and blocking the last stage of hyaluronic acid formation from glucose metabolites [54]. This drug has been approved for biliary spasm treatment, but it can cause diarrhea, followed by hypokalemia. There are currently no studies on the effectiveness of 4-methylumbelliferone in the patients with COVID-19.

Other drugs for pathogenetic therapy. Among the most accessible and frequently used agents in the therapy of "cytokine storm", preparations of monoclonal antibodies against IL-6 (siltuximab) or its receptors (tocilizumab, sarilumab), as well as glucocorticosteroids (GCSs), should be singled out. IL-6 inhibitors have not been approved for the treatment of COVID-19 yet, but a number of non-placebo-controlled and observational trials in the patients with severe COVID-19 and ARDS indicate the significant potential of these drugs (primarily tocilizumab) [55]. Several metaanalyses of the tocilizumab efficacy for COVID-19 have been published. One of the recently published metaanalyses evaluated the results of controlled and uncontrolled trials separately [56]. It included 16 controlled studies with a total amount of 2,545 patients and showed a 55% reduction in mortality with tocilizumab therapy, compared to controls (the odds ratio of 0.453, 95% CI 0.376–0.547, p<0.001). In 18 uncontrolled trials involving 886 people, the death rate from severe COVID-19 ranged from 0% to 42.4%.

However, this meta-analysis did not include the unpublished results of the COVACTA randomised controlled study that showed no reduction in mortality from COVID-19 with tocilizumab compared with standard therapy. This fact questioned the potential efficacy of the drug and the ethical basis for continuing other studies [56]. However, several RCSs are still ongoing to evaluate the efficacy of tocilizumab in the patients with the novel coronavirus infection (ClinicalTrials.gov identifiers: NCT04409262, NCT04372186, NCT04356937, NCT04412772).

There remains a controversial issue regarding the use of glucocorticosteroids in COVID-19. There is evidence of both improved symptoms and a reduced mortality with the administration of this group of drugs, and the studies showing that treatment with corticosteroids for COVID-19 is either not beneficial or harmful [57]. The results of the RECOVERY randomised controlled study have now been published [58]. The study included 2,104 patients who had been prescribed dexamethasone at the dose of 6 mg/per day for 10 days, and 4,321

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patients – standard therapy. Within 28 days, the percentage of deaths in the first group was 21.6%, in the second – 24.6%. Among the patients receiving invasive mechanical ventilation, the death rate was lower than in the standard therapy group (29.3% versus 41.4%; the frequency ratio of 0.64; 95% CI, 0.51-0, 81) in the dexamethasone group. Among the patients receiving oxygen without invasive mechanical ventilation (23.3% versus 26.2%; the frequency ratio of 0.82; 95% CI 0.72 to 0.94), but not among those who did not receive any respiratory support (17.8% versus 14.0%; the frequency ratio of 1.19; 95% CI 0.91 to 1.55). Based on the available data, it can be concluded that the administration of dexamethasone is recommended in small doses only for patients in a severe condition and requiring respiratory support. A randomised controlled study is currently underway to investigate the efficacy of a high-dose of dexamethasone (16 mg / per day) in the treatment of ARDS in COVID-19 [59].

CONCLUSION

Thus, understanding the pathogenetic mechanisms of the development of a "cytokine storm" in COVID-19 opens the way for the study of new pharmacological targets and a further development of drugs that can prevent complications and reduce mortality. The efficacy and safety of most drugs for the treatment of COVID-19 remains to be studied in well-designed clinical trials.

However, already at this stage, the instruments for the treatment of pathological processes induced by a viral infection, seem to be a more reliable solution in the long term, due to the fact that viral target proteins have variability and species specificity.

This significantly limits the use of etiotropic therapy, while the typical pathological processes of the immune system's response to an infectious agent are stable, which makes it possible to use drugs without fear of therapeutic resistance.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

V.I. Petrov – review planning and editing; A.A. Amosov – review writing; A.S. Gerasimenko – review writing; O.V. Shatalova – review writing; A.V. Ponomareva – collecting materials for the review; A.N. Akinchits – review planning and editing; I.S. Kulakova – collecting materials for the review; V.S. Gorbatenko – collecting materials for the review.

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REVIEWS, LECTURES

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PHARMACOGENETIC BASES OF INDIVIDUAL SENSITIVITY AND PERSONALIZED ADMINISTRATION OF ANTIPLATELET THERAPY IN DIFFERENT ETHNIC GROUPS

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Cardiovascular diseases (CVDs) are the leading cause of disability and mortality worldwide. Increased thrombosis is the trigger point for the development of various CVDs and their complications, and therefore, therapy with P2Y12-receptor inhibitors is always pathogenetically justified and vital. However, according to the various data, 10-25% of patients treated with clopidogrel have "resistance" to antiplatelet therapy. The causes for the formation of resistance are still not clear. There is no generally accepted, standard methodology for determining resistance to antiplatelet agents. In addition, there are no methodological approaches to identify the patients with resistance to antiplatelet drugs, and standardized schemes for correcting a low sensitivity to these drugs.

The aim of this review was to summarize the available results of foreign and domestic studies devoted to the investigation of the effectiveness and safety problems of antiplatelet drugs administration from the point of view of the genetic predisposition to changes in their metabolism.

Materials and methods. For the review, the following information from scientific literature represented in open and accessible sources for the period of 1996-2020, was used: pharmgkb.org, PubMed, Scopus, Web of Science Core Collection, Elibrary. Search queries – "Genetic features+antiplatelet therapy+ethnic groups", "CYP2C19+clopidogrel+antiplatelet therapy effectiveness"; "Stent retrombosis+CYP2C19 polymorphism+ residual platelet reactivity" and "CYP2C19 polymorphism+ethnic groups+clopidogrel resistance" in both Russian and English equivalents. All these data are placed in electronic databases.

Results. Currently, the problem of the resistance formation to antiplatelet drugs is studied insufficiently. The best thought-out issue is the research of the effect of the polymorphic alleles carriage of the CYP2C19 gene on the residual platelet reactivity in the patients administrated with dual antiplatelet treatment, including clopidogrel. In general, the analysis of open literature sources indicates the presence of a statistically significant association between the carrier of slow alleles of the CYP2C19 gene and the residual platelet reactivity, clinically manifested by thrombosis and adverse cardiovascular events. The occurrence frequency of polymorphic carriage of the CYP2C19 gene varies in different ethnic groups, so it cannot be extrapolated to individual subjects, peculiar in the ethnic diversity.

Conclusion. To develop preventive and predictive measures aimed at overcoming resistance to antiplatelet agents, as well as working out methodological approaches to personalized prescribtion of this group drugs, a further investigation with the expansion of the search for causes and the study of the other genes participation of the cytochrome P450 system, is required.

Keywords: antiplatelet agents; clopidogrel; pharmacogenetics; ethnic groups; resistance to antiplatelet therapy

Abbreviations: CVD – cardiovascular diseases; CVC – cardiovascular pathology; PCI – percutaneous coronary intervention; ASA – acetylsalicylic acid; CVD – cardiovascular complication; MI – myocardial infarction; ACS – acute coronary syndrome; CHD – coronary heart disease.

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ФАРМАКОГЕНЕТИЧЕСКИЕ ОСНОВЫ ИНДИВИДУАЛЬНОЙ ЧУВСТВИТЕЛЬНОСТИ И ПЕРСОНАЛИЗИРОВАННОГО НАЗНАЧЕНИЯ АНТИАГРЕГАНТНОЙ ТЕРАПИИ В РАЗЛИЧНЫХ ЭТНИЧЕСКИХ ГРУППАХ

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Сердечно-сосудистые заболевания (ССЗ) являются ведущей причиной инвалидности и смертности населения во всем мире. Повышенное тромбообразование является пусковым моментом развития различных ССЗ и их осложнений, в связи с чем, терапия ингибиторами P2Y12-рецепторов всегда является патогенетически обоснованной и жизненно необходимой. Однако, по разным данным, у 10-25% пациентов, получающих клопидогрел, отмечается «резистентность» к антиагрегантной терапии. Причины формирования резистентности до сих пор не ясны. Общепризнанной, стандартной методики определения резистентности к антиагрегантам не существует. Кроме того, нет методологических подходов по выявлению пациентов с невосприимчивостью к антиагрегационным препаратам и стандартизированных схем коррекции низкой чувствительности к ним.

Цель. Целью написания данного обзора было резюмировать имеющиеся результаты зарубежных и отечественных исследований, посвященных изучению вопросов эффективности и безопасности назначения антитромбоцитарных препаратов с позиции генетической предрасположенности к изменению их метаболизма.

Материалы и методы. Для обзора использовали сведения научной литературы из открытых и доступных источников за период 1996–2020 гг., размещенных в электронных базах данных: pharmgkb.org; PubMed; Scopus; Web of Science Core Collection; Elibrary. Поисковые запросы – «генетические особенности+антиагрегационная терапия+этнические группы», «CYP2C19+клопидогрел+эффективность антитромбоцитарной терапии»; «ретромбоз стента+полиморфизм CYP2C19+ остаточная реактивность тромбоцитов» и «полиморфизм CYP2C19+этнические группы+резистентность к клопидогрелу» как в русском, так и английском эквиваленте.

Результаты. Проблема формирования резистентности к антиагрегационным препаратам в настоящее время изучена недостаточно. Наиболее проработанным вопросом является изучение влияния носительства полиморфных аллелей гена CYP2C19 на остаточную реактивность тромбоцитов, у пациентов получающих двойную антиагрегационную терапию, включающую клопидогрел. Анализ открытых литературных источников, в целом, свидетельствует о наличии статистически значимой ассоциативной связи между носительством медленных аллелей гена CYP2C19 и остаточной реактивностью тромбоцитов, клинически проявляющуюся тромбозом и неблагоприятными сердечно-сосудистыми событиями. Частота встречаемости полиморфного носительства гена CYP2C19 варьирует в разных этнических группах, поэтому не может быть экстраполирована на отдельные субъекты, отличающиеся этническим разнообразием.

Заключение. Для разработки превентивных и предиктивных мер по преодолению резистентности к антиагрегантам, а также методологических подходов к персонализированному назначению препаратов этой группы, требуются дальнейшие исследования, с расширением поиска причин и изучением участия других генов системы цитохрома P450.

Ключевые слова: антиагреганты; клопидогрел; фармакогенетика; этнические группы; резистентность к антиагрегантной терапии

Список сокращений: ССЗ – сердечно-сосудистые заболевания; ССП – сердечно-сосудистая патология; ЧКВ – чрезкожное коронарное вмешательство; АСК – ацетилсалициловая кислота; ССО – сердечно-сосудистые осложнения; ИМ – инфаркт миокарда; ОКС – острый коронарный синдром; ИБС – ишемическая болезнь сердца.

INTRODUCTION

The modern world is in the midst of a global epidemy of infectious and non-infectious diseases [1]. The leading cause of mortality and disability of the population is currently cardiovascular diseases (CVDs). In the Russian Federation, the pathology of the circulatory system has been diagnosed in 27355.3 people per 100 thousand of the adult population; the diseases of the cardiovascular system are 56.8% as the cause of death [2].

Cardiovascular pathology (CVP) significantly disrupts patients' habitual lifestyles, contributes to disability and medical and social dysadaptation. The role of

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excessive platelet functioning in the pathogenesis of cardiovascular complications is, to a great extent, decisive since the systemic nature of microcirculatory disorders entails further, more irreversible consequences. In this connection, pathogenetic, antiplatelet therapy of CVDs is carried out for a long time, while the prerequisites for its implementation are efficiency and safety.

In clinical practice, it is quite common to observe patients with a lack of susceptibility even to double antiaggregatory therapy. Such patients develop serious cardiovascular events: sudden deaths, myocardial infarctions (MIs), ischemic strokes, unstable angina pectoris, and stent thrombosis, which can be repeated after percutaneous coronary intervention (PCI) [3].

The lack of the effect on therapy with acetylsalicylic acid (ASA) occurs, on average, in 54.8% of patients, and with clopidogrel – in 10–25% of patients [4–6]. As shown by numerous domestic and foreign studies, the risk of developing myocardial infarctions (MIs), episodes of unstable angina pectoris, ischemic strokes in the patients with resistance to antiplatelet therapy is 2.5–3.5 times higher than in the patients sensitive to it. The studies carried out demonstrate different ethnic sensitivity to clopidogrel, due to the variability in the frequency of occurrence of the slow alleles of the CYP2C19 gene.

In the authors' opinions, in this connection, the study of the genetic causes of the resistance development in various ethnic grops, has a promising scientific and practical value.

THE AIM of this review was to summarize the available results of foreign and domestic studies devoted to the investigation of the effectiveness and safety problems of antiplatelet drugs administration from the point of view of the genetic predisposition to changes in their metabolism.

MATERIALS AND METHODS

For the review, the following information from scientific literature represented in open and accessible sources for the period of 1996–2020, was used: pharmgkb.org, PubMed, Scopus, Web of Science Core Collection, eLIBRARY. Search queries – "Genetic features+antiplatelet therapy+ethnic groups", "CYP2C19+clopidogrel+antiplatelet therapy effectiveness"; "Stent retrombosis+CYP2C19 polymorphism+ residual platelet reactivity" and "CYP2C19 polymorphism+ethnic groups+clopidogrel resistance" in both Russian and English equivalents. All these data are placed in electronic databases.

RESULTS AND DISCUSSION

Clopidogrel metabolism

Most often, clopidogrel in combination with acetylsalicylic acid is prescribed as antiplatelet therapy. Clopidogrel is a prodrug, which requires its transformation in the liver to an active metabolite that can have a disaggregation effect. The absorption of the drug in the intestine occurs with the participation of P-glycoprotein, the synthesis of which is regulated by the MDR1 (ABCB1) gene. In the case of carriage of polymorphic alleles of the ABCB1 gene (alleles CC, CT, TT), the activity of clopidogrel during its absorption can change [7]. Approximately 85% of the absorbed drug is deactivated under the action of liver enzymes, while 15%, with the participation of cytochrome P450 isoenzymes CYP1A2, CYP2B6 and CYP2C19, are converted into an intermediate metabolite 2-oxo-clopidogrel (thiolactone). Further, from the intermediate inactive metabolite, mainly with the participation of CYP2C19, the active compound R130964 is formed. It inhibits platelet aggregation through irreversible blockade of ADP P2Y12 on the platelet surface. The best known is the carriage of polymorphic alleles CYP2C19, associated with a complete loss or decrease in the function of the enzyme, in which the formation of an active metabolite of clopidogrel does not occur [8–10]. The involvement of other cytochrome P450 enzymes requires a further in-depth study and systematization.

A number of alleles associated with changes in the activity of the CYP2C19 enzyme, were revealed by methods of genetic identification: a complete loss, for example, CYP2C19*2, CYP2C19*3, CYP2C19*4, CYP2C19*5, CYP2C19*6, CYP2C19*7, CYP2C19*8; a decrease in activity, for example, CYP2C19*9, CYP2C19*11, CYP2C19*13; or an increased activity – CYP2C19*17. In the case of deprivation or a function loss of the CYP2C19 enzyme, the formation of active metabolites of clopidogrel does not occur. Clinically, it can manifest itself by the activation of the thrombus formation process. In the case of carriage of a polymorphic allele responsible for the rapid rate of metabolic reactions, some individuals may experience undesirable side effects associated with an excessive disaggregation activity, for example, varying degrees of severity of hemorrhage [11].

Experience of researchers from other countries

Foreign pharmacokinetic and pharmacodynamic studies have demonstrated a wide variability in the concentration of the active metabolite of clopidogrel and the variability in suppression of the platelet function after taking clopidogrel at a standard dose. There are significant differences in the distribution of CYP2C19 polymorphic alleles. Moreover, the differences concern both separate individuals and ethnic populations living in designated areas [12–14].

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Table 1 – Summary information on the analysis of literary sources

COUNTRY	TYPE OF RESEARCH	SUMMARY / CONCLUSIONS	reference to the literature source
USA	Randomized, double-blind, active control study (reactivity assessment using Verify Now analysis – impact on thrombosis and safety [GRAVITAS]). 2,214 patients with high reactivity to treatment in 12-24 hours after PCI, with stents, in 83 centers in North America between July 2008 and April 2010.	It has been established that the problem of clopidogrel resistance is not solved by increasing the dose of the drug. Genetic testing is required for the carriage of polymorphic alleles of the CYP2C19 gene 2 *; 3 * with a loss of the enzyme functional activity.	[15]
USA	Genome-wide, associative study of platelet reactivity and cardiovascular events in patients receiving clopidogrel (GWAS). The study involved 2,750 people of European ancestry whose DNA had been genotyped.	The response to clopidogrel is highly variable. The GWAS study did not identify any other SNP, except CYP2C19 * 2, the value of which would have reached a genome-wide significance.	[21]
USA	A prospective, randomized, controlled study of antiplatelet therapy, taking into account the association of the CYP2C19 genotype and clinical evidence.	The developed document is an update of the Consortium guidelines on the clinical use of the results of testing the CYP2C19 genotype for patients requiring antiplatelet therapy. The document indicates the presence of interindividual, interethnic variability in the frequency distribution of polymorphic CYP2C19 alleles.	[32]
GREAT BRITAIN	Associated clinical study from April 1, 1996 to April 1, 2008, 259 young patients (aged <45 years).	The genetic variant of CYP2C19 * 2 is the main factor determining the prognosis in young patients receiving clopidogrel treatment after myocardial infarction.	[22]
GREAT BRITAIN	Genome-wide, associative clinical observation in patients with myocardial infarction (MI) with ST-segment elevation and without ST-segment elevation, n = 2,208 people who received clopidogrel.	Patients – carriers of two alleles of the CYP2C19 gene * 2; * 3 – were more at risk of developing an "endpoint" than in the group of patients without polymorphic carriage.	[23]
POLAND	Position paper of the working group on antiplatelet drug resistance, appointed by the section of cardiovascular interventions of the Polish cardiological society, approved by the working group on thrombosis of the European Society of Cardiology (including 91 sources).	Studies have shown significant interindividual, ethnic sensitivity to clopidogrel. In addition, the polymorphisms in the genes of P-glycoprotein and the purinergic receptor P2Y12 (a receptor for the action of clopidogrel) and their role in the reactivity of clopidogrel were studied. It was concluded that considering the ABCB1 genotype in addition to CYP2C19, makes it possible to better predict the development of clopidogrel resistance.	[16]
GERMANY	In Germany, 2C19 681G> cytochrome P450 polymorphism and the phenomenon of high platelet reactivity against the background of clopidogrel, were studied in 797 patients in 2008.	It has been established that carriers of polymorphic, non-functional CYP2C19 alleles are associated with increased residual platelet activity and an unfavorable clinical outcome of planned PCIs during the first year.	[37]
CHINA	Genome-wide, associative clinical study investigating the effect of the carriage of polymorphic alleles CYP2C19 * 2, ABCB1 and PON1 on the pharmacodynamics of clopidogrel and clinical outcomes in 670 Chinese patients after PCI.	The results indicate the ethnic specificity of the prevalence of the CYP2C19 * 2 allele. The incidence of CYP2C19 * 2 in the Chinese is higher than in the Caucasians, therefore, the cases of ineffectiveness of antiplatelet therapy and the use of PCI in the Chinese ethnic group are comparable.	[34]
CHINA	Comparative analysis of the frequency of polymorphic carriage of CYP2C19 in comparison with Caucasian and Eastern ethnic groups.	The analysis of the CYP2C19 genotypes in 107 Thais showed that the allele frequencies for CYP2C19 * 1, CYP2C19 * 2 and CYP2C19 * 3 have ethnic specificity in the distribution. The frequencies of defective CYP2C19 alleles in the Thais, especially CYP2C19 * 3, were lower than in other eastern populations.	[36]

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COUNTRY	TYPE OF RESEARCH	SUMMARY / CONCLUSIONS	reference to the literature source
JAPAN	Associative clinical and pharmacogenetic examination of 114 patients who underwent PCI.	The authors have concluded that not only the interindividual difference in the carriage of polymorphic CYP2C19 alleles in the Japanese population affects the sensitivity to clopidogrel, but also the level of hyperlipidemia. The authors have developed a multifactorial algorithm for predicting an individual response to clopidogrel therapy.	[29]
SOUTH KOREA	16 prospective cohort studies, including 7,035 patients carrying ≥ 1 CYP2C19 LOF allele (loss of function), and 13,750 patients with the wild-type genotype were included into meta-analysis.	Stratified analysis by ethnicity of the study population showed a higher chance of adverse clinical events in the Asian population with options LOFCYP2C19 (or 1.89, 95% CI 1.32-2.72) compared with the Western population (or 1.28, 95% CI 1.00–1.64).	[33]
SPAIN	Comparative study of the occurrence frequency of polymorphic genes CYP2C8, CYP2C9 and CYP2C19 in the ethnic groups of the Spaniards (n = 282) and Ecuadorian mestizos (n = 297).	Ethnic specificity in the distribution of occurrence frequencies of polymorphic alleles CYP2C19, has been established. The frequency of CYP2C19 * 17 alleles was higher in the Ecuadorians than in the Spaniards (P <0.001), and the frequency of CYP2C19 * 3 was the same in the studied groups. There was a higher activity of CYP2C8, CYP2C9 and CYP2C19 in the Ecuadorian mestizos, in contrast to the Spaniards.	[40]
CANADA	CYP2C19-associated polymorphism of mephenytoin in the Inuit population living in Canada (n = 152), has been investigated.	Ethnic specificity in the distribution of occurrence frequencies of polymorphic alleles CYP2C19 has been established. No CYP2C19 * 2 polymorphism has been found in this ethnic group. In terms of the frequency of phenotypes and the molecular basis of polymorphism, the Canadian Inuits are close to the Caucasians, and not to the Asian ethnic groups.	[41]
CROATIA	200 blood samples to study the prevalence of CYP2C9, CYP2C19 and CYP2D6 allelic variants in the Croatian ethnic group, have been genotyped.	For CYP2C19, the most frequent alleles were CYP2C19 * 1 and CYP2C19 * 2, with frequencies of 0.85 and 0.15. The occurrence frequency of polymorphic alleles CYP2C19 in the Croatian ethnic group was comparable to the Central European and Mediterranean populations.	[43]
SINGAPORE	The functional significance of SNP CYP2C19 * 2, * 3, * 17 and in Asian patients treated with clopidogrel and the prevalence of functionally significant polymorphisms among 300 Chinese, Malays and Asian Indians, have been investigated.	Interethnic differences in the frequency distribution of CYP2C19 genotypes have been established. To study the response to clopidogrel, it was proposed to study the CYP2C19 * 2 and * 3 polymorphisms, but not * 17 in the Chinese, and the CYP2C19 * 2 and * 17 polymorphisms, but not * 3 in the Indians. All three polymorphisms must be genotyped in the Malays.	[45]
RUSSIA	The occurrence frequency of polymorphic alleles CYP2C19 in patients of the central part of Russia and Siberia has been comparatively evaluated.	Interethnic variability in the distribution of polymorphic alleles of the CYP2C19 gene has been revealed.	[47]
RUSSIA	The occurrence frequency of polymorphic alleles of metabolism genes and transport proteins in three ethnic groups of Dagestan has been determined.	Interethnic variability in the distribution of polymorphic alleles of the CYP2C19 gene has been revealed.	[14]
RUSSIA	Work has been carried out to assess the effect of polymorphisms of the CYP2C19, ABCB1 genes and a low effect of the CYP3A4 isoenzyme on the development of complications after stent implantation in patients with ACS.	There was an increased laboratory resistance to clopidogrel (PRU> 208) in carriers of polymorphic alleles CYP2C19 * 2: 53.8% versus 16.2%, which, however, did not affect the frequency of stent thrombosis.	[49]

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COUNTRY	TYPE OF RESEARCH	SUMMARY / CONCLUSIONS	reference to the literature source
RUSSIA	Clinical observation and genetic testing of 399 patients with CVP have been carried out. for 18 months.	No statistically significant relationship has been found between the level of residual platelet reactivity and the carriage of polymorphic markers of the CYP2C19 gene.	[50]
RUSSIA	The prevalence of allelic variants of the CYP2C19 gene *1, *2, *3, *17 has been studied in patients receiving clopidogrel – representatives of the West Siberian and Far Eastern regions.	Depending on the degree of platelet aggregation, groups of patients with different sensitivity to clopidogrel have been identified.	[51]

The randomized, double-blind, multicenter, controlled study GRAVITAS made it possible to draw several significant conclusions necessary for the development of a targeted antiplatelet therapy strategy. First, it was found out that there is no benefit from the use of a double dose of clopidogrel in the patients whose platelet reactivity increases sharply after percutaneous coronary intervention (PCI) [15]. Second, the study made it possible to expand pharmacogenetic testing for the CYP2C19 enzyme. 1152 blood samples were examined, 40 polymorphisms including CYP2C19 *2, *3 *4, *5 *6, *7, *8, and *17; ABCB1 and PON1, were studied. The results showed that patients with one or two polymorphic alleles of the CYP2C19 gene, in which their functional activity is lost, do not react to a double dose of clopidogrel at all. An 11-fold increase in the risk of a sustained increase in platelet reactivity within 30 days was found out in the patients who were homozygous carriers of the CYP2C19 * 2 gene, compared with the patients who had a functionally active wild type of the gene. Heterozygotes also retained a high platelet reactivity – up to 62% compared to the carriers of the wild, fast allele [15].

In Poland, interindividual variability of response to the oral administration of antiplatelet drugs was studied by Kuliczkowski et al. A position document of the working group on the antiplatelet drug resistance was developed, administrated by the section of cardiovascular interventions of the Polish Cardiological Society and also approved by the working group on thrombosis of the European Society of Cardiology [16]. The position document united and summarized all the available research results in the field of atherothrombosis and also made it possible to state a high percentage of clinical failures in the patients receiving double antiaggregation therapy, which predetermined the search relevance for individual genetic causes of resistance to antiaggregatory drugs. The carried out studies have proven a significant interindividual, ethnic sensitivity to clopidogrel.

CYP2C19*2 was found most frequently in Caucasian, African American and Asian peoples [17]. The frequency of occurrence of CYP2C19*2 alleles in Asian populations (~ 30%) was significantly higher than in the Caucasians (~13%) and African Americans (18%) [18, 19]. In the study by Sorich Michael J. et al., an association between the carriage of the CYP2C19 loss of function allele and major cardiovascular events was established. Moreover, the PCI frequency was significantly different in the ethnic groups of Europeans and Asians [20].

In a genome-wide, associative study (GWAS) of the platelet reactivity and cardiovascular events in the patients treated with clopidogrel, conducted by the International Pharmacogenetic Consortium (IPGC), it was also shown that the response to clopidogrel has a significant variability. At the same time, according to the authors, alleles of the loss of CYP2C19 function make up only a certain part in the structure of the reasons for the low response to the drug. The study involved 2,750 people of the European ancestry, whose DNA was genotyped. The GWAS study did not reveal any other SNP, except CYP2C19 * 2, the value of which would have reached a general genome significance. At the same time, in the subgroups of ischemic heart disease, percutaneous coronary intervention and acute coronary syndrome, mutations in SCOS5P1, CDC42BPA and CTRAC1 showed a general genome significance associated with the occurrence of cardiovascular death, myocardial infarction or a stroke, which requires comprehension and a further research in this direction [21].

The CYP2C19 genotype was associated with the fact of the “end point” in the form of cardiovascular death, non-fatal myocardial infarction in young patients with myocardial infarction and receiving clopidogrel at the dose of 75 mg/daily. It turned out that in the patients – carriers of CYP2C19*2 (28%) – the risk of recurrence of an acute coronary event during the first year was several times higher than in the patients – carriers of the wild type. Moreover, the authors positioned the polymorphism of the CYP2C19 genotype as the only significant predictor of the primary outcome in this patient population [22].

In England, a study was conducted in patients with myocardial infarction (MI) with a ST-segment elevation and without it (n = 2208 people) who received clopidogrel [23]. All patients underwent genotyping for the following genes – CUR2C19; CYP3A5; ABCB1; P2Y12, P2RY12; ITGB3 (IIB-IIIA receptor). For CYP2C19, the frequencies of the CYP2C19 alleles *2, *3, *4 and *5 were studied. In this study, the criterion of the “endpoint”, or

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the primary outcome, was used. It included death from any cause (strokes, myocardial infarction, stent thrombosis, etc.) during the first year after the development of myocardial infarction. In the group of patients with the presence of cardiovascular events and complications, the frequency of single nucleotide polymorphisms in the CYP3A5, P2RY12 and ITGB3 genes was significantly higher than in the group without the "endpoint". The patients carrying two alleles of the CYP2C19 gene were more at risk of developing an "endpoint" than in the group of patients without a polymorphic carriage [23].

A research group under Sibbing D., Stegherr J., Latz W.'s direction examined 772 patients who had been stented and were receiving dual antiplatelet therapy (aspirin and clopidogrel). All patients received clopidogrel 600 mg/daily as a loading dose and 75 mg/daily as a maintenance dose. Alleles CYP2C19*1 and *2 were genotyped. The primary endpoint or probable stent thrombosis was determined within 6 months after stenting. It turned out that in the patients carrying at least one variant of the CYP2C19*2 allele, the risk of recurrent thrombosis was much higher than in the patients carrying functionally active alleles [24].

Other randomized, controlled trials have shown that the patients who had undergone PCIs and were receiving clopidogrel at the dose of 300 mg/daily followed by a maintenance dose of 75 mg/daily, had a higher incidence of adverse cardiovascular outcomes (death from cardiovascular causes) and re-thrombosis of the stent, in the case of carriage of polymorphic genes encoding CYP2C19, CYP2C9, CYP2B6, CYP3A5, CYP3A4 and CYP1A2. Moreover, it was found out that the most pronounced relationship was between the carriage of polymorphic alleles of the isoenzyme CYP2C19 and death [25, 26].

The problem of searching for the causes of the resistance development to clopidogrel was studied in Portugal. According to the above studies, the patients with carriage of CYP2C19*2 and *17 had a poorer medium-term prognosis of ischemic events compared to other diplotypes. This fact also reflects the specificity in establishing associations with clinical outcomes of ischemic events [27].

Separate studies were devoted to the investigation of the polymorphism of P-glycoprotein – the ABCB1 gene responsible for the adsorption of clopidogrel in the gastrointestinal tract after the oral administration. Thus, it was shown that a decrease in the concentration of clopidogrel after a single dose of 300 or 600 mg, was observed in the patients homozygous for the ABCB1 variant of the 3,435T allele [28].

In Japan, a study was conducted by Miura G. et al. The aim was to investigate the genetic and non-genetic factors responsible for the antiplatelet effects of clopidogrel in Japanese patients undergoing coronary stent implantation [29]. It was concluded that the frequency of CYP2C19 polymorphic carriage in Japanese patients is close to the frequency of the identical single nucleotide substitutions in Asians. The researchers have assotiated the antiplatelet effects of clopidogrel in the steady state not only with CYP2C19 genotypes, but also with several nongenetic, modifiable factors, including dyslipidemia. Based on their own research results, the authors proposed a multifactorial algorithm for predicting individual responses to clopidogrel therapy [29].

In the pharmacoeconomic study by Jiang Minghuan, You Joyce H.S. et al., lifelong health costs were modeled, taking into account the quality-adjusted life years (QALYs) of the three antiplatelet regimens, in hypothetical 60-year-old ACS patients after PCIs. The first model included the administration of clopidogrel at the dose of 75 mg daily, the second – clopidogrel at the dose of 225 mg daily, and the third model suggested a universal alternative antiplatelet therapy (prasugrel or ticagrelor). It has been proved by a mathematical method that clopidogrel therapy at the dose of 75 mg daily is more cost-efficient only when the prevalence among the population of CYP2C19 alleles with a loss of function is less than 2.6%. That explains the relevance and the need for further population genetic studies [30].

In the study by Hokimoto Seiji, Akasaka Tomonori et al. it was found out that the simultaneous use of esomeprazole with clopidogrel does not cause a decrease in the antithrombotic efficacy of clopidogrel or an increase in the risk of cardiovascular events, regardless of the CYP2C19 genotype. These results are interesting from a research and practice belief, since they indicate a less significant role of modifiable factors, such as drug interactions, on the effectiveness of antiaggregatory therapy [31].

In the United States, clinical guidelines on the implementation of pharmacogenetic studies of the CYP2C19 gene in the clopidogrel therapy process, were developed by a consortium of physicians [32]. The document was worked out on the basis of the fundamental research showing that alleles of CYP2C19 function loss, disrupt the formation of active metabolites of clopidogrel which leads to a significant decrease in platelet inhibition. Ineffective CYP2C19 alleles increase the risk of serious adverse cardiovascular events among the patients receiving clopidogrel with ACS undergoing PCIs. Appropriate indications for genotype-directed antiplatelet therapy of CYP2C19 have been developed and recommendations for specific CYP2C19 alleles have been refined.

A group of South Korean scientists conducted a comparative meta-analysis of CYP2C19 gene polymorphism and the risk of adverse clinical outcomes in the patients with an ischemic heart disease of various ethnic groups who were receiving clopidogrel [33]. The meta-analysis comprised 16 prospective cohort studies, including 7,035 patients carrying ≥ 1 CYP2C19 LOF allele (loss of function) and 13,750 patients with the wild-type genotype. The results of the studies from the central and eastern parts of Europe turned out to be identical, while the ones from the western countries were contradictory.

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A stratified analysis by ethnicity of the study population showed a higher chance of adverse clinical events in the Asian population with variants LOFCYP2C19 (or 1.89, 95% CI 1.32–2.72) compared with the Western population (or 1, 28, 95% CI 1.00–1.64). In addition, the carriers of ≥1 CYP2C19 LOF allele had twice as high mortality and stent thrombosis compared with the wild-type homozygotes.

According to the Chinese researchers Tang Xiao-Fang, Wang Jing, Zhang Jia-Hui et al., the prevalence of the CYP2C19 * 2 allele in the Chinese is higher than in the Caucasians, therefore, the cases of ineffectiveness of antiplatelet therapy and the use of PCIs in the Chinese ethnic group are comparably more. The authors studied the effect of carriage of polymorphic alleles CYP2C19 * 2, ABCB1 and PON1 on clopidogrel pharmacodynamics and clinical outcomes in 670 Chinese patients after PCIs. It turned out that only alleles of CYP2C19 function loss had a dose-dependent effect on the pharmacodynamics of clopidogrel. However, the role of the ABCB1 and PON1 genotypes in the antiplatelet effect of clopidogrel has not been established [34]. The studies have been carried out to research the activity of clopidogrel in 183 Chinese patients with a stroke. After loading with clopidogrel at the dose of 300 mg and seven maintenance doses of clopidogrel at the dose of 75 mg, the platelet function was assessed. According to the results of the study, it was established that both CYP2C19 alleles (*2 and *3) are significantly associated with a maximum platelet aggregation and a low response to clopidogrel [35]. A comparative analysis of the frequency of polymorphic CYP2C19 carriage was carried out in the Thai population and in the Caucasian and Eastern ethnic groups [36]. The analysis of the CYP2C19 genotypes in 107 Thais showed that the allele frequencies for CYP2C19*1, CYP2C19*2 and CYP2C19*3 were 0.71 (95% CI 0.65–0.77), 0.27 (95% CI 0.21–0.33) and 0.02 (95% CI 0.01–0.05), respectively. The frequencies of the defective CYP2C19 alleles in the Thais, especially of CYP2C19*3, were lower than in other eastern populations.

In 2008, 797 patients in Germany studied 2C19 681G> cytochrome P450 polymorphism and the phenomenon of high platelet reactivity in the presence of clopidogrel. It was found out that carriers of polymorphic, non-functional CYP2C19 alleles are associated with increased residual platelet activity and unfavorable clinical outcome of planned PCIs during the first year. Moreover, it was found out that the residual platelet aggregation in the initial state did not differ significantly between the genotypes [37].

In 2008, 2C19 681G> polymorphism of P450 cytochrome and a phenomenon of a high platelet reactivity against the background of clopidogrel, were studied in 797 patients in Germany. It was found out that carriers of polymorphic, non-functional CYP2C 19 alleles are associated with an increased residual platelet activity and unfavorable clinical outcome of the planned PCIs during the first year. Moreover, it was found out that the residual platelet aggregation did not differ significantly between genotypes in the initial state [37].

In Poland, a group of scientists studied a platelet function in 105 patients with ACS who had received PCIs. After ACS, the patients were followed up for 12 months. During the first year of follow-up, two out of 11 patients who were carriers of polymorphic alleles 2*CYP2C19, had a recurrent development of ACS [38].

Similar studies were carried out in Florence [39]. The role of the CYP2C19*2 polymorphism in the occurrence of stent thrombosis (ST), or adverse cardiovascular events during a 6-month follow-up after PCIs with stent implantation, against the background of dual antiplatelet therapy, was assessed. In the patients with stent thrombosis or a combination of thrombosis and sudden death, a higher prevalence of carriers of the slow CYP2C19 alleles was noted.

A comparative study of the occurrence frequency of polymorphic genes CYP2C8, CYP2C9 and CYP2C19 in the ethnic groups of the Spaniards (n=282) and Ecuadorian mestizos (n = 297) has been conducted by foreign researchers. Blood samples were genotyped for alleles CY2C8*3, CYP2C9*2, CYP2C9*3, CYP2C19*2 and CYP2C19*3, CYP2C19*17 [40]. It turned out that the frequency of CYP2C19*17 alleles was higher in the Ecuadorians than in the Spaniards (P <0.001), and the frequency of CYP2C19*3 was the same in these two populations (P>0.05). Other allelic variants were found out at significantly lower frequencies in the Ecuadorians than in the Spaniards (P<0.05). There was a higher activity of CYP2C8, CYP2C9 and CYP2C19 in Metis-Ecuadorians, in contrast to the Spaniards, which, according to the authors, may mean differences in the dosage requirements for the drugs metabolized by these cytochromes, and should be also considered in the studies associated with the alleles and diseases. [40].

The CYP2C19-associated mephenytoin polymorphism was investigated in the Inuit population living in Canada (n = 152). The frequency of the polymorphic CYP2C19*1 allele, determined in unrelated subjects, was 0.12 (95% CI: 0.07–0.17). CYP2C19*2 was not detected in this ethnic group. It was concluded that Canadian Inuits resemble Caucasian rather than Asian populations, both in phenotype frequency and in the molecular basis of polymorphism [41].

A group of Iranian scientists studied the effect of polymorphism of the P2Y12, CYP3A5 and CYP2C19 genes on platelet reactivity in the postoperative period. All patients included in the study received aspirin at the dose of 80-325 mg one week before PCI. After taking 600 mg of clopidogrel at 2 hours, 24 hours and 30 days after PCIs, platelet aggregation was measured by a turbidimetric aggregation analysis with two different concentrations of ADP. The work showed that the maximum resistance to clopidogrel was observed 2 hours after taking the loading dose of the drug. At the same time, an asso-

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ciative relationship between the carriage of polymorphic CYP2C19, CYP3A5, and P2Y12 alleles and a clopidogrel reactivity in the Iranian population was not established. According to the authors, this indicates a greater influence of non-genetic, modifiable traits on the variability of the response to clopidogrel [42].

In 2003, 200 blood samples were genotyped in Croatia to study the prevalence of allelic variants CYP2C9, CYP2C19 and CYP2D6. The allele frequencies for CYP2C9*1 (wt), CYP2C9*2, and CYP2C9*3 were 0.74, 0.165, and 0.095, respectively. For CYP2C19, the most frequent alleles were CYP2C19*1 and CYP2C19*2, with frequencies of 0.85 and 0.15. For CYP2D6, the most common alleles were CYP2D6*1 (the frequency of 0.765), CYP2D6*2 (0.04), CYP2D6*3 (0.0275), CYP2D6*4 (0.14), CYP2D6*5 (0.01) and CYP2D6*6 (0.015). The study showed that in the Croatian ethnic group, the prevalence of allelic variants and predicted genotypes corresponds to other European populations and can be interpolated between the values for the Central European and Mediterranean populations [43].

In Mexico, the polymorphism of the ABCB1, CYP3A5, CYP2C19, and P2RY12 genes was studied [44]. Polymorphisms ABCB1 T3435C, CYP3A5 V3 A6986G, P2RY12 G52T, P2RY12 C34T, CYP2C19 V2 and V3 (positions g681a and G636A, respectively) were analyzed using 5' exonuclease genotyping. The CYP2C19 * 3 G636A allele was not identified in the Mexican mestizo population. However, in the study group, significant differences (P<0.05) were observed in the distribution of polymorphisms T3435C, A6986G, G681A, G52T, and C34T in comparison with the recorded frequencies in the populations of Indians of South America, the Caucasus, Asia and Africa [44]

The clinical significance of SNPS CYP2C19*2, *3, *17 and PON1 Q192R in the Chinese, Malays and Asian Indians was studied. The results were obtained indicating the presence of pronounced interethnic variability in the formation of the response to clopidogrel. The authors concluded that in the Chinese, the CYP2C19*2 and *3 polymorphisms but not *17 must be studied. While in the Indians, polymorphisms CYP2C19*2 and *17, but not *3 have a great clinical significance in the formation of resistance to clopidogrel. In the Malays, the authors propose to study all three polymorphisms for the individual selection of the dose of clopidogrel [45].

In France, within the framework of the creation of the French myocardial infarction register (FAST-MI), the modification of the P2RY12 receptor and the effect of the carriage of polymorphic sequences of this gene on the clinical efficacy of clopidogrel were studied. Associative connections were not established [46]. Apparently, the carriage of this polymorphism cannot be considered as an independent cause of clinical failures of antiplatelet therapy. It seems promising and most important to study the frequency of carriage of polymorphic alleles P2RY12, ABCB1 (C3435T, G2677T, and C136T) in combination with the study of CYP2C19 gene polymorphism both in populations and in separate individuals.

Results of Russian studies

In the Russian Federation, the frequency of occurrence of CYP2C19 polymorphisms in the Russian and Nogai populations has been studied. The frequency of occurrence of polymorphic CYP2C19 alleles in patients from the central part of Russia and Siberia has been comparatively evaluated [47]. The occurrence frequency of polymorphic alleles of metabolism genes and transport proteins in three ethnic groups of Dagestan has been determined [14]. The studies have demonstrated a wide interethnic variability in the distribution of polymorphic alleles of the CYP2C19 gene.

The work to assess the influence of polymorphisms of the CYP2C19, ABCB1 genes and a low activity of the CYP3A4 isoenzyme on the development of complications after stent implantation in the patients with ACS, has been carried out [48]. There was an increased laboratory resistance to clopidogrel (PRU>208) in carriers of polymorphic alleles CYP2C19*2: 53.8% vs. 16.2%, which, however, did not affect the incidence of stent thrombosis. The same changes concerned the study of CYP2C19*17 gene polymorphism.

The effect of CYP2C19 gene polymorphism on platelet reactivity, pharmacokinetics and pharmacodynamics of clopidogrel, the incidence of cardiovascular events and complications, has been studied [49]. As a result of genotyping, 39 out of 55 people were identified with the genotype (CYP2C19*1 / *1), 14 were heterozygous carriers (CYP2C19*1 / *2) and 2 patients were with the CYP2C19*2 / *2 genotype. It was found out that patients with a clinical resistance to clopidogrel, confirmed by laboratory tests, had a higher risk of ischemic complications.

Clinical observation and genetic testing of 399 patients with CVPs [50] have been conducted by Komarov A.L. et al. for 18 months. The main inclusion criteria were: a stable manifestation of ischemic heart disease, myocardial revascularization, an ACS episode more than a month ago, clopidogrel monotherapy (n = 83), or clopidogrel + ASA (n = 316). In this case, the daily dose of clopidogrel and ASA was 75-150 mg of each drug. The following parameters were selected as primary endpoints: death from cardiovascular causes, ACS, ischemic strokes, transient ischemic attack (TIA), coronary artery revascularization, and cases of any bleeding according to TIMI (Thrombolysis In Myocardial Infarction). The residual platelet reactivity was determined by the Born turbodimetric method in 3 and 6 months. There was no statistically significant relationship between the level of residual platelet reactivity and the carriage of polymorphic markers of the CYP2C19 gene.

The prevalence of allelic variants of the CYP2C19 gene *1, *2, *3, *17 has been studied in the patients receiving clopidogrel – representatives of the West Sibe-

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rian and Far Eastern regions. Depending on the degree of the platelet aggregation, the groups of patients with different sensitivity to clopidogrel, were identified. In this study, a statistically significant relationship between the CYP2C19*2 polymorphic variant and the change in the aggregation after taking clopidogrel, was established [51].

CONCLUSION

The analysis of the literature sources devoted to the study of the carriage of polymorphic cytochrome P450 genes involved in the metabolism of antiplatelet drugs, indicates the multifactoriality of candidate genes and the heterogeneity of the polymorphic alleles distribution of the CYP2C19 genes in ethnic groups. This creates preconditions for the need for fundamental, populations studies of hereditary sensitivity to antiaggregatory drugs in various subjects of the Russian Federation. In many countries, based on the results of determining the genotypes of CYP2C19, appropriate indications for genotype-directed antiplatelet therapy have already been developed, and recommendations for specific alleles of CYP2C19 have been refined. At the same time, we consider it necessary to further study the influence of the carriage of other polymorphic alleles of the cytochrome P450 genes – CYP1A2 and CYP2B6, as well as the polymorphism of platelet receptors on the formation of resistance to antiaggregation therapy. Based on the results of the CYP2C19 genotypes determination, appropriate indications for genotype-directed antiplatelet therapy have already been developed, and recommendations for specific CYP2C19 alleles have been refined in many countries.

At the same time, the effect of carriage of other polymorphic alleles of the cytochrome P450 genes – CYP1A2 and CYP2B6, as well as platelet receptor polymorphism, on the formation of resistance to antiaggregation therapy, is necessary to be studied further.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

B.I. Kantemirova – planning and writing the review; E.A. Orlova – collecting the material and editing the review; O.S. Polunina – review editing; E.N. Chernysheva – collecting the material for the review; M.A. Abdullaev – collecting the material for the review; D.A. Sychev – planning, working out the concept of the article.

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PHLEBOPROTECTORS BASED ON FLAVONOIDS: DOSAGE FORMS, BIOPHARMACEUTICAL CHARACTERISTICS, TECHNOLOGICAL FEATURES

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Micronized purified flavonoid fraction (MPFF) is the original phlebotropic drug. Its marketed form (Detralex®) consists of 90% diosmin and 10% of other flavonoids. Calculated as hesperidin, it is the most widely used drug today. Diosmin and hesperidin, which are parts of the majority of venoactive drugs, are sparingly water-soluble compounds, and this feature can effect on their clinical efficacy. One of the ways to increase the solubility of these compounds leading to an increase in bioavailability, is the micronization of the active ingredients.

The aim of the investigation is a comparative determination of the dynamics and dissolution efficiency of the drugs containing bioflavonoid fractions in the dissolution test, as well as the analysis of the micronization degree and its impact on technology and biopharmaceutical parameters.

Materials and methods. A biopharmaceutical release profile was determined using HPLC. Disintegration, characteristics of the shape and size of the tablets' particles were determined according to the methods of the State Pharmacopoeia of the XIV edition.

Results. The objects created with the use of diosmin and hesperidin, have been considered in detail. The role of technological solutions in relation to the corresponding dosage forms is notified. Detailed biopharmaceutical characteristics have been established with a choice of HPLC-based release control methodology. All the drugs in this group have a low water solubility leading to the maximum bioavailability for Detralex® which is about 1.26%; and no more than 0.2% for other analyzed models.

Conclusion. Detralex® dominates among the analyzed objects (tablets) in terms of the release rate. With regard to the overall quantitative indicators of release, the actual numbers are quite low, which is associated with the poor water solubility of the active substances.

Keywords: diosmin; hesperidin; Detralex® tablets; release profile; HPLC

Abbreviations: CVD – chronic venous diseases; GIT – Gastrointestinal tract; SRMR of the RF – State Register of Medicinal Remedies of the Russian Federation; MCC – microcrystalline cellulose

ФЛЕБОПРОТЕКТОРЫ НА БАЗЕ ФЛАВОНОИДОВ: ЛЕКАРСТВЕННЫЕ ФОРМЫ, БИОФАРМАЦЕВТИЧЕСКАЯ ХАРАКТЕРИСТИКА, ТЕХНОЛОГИЧЕСКИЕ ОСОБЕННОСТИ

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RESEARCH ARTICLE

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Микронизированная очищенная флавоноидная фракция (МОФФ) – оригинальный флеботропный препарат, и его выпускаемая на рынок форма (Детралекс®) состоит из 90% диосмина и 10% – другие флавоноиды, в пересчете на гесперидин и является наиболее широко используемым в настоящее время лекарственным препаратом. Диосмин и гесперидин, входящие в состав большинства веноактивных лекарственных средств, являются труднорастворимыми в воде соединениями, что может сказываться на их клинической эффективности. Одним из способов повышения растворимости данных соединений, который приводит к повышению биодоступности, является микронизация действующих веществ.

Цель. Сравнительное определение динамики и результативности растворения лекарственных препаратов, содержащих биофлавоноидные фракции, при проведении теста растворения, а также анализ степени микронизации и ее влияние на технологию и биофармацевтические показатели.

Материалы и методы. Биофармацевтический профиль высвобождения определяли с помощью ВЭЖХ. Распадаемость, характеристика формы и размера частиц таблеток определяли согласно методикам Государственной Фармакопеи XIV издания.

Результаты. Подробно рассмотрены объекты, созданные с использованием диосмина и гесперидина. Отмечена роль технологических решений в отношении соответствующих лекарственных форм. Установлена подробная биофармацевтическая характеристика с выбором методики для контроля высвобождения на основе ВЭЖХ. Все препараты данной группы обладают небольшой растворимостью в воде, что приводит к максимальной биодоступности для Детралекса®, составляющей около 1,26%; для других анализируемых моделей – не более 0,2%.

Заключение. Среди проанализированных объектов (таблетки) по степени высвобождения доминирует Детралекс®. Что касается общих количественных показателей высвобождения, то фактические числа довольно низкие, что связано с плохой растворимостью в воде действующих веществ.

Ключевые слова: диосмин; гесперидин; таблетки Детралекс®; профиль высвобождения; ВЭЖХ

Список сокращений: ХЗВ – хронические заболевания вен; ЖКТ – желудочно-кишечный тракт; ГРЛС – Государственный реестр лекарственных средств РФ; МКЦ – микрокристаллическая целлюлоза

INTRODUCTION

Chronic venous diseases (CVD) take a distinctive place among the diseases with a substantial social impact. They are manifested clinically by variable signs and symptoms that generally worsen the patients' quality of life. These are, first of all, venous outflow disorders, feelings of leg fatigue, heaviness and tightness, leg swelling and pain after prolonged standing or sitting, and, finally, trophic skin disorders including venous ulcers [1–4].

On the pharmaceutical market, there is a wide variety of drugs available for the treatment of CVD, including phleboprotectors. The drugs based on flavonoids and flavonoid complexes, mainly diosmin and hesperidin, are prevailing among them [1, 5, 6]. The primary mode of action of diosmin is capillary protection. In addition, diosmin has anti-inflammatory, antioxidant and antimutagenic effects, as well as it improves the rheological properties of blood and lymphatic drainage, which substantiate its use in the pharmacotherapy of CVD [1, 7–9]. One of the options for producing diosmin is splitting of another flavonoid, hesperidin, similar in its structure and pharmacological properties. Therefore, their combination in one drug is undoubtedly effective, which has been proven over the years of using this composition in the Detralex® drug [5, 11].

As for the physicochemical properties of the active substances, it is the structure of diosmin and hesperidin molecules that determines their practical insolubility in water. Therefore, to increase solubility and, accordingly, enhance bioavailability, a number of effective technological methods such as changing the particle size of the active substance by micronization, are used [12].

Currently, Detralex® holds a leading position among venotonics on the pharmaceutical market of the Russian Federation due to its proven efficacy associated with the presence of a micronized purified flavonoid fraction in its formula. As a result, in the long term, this drug reduces the severity of CVD symptoms, which makes it appropriate for both the treatment of CVD and the prevention of the disease progression [4].

In terms of many pharmacokinetic parameters, the effectiveness of Detralex® is enhanced due to the manufacturing features of this pharmaceutical form [12]. The active complex of Detralex® is composed of a micronized fraction of flavonoids containing 90% of diosmin and 10% of other active flavonoids equivalent to hesperidin (hesperidin, diosmetin, linarin and isorhoifolin). These components contribute significantly to the activity of the drug [13]. The oral intake of these substances in a finely dispersed state, including the form of micronized complexes, results in the creation of a larger surface of the solid phase and increases the rate and degree/completeness of adsorption in the gastrointestinal tract, which makes it possible to achieve a greater therapeutic effect. There are examples in pharmaceutical practice, such as the micronization of glibenclamide, which significantly increased its pharmacodynamic effects as a hypoglycemic agent used in the treatment of type 2 diabetes mellitus [14].

The drug absorption rate depends on the rate of release of its active substance(s), i.e. its extraction from the solid dosage form. In its turn, the release rate is influenced by a Powder Fineness, and this may require the evaluation of differences in the clinical efficacy between the drugs with the same active substance, as evidenced by the pharmacokinetic parameters [4, 13].

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Therefore, **THE AIM** of these studies was to compare dissolution profiles and effectiveness of the agents containing bioflavonoid fractions, in the dissolution test, as well as to analyze the degree of their micronization and its impact on the manufacturing process and biopharmaceutical parameters. This required using a sufficient number of buffer solutions, study time points and samples for each point.

The following drugs were used as reference objects: Detralex® (tablets 1.0 each)[1] and tablet forms, marked as A, B, C, D, X, Y, and Z (see Materials and Methods), which contain only diosmin or a combination of diosmin and hesperidin as active substances, according to the State Register of Medicines of the Russian Federation [11].

MATERIALS AND METHODS

A biopharmaceutical release profile is a parameter characterizing the rate of release of an active substance (in our case, diosmin and hesperidin) from a dosage form (tablets) in a certain period of time.[2]

The following models were analyzed: Detralex® (tablets 1.0 g) [22], as well as tablet forms of drugs: A (LP-003561), B (LP-005365), C (LP-005215), D (LP-004167), X (LP-003371), Y (LP-002517), Z (P N016081/01), according to the official website of the State Register of Medicines [11].

The release of active substances was studied the following conditions: 0-1 hour (hydrochloric acid solution pH=1.5), 1–4 hours (phosphate buffer solution pH=4.5), and 4–24 hours (phosphate buffer solution pH=7.2). The volume of the medium was 900 ml, the rotation speed was 100 rpm, and the ambient temperature was 37±0.5°C [5].

HPLC studies were carried out using the UltiMate 3000 system (Dionex, USA) with a spectrophotometric detector covering the operating wavelength range from 190 to 900 nm. The data were recorded and processed using the chromatographic data collection and the processing system Chromeleon, version 7 (Dionex, USA).

Centrifugation of the samples before the HPLC analysis was carried out on a laboratory centrifuge with SIGMA 2-16P accessories (SIGMA Laborzentrifugen GmbH, Germany). Before the analysis, the test solutions were filtered using the 25 mm syringe filters with a 0.2 µm nylon membrane (Phenomenex, USA). All sample solutions were centrifuged at 8000 rpm for 3 min. before placing them into the device.

The samples were weighed on a laboratory electronic balance LV 210-A (ZAO Sartogosm, St. Petersburg, Russia).

The pH level of the solutions was measured using a pH-meter pH-150MI (OOO "Izmeritel'naya tekhnika", Moscow, Russia).

The chromatographic conditions

The mobile phase – acetonitrile: 0.05 M phosphoric acid (23:77), a stainless steel chromatographic column Luna C18 (2) 250×4.6 mm with a particle size of 5 µm, a flow rate 0.9 mL/min, the temperature of the column 25°C, the detection at 280 nm, the injection volume 20 µl, the analysis time – over 10 minutes.

Disintegration of the tablets was determined according to the methods of the State Pharmacopoeia (14th ed., Vol. 2, Chapter.1.4.2.0015.15) [23].

Characterization of the shape and size of the tablet particles was carried out according to the State Pharmacopoeia, Chapter.1.2.1.0009.15 "Optical microscopy", using the modular brightfield research microscope B-1000BF (Optima spectator 40×400) equipped with a digital camera with a resolution of 16 MP. The microscopy of the tablets matrix of the studied drugs was carried out after a spontaneous disintegration of the tablets in an aqueous solution at pH 6.8. Herewith, the sizes of the particles in the tablet suspension, conisting of the flavonoid fraction and excipients, were measured [5].

The particle shape and size were measured using the MOV-1-16x ocular screw micrometer, an attachment to the Optima spectator 40×400 ocular microscope.

The particle size was determined by the formula [16]:

$$t = \frac{II - I}{\beta},$$

where: t is the particle size, mm; II–I is the difference between two readings on the scales of the ocular microscope, mm; β is the linear magnification of the objective lens 15×40=600.

RESULTS AND DISCUSSION

The biopharmaceutical profiles of the studied tablets are presented in Table 1.

The 24-hour release rates of the active substances were found to be 1.26% for Detralex® tablets, 0.11% for drug A, 0.066% for drug B, 0.103% for drug C, 0.106% for drug D, 0.075% for drug X, 0.033% for drug Y, and 0.202% for drug Z.

The comparisons of the biopharmaceutical profiles of all the studied drugs are presented in Fig. 1.

The presented data indicate that the main constituents of Detralex®, as well as the features of its manufacturing technology (micronization), provide a higher bioavailability of diosmin and hesperidin (1.26%) compared to the analyzed drugs (not more than 0.2%). The results of the analysis allows us to draw conclusions about the differences in the release rate of the active substances from the studied drugs (Fig. 2). If arbitrarily take the release rate of the active substances from Detralex® equal to 1 as the highest value, then the 24-hour release rates for drugs Z, A, C and D, X, B and Y will be 6, 11, 12, 17, 19 and 38 times lower, respectively.

[1] Detralex® tablets indications of use. Tyubik.Net. Available from: https://grls.rosminzdrav.ru/grls.aspx?s=%u0434%u0435%u0442%u0440%u0430%u043b%u0435%u043a%u0441&m=tn (date of access 01 Jan 2020). Russian

[2] State Register of Medicines of the Russian Federation. Available from: http://grls.rosminzdrav.ru/Default.aspx (date of access: 15 March 2020). Russian

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Therefore, to increase the diosmin and hesperidin bioavailability determined *in vitro* and *in vivo,* most of the flavonoid-based drugs require using additional technological approaches. For example, a clinical study of Garner et al. [17] provides evidence of the benefits of micronization in improving the absorption of poorly soluble diosmin. The absorption of micronized forms of diosmin was significantly more effective than the one of non-micronized forms ($P<0.001$), which was confirmed by the data on the accumulated radiation detected in the urine in the period up to 168 hours (with a predominance during the first 24 hours).

Taking into account the reliability of the urine data for studying the minimum absorbed fraction, the obtained results clearly demonstrate that a decrease in the particle size provides a more complete absorption of diosmin [18]. Such an approach improves the efficacy of treating patients with CVD and hemorrhoids, as well as reduces the side effects caused by a high concentration of unabsorbed flavonoids.

When performing the relevant biopharmaceutical tests, the requirements for *in vitro* bioequivalence studies in the frame of the biowaiver procedure were complied [1, 19, 20], which made it possible to use this procedure to assess the bioequivalence of Detralex® tablets and drugs of a similar composition and clinical purpose [16, 21, 22].

To identify the causes of the differences between the studied drugs in the release rate, additional studies were carried out by imaging tablet particles in the solution (using optical microscopy).

Comparative characteristics of the shape and size of the studied tablets' particles

As Fig. 2 shows, after disintegration, the tablet B particles consist mainly of the micronized flavonoid fraction conglomerates with binders gelatin and carboxymethyl starch (additional solvents of hydrophobic compounds) and partially free microcrystals of flavonoids. Their size ranges from 83.4×10^{-3} mm to 0.8×10^{-3} mm in length and from 56.2×10^{-3} mm to 0.42×10^{-3} mm in width, which indicates a marked heterogeneity of the microscopic structure of the drug.

As Fig. 3 shows, after disintegration, the tablet C particles consist mainly of free microcrystals of flavonoids, talc and microcrystalline cellulose and partially of conglomerates of gelatin binder with micronized flavonoid fraction.

All discovered substances, except flavonoids, are excipients and play the role of additional solvents of hydrophobic flavonoids. Particle sizes range from 31.2×10^{-3} mm to 0.8×10^{-3} mm in length and from 21.15×10^{-3} mm to 0.30×10^{-3} mm in width, which indicates the isodiametric nature of crystals and a large spread in particle size.

As Fig. 4 shows, after disintegration, the tablet Y particles consist mainly of free microcrystals of flavonoids and microcrystalline cellulose. Particle sizes are uniform and relatively equal in diameter, and range from 11.3×10^{-3} mm to 5.2×10^{-3} mm.

As Fig. 5 shows, after disintegration, the tablet D particles consist mainly of conglomerates of hypromellose and sodium carboxymethyl starch binders (the substances that improve the solubility of hydrophobic compounds) with non-micronized flavonoid particles. The mass of the particles includes a small amount of free microcrystals of flavonoids, talc and microcrystalline cellulose. Particle sizes range from 12.1×10^{-3} mm to 1.8×10^{-3} mm. The shape of the particles is generally elongated with a wide variation in size.

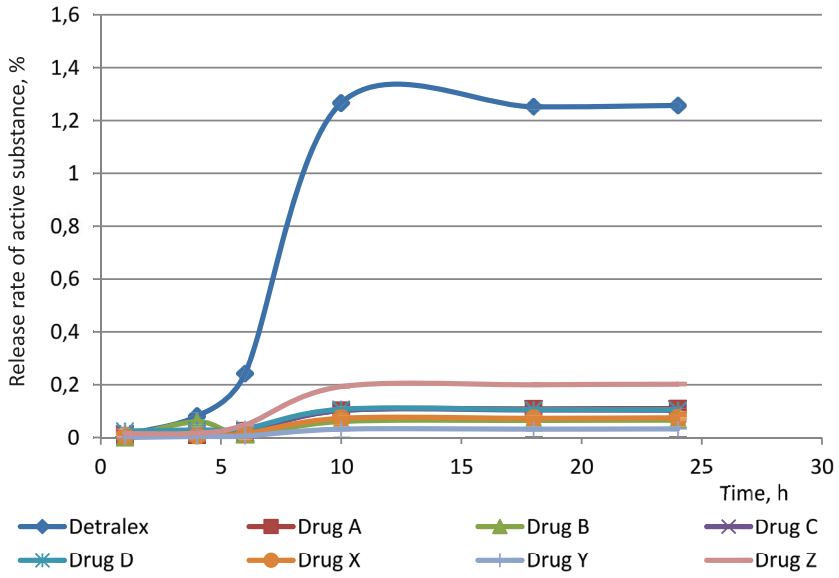

Figure 1 – Comparisons of biopharmaceutical profiles of the studied drugs containing diosmin and hesperidin

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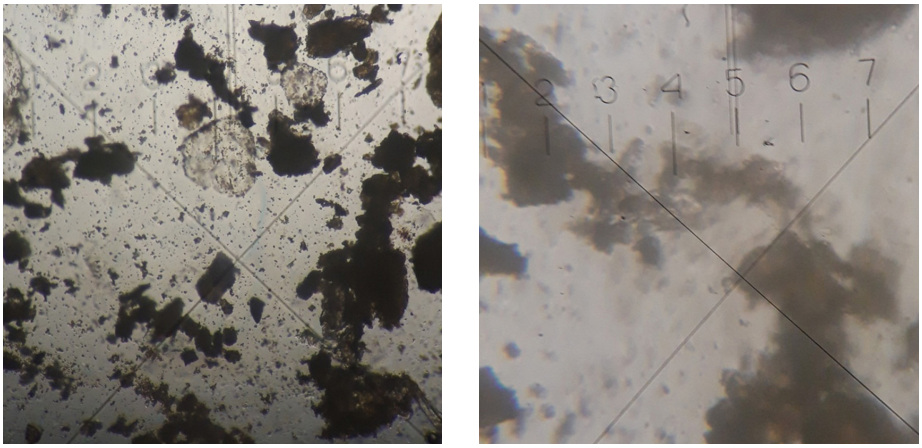
Table 1 – Biopharmaceutical profiles of Detralex® and tablets A, B, C, D, X, Y, and Z

Time	1 h	4 h	6 h	10 h	18 h	24 h
1	2	3	4	5	6	7
Detralex®						
Substance release, %	0.01	0.08	0.242	1.262	1.236	1.264
	0.009	0.078	0.236	1.284	1.288	1.293
	0.009	0.076	0.244	1.285	1.267	1.214
	0.011	0.085	0.252	1.298	1.174	1.194
	0.01	0.092	0.238	1.226	1.284	1.302
	0.01	0.076	0.239	1.239	1.263	1.273
Mean	**0.01**	**0.081**	**0.242**	**1.266**	**1.252**	**1.257**
Drug A						
Substance release, %	0.009	0.011	0.022	0.094	0.088	0.095
	0.0075	0.009	0.036	0.105	0.124	0.087
	0.0068	0.009	0.019	0.116	0.126	0.135
	0.0078	0.0085	0.024	0.084	0.115	0.114
	0.0081	0.012	0.035	0.094	0.096	0.124
	0.0065	0.011	0.021	0.123	0.105	0.108
Mean	**0.0076**	**0.01**	**0.026**	**0.103**	**0.109**	**0.110**
Drug B						
Substance release, %	0.0005	0.008	0.012	0.068	0.072	0.074
	0.0008	0.006	0.009	0.074	0.087	0.078
	0.0010	0.008	0.009	0.053	0.056	0.053
	0.0005	0.004	0.014	0.049	0.054	0.058
	0.0004	0.006	0.016	0.058	0.059	0.069
	0.0005	0.006	0.012	0.065	0.062	0.066
Mean	**0.0006**	**0.060**	**0.012**	**0.061**	**0.065**	**0.066**
Drug C						
Substance release, %	0.014	0.018	0.024	0.104	0.117	0.106
	0.017	0.014	0.018	0.114	0.101	0.118
	0.015	0.016	0.019	0.094	0.093	0.112
	0.014	0.014	0.026	0.092	0.098	0.092
	0.012	0.018	0.021	0.111	0.114	0.094
	0.014	0.019	0.018	0.097	0.102	0.097
Mean	0.014	0.016	0.021	0.102	0.104	0.103
Drug D						
Substance release, %	0.024	0.028	0.029	0.098	0.124	0.106
	0.018	0.024	0.033	0.118	0.111	0.124
	0.031	0.036	0.036	0.114	0.102	0.116
	0.026	0.028	0.041	0.096	0.094	0.091
	0.026	0.026	0.028	0.111	0.094	0.103
	0.022	0.031	0.034	0.105	0.110	0.097
Mean	**0.025**	**0.029**	**0.034**	**0.107**	**0.106**	**0.106**
Drug X						
Substance release, %	0.0005	0.009	0.012	0.067	0.077	0.077
	0.0008	0.006	0.016	0.078	0.068	0.078
	0.0006	0.008	0.009	0.079	0.075	0.075
	0.0008	0.008	0.018	0.086	0.076	0.069
	0.0004	0.006	0.018	0.061	0.071	0.081
	0.0005	0.008	0.014	0.065	0.073	0.068
Mean	**0.006**	**0.0075**	**0.015**	**0.073**	**0.073**	**0.075**
Drug Y						
Substance release, %	0	0.0035	0.0062	0.028	0.035	0.034
	0	0.0038	0.0074	0.028	0.038	0.028
	0	0.0048	0.0058	0.029	0.033	0.036
	0	0.0052	0.0064	0.039	0.029	0.031
	0	0.0044	0.0058	0.036	0.026	0.038
	0	0.0048	0.0056	0.031	0.030	0.031
Mean	**0**	**0.0044**	**0.0062**	**0.032**	**0.032**	**0.033**
Drug Z						
Substance release, %	0.014	0.014	0.054	0.188	0.214	0.198
	0.016	0.018	0.050	0.212	0.197	0.206
	0.008	0.015	0.048	0.186	0.203	0.209
	0.018	0.016	0.044	0.189	0.187	0.211
	0.016	0.021	0.048	0.198	0.197	0.193
	0.015	0.018	0.046	0.185	0.201	0.197
Mean	**0.015**	**0.017**	**0.048**	**0.193**	**0.200**	**0.202**

1. Drug B (tablets, MPFF, 500 mg)

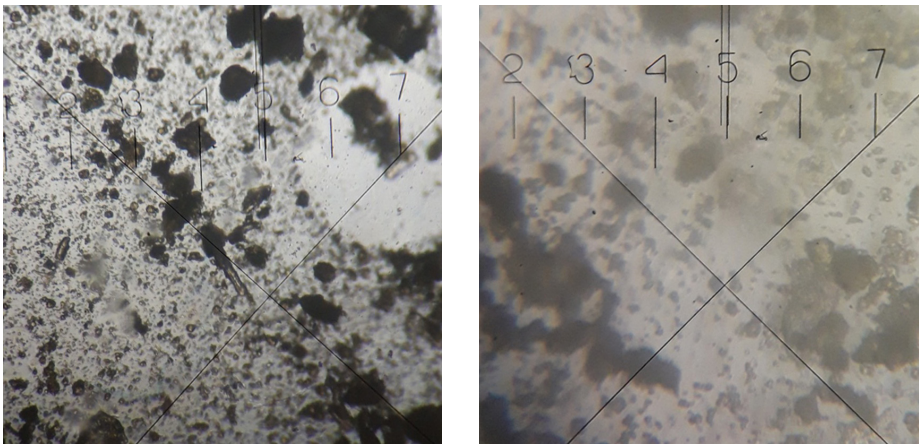


a) b)

Figure 2 – Micrographs of the drug B

Note: a) magnification 15×8; b) magnification 15×40

2. Drug C (tablets, MPFF, 1000 mg)

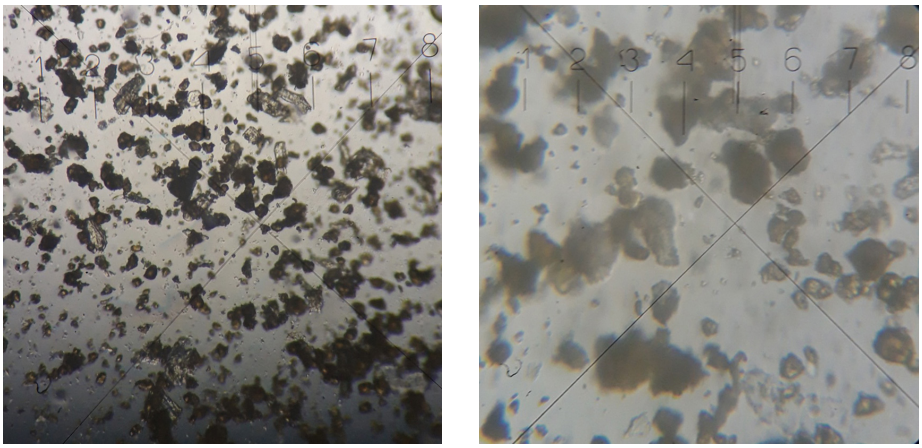


a) b)

Figure 3 – Micrographs of the drug C

Note: a) magnification 15×8; b) magnification 15×40

3. Drug Y (tablets, diosmin, 600 mg)



a) b)

Figure 4 – Micrographs of the drug Y

Note: a) magnification 15×8; b) magnification 15×40

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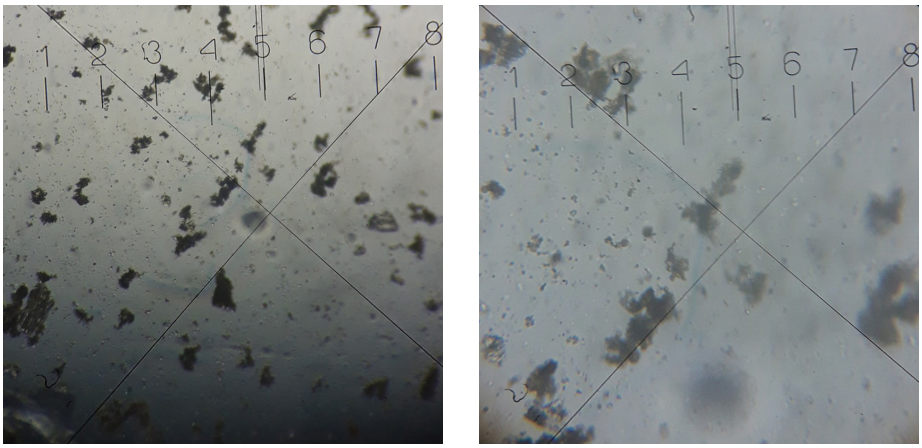
4. Drug D (tablets, diosmin 450 mg + hesperidin 50 mg)

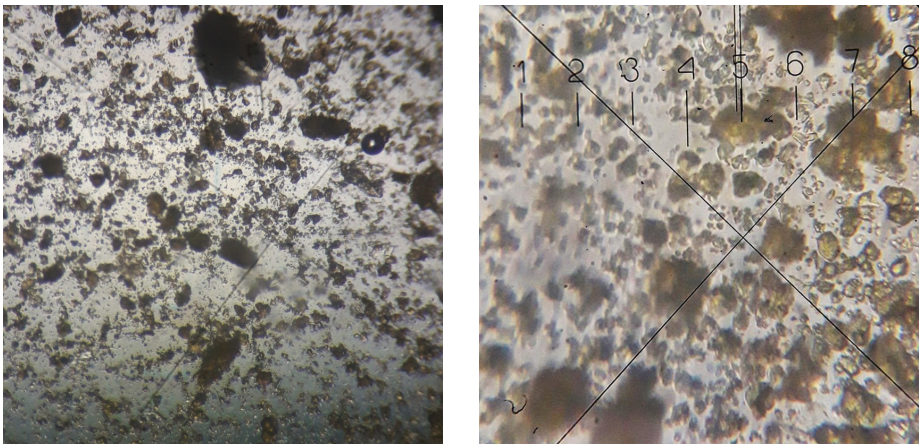


a) b)

Figure 5 – Micrographs of the drug D

Note: a) magnification 15×8; b) magnification 15×40

5. Drug Z (tablets, diosmin, 600 mg)

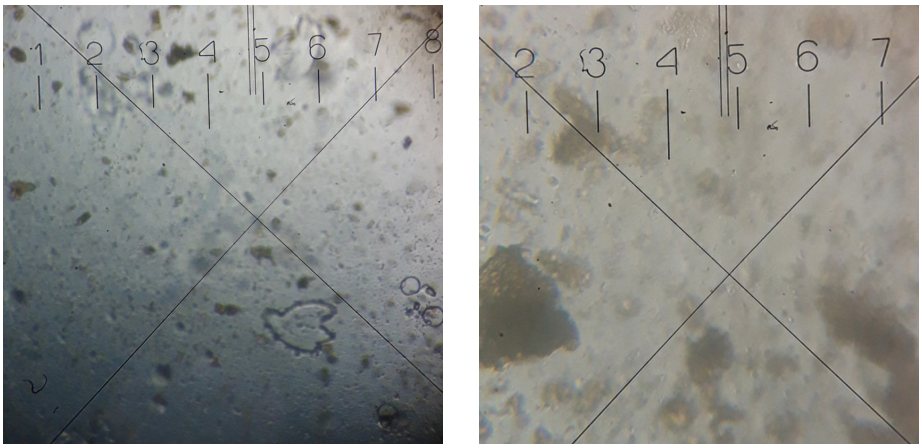


a) b)

Figure 6 – Micrographs of the drug Z

Note: a) magnification 15×8; b) magnification 15×40

6. Drug X (tablets, MPFF, 500 mg)

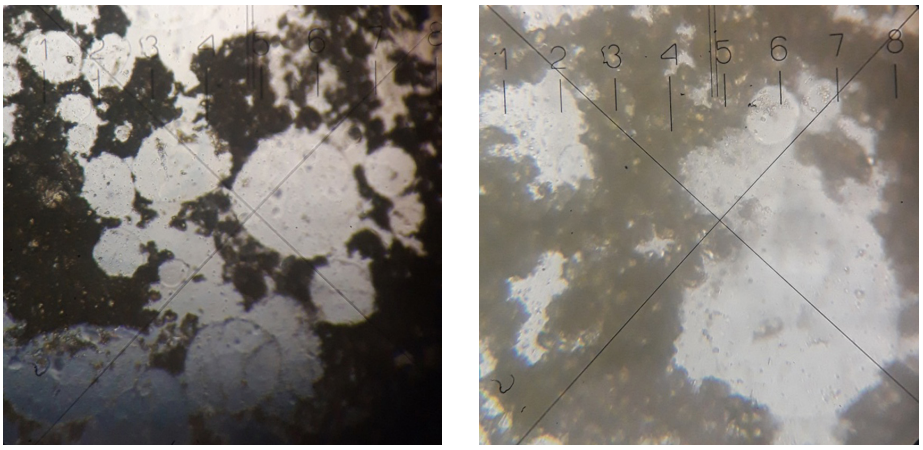


a) b)

Figure 7 – Micrographs of the drug X

Note: a) magnification 15×8; b) magnification 15×40

7. Drug A (tablets, 1000 mg: diosmin 900 mg + hesperidin 100 mg)

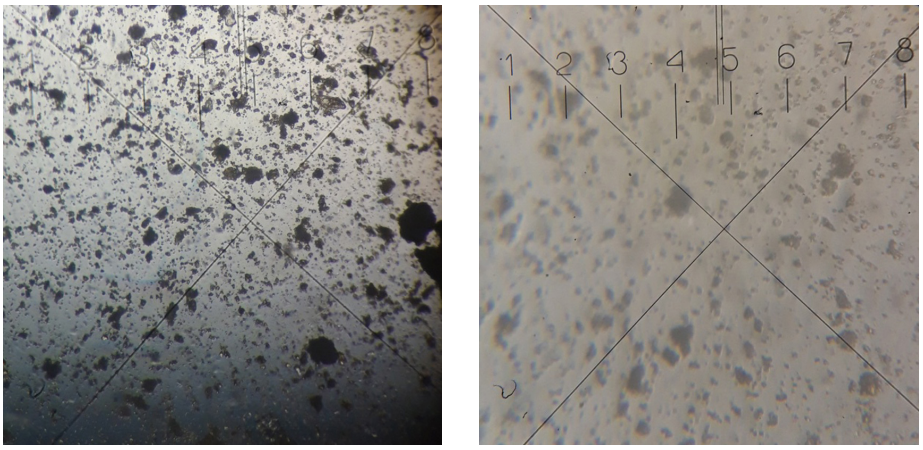


a) b)

Figure 8 – Micrographs of the drug A

Note: a) magnification 15×8; b) magnification 15×40

8. Detralex® (tablets, 1000 mg)



a) b)

Figure 9 – Micrographs of the Detralex® tablets

Note: a) magnification 15×8; b) magnification 15×40

Table 2 – Comparison of the optical microscopy gained results

Drug	Active substance(s)	Dose(s), mg	Particle size, mm	Micronization
A	Diosmin, hesperidin	900/100	$50,1–12,0\times10^{-3}$	No
B	MPFF	500	$83,4–0,8\times10^{-3}$ $56,2–0,42\times10^{-3}$	Yes
C	MPFF	1000	$31,2–0,8\times10^{-3}$ $21,15–0,3\times10^{-3}$	Yes
Y	Diosmin	600	$11,3–5,2\times10^{-3}$	No
D	Diosmin, hesperidin	450/50	$12,1–1,8\times1^{0-}3$	No
Z	Diosmin	600	$18,2–1,2\times10^{-3}$	No
X	MPFF	500	$21,1–1,0\times10^{-3}$	Yes
Detralex®	MPFF	1000	$3,3–0,8\times1^{0-}3$	Yes

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As Fig. 6 shows, after disintegration, the tablet Z particles consist mainly of free microcrystals of flavonoids, as well as inclusions of excipients microcrystalline cellulose, talc and aerosil, which improve the solubility of active flavonoids. The particles are heterogeneous in size, but have a similar diameter ranging from 18.2×10^{-3} mm to 1.2×10^{-3} mm.

As Fig. 7 shows, after disintegration, the tablet X particles consist mainly of conglomerates of micronized flavonoid fraction with binding agents carboxymethyl starch and povidone K 30, as well as inclusions of an excipient microcrystalline cellulose, which act as additional solvents of free microcrystals of flavonoids also visible in the micrograph. The particles' sizes are sharply inhomogeneous, isodiametric (relatively equal in diameter) and range from 21.1×10^{-3} mm to 1.0×10^{-3} mm.

As Fig. 8 shows, after disintegration, the tablet A particles consist mainly of conglomerates of non-micronized flavonoids with binders sodium carboxymethyl starch and gelatin, with small inclusions of excipients (microcrystalline cellulose and talc), apparently, to improve the solubility of active hydrophobic substances. The particles are represented by hardly disintegrating granules sized from 50.1×10^{-3} mm to 12.0×10^{-3} mm, which confirms the lack of micronization in the manufacturing process and, as a result, affects absorption and reduces the therapeutic efficacy of this drug.

As Fig. 9 shows, after disintegration, the Detralex® tablet particles consist mainly of free microcrystals of flavonoids and partially of microcrystalline cellulose. The particle size is uniform, isodiametric and ranges from 3.3×10^{-3} mm to 0.8×10^{-3} mm. It should be notified that microcrystalline cellulose (MCC) is used as a special carrier that promotes a more complete dispersion of hydrophobic compounds. This excipient is indifferent to the body, however, in combination with sodium carboxymethyl starch, a gel-like structure is formed, which makes it possible to significantly increase the dispersed phase of the drug and, therefore, the absorption area. MCC accepts a large amount of parietal water in the intestine, creating optimal conditions for the dissolution of drug microcrystals [12, 23–25].

For the convenient comparison of the optical microscopy data, the summarized results have been presented in Table 2. Out of the four drugs with micronized flavonoid fraction, the drug C and Detralex® have a dose of 1000 mg, while drugs B and X have a dose of 500 mg. Among all the four drugs, Detralex® is predominant with the smallest particle size and uniformity (3.3×10^{-3} to 0.8×10^{-3}), while the other three drugs have large heterogeneous particles.

Therefore, among the four drugs with non-micronized flavonoid fraction, two (A and D) contain two active substances (diosmin and hesperidin) at practically similar maximum doses (900/100 and 450/50 mg, respectively), and the other two drugs (Y and Z) contain only diosmin at the dose of 600 mg. The smallest particle size was found out in two drugs (Y and D), with relatively homogeneous particles in drug Y containing diosmin ($11.3–5.2\times10^{-3}$) and heterogeneous particles of an elongated shape with a wide scatter in size in drug D containing diosmin and hesperidin ($12.1–1.8\times10^{-3}$).

CONCLUSION

The release rates of the active substances of all the studied drugs confirm their poor water-solubility.

The studied biopharmaceutical profiles and, accordingly, revealed differences in the release rates suggest that the Detralex® formula, as well as the features of its manufacturing process (micronization) provide, in a comparative aspect, a higher bioavailability of diosmin and hesperidin. After 24 hours, it is at least 6 times larger than that of the other studied drugs (in general, no more than 0.2%), in particular of the drug Z, and, as a maximum, 38 times larger than that of the drug Y.

The optical microscopy data showed a key advantage of Detralex® over the other studied drugs due to the smallest and most uniform particle size, which indicates a high quality of this drug micronization ~~of~~ and its undoubted compliance with the GMP requirements and a beneficial effect on the drug release.

The release rates measured in this study, confirm a poor water-solubility of flavonoid compounds of all the analyzed tablet preparations and show that Detralex® is predominant among all the other studied drugs due to a more than 6 times better release rate after 24 hours. Its optimal release is also confirmed by microscopic studies and is determined by a high-quality micronization of active hydrophobic substances.

Despite the presence of excipients which can form complexes promoting better solubility, in the all the studied tablet preparations, the micronization technology used in the production of Detralex® determine its superior dissolution characteristics. Therefore, the use of micronization currently seems to be the optimal solution in terms of cost effectiveness and suggests certain advantages of this drug over the other studied preparations as far as biopharmaceutical characteristics are concerned.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

E.F. Stepanova – selection of the optimal variant of biopharmaceutical researches; introduction, conclusion and annotation writing;
I.P. Remezova – performing biopharmaceutical researches, review writing;
A.M. Shevchenko – production of dosage forms, comparative evaluation;
A.V. Morozov – performing biopharmaceutical researches;
V.K. Maltseva – selection of the optimal variant of biopharmaceutical researches, results and discussion writing.

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RESEARCH ARTICLE

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APPLICATION OF MULTIPOTENT MESENCHYMAL STEM CELL SECRETOME IN THE TREATMENT OF ADJUVANT ARTHRITIS AND CONTACT-ALLERGIC DERMATITIS IN ANIMAL MODELS

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The therapeutic effect of multipotent mesenchymal stem cells has been proven on various disease models. One of the mechanisms is the paracrine effect of the cells on the surrounding tissues.

The aim. To investigate the secretome effectiveness of the multipotent mesenchymal stem cells in the treatment of adjuvant arthritis and contact-allergic dermatitis in Wistar rats.

Materials and methods. Adjuvant arthritis was simulated in 26 female rats by the administration of Freund's complete adjuvant and then treated with the administration of 100 µl of multipotent mesenchymal stem cell secretome or saline. Contact-allergic dermatitis was modeled on 30 female rats by applying 200 µl of an oil solution of dinitrofluorobenzene to the skin on days 1, 5 and 6. Then the rats were treated with fluocinolone ointment (a positive control), baby cream (a negative control), baby cream with a secretome of native multipotent mesenchymal stem cells or from the cells processed with dexamethasone.

Results. Judging by the indicators of the longitudinal and transverse dimensions of the paws in rats and a histological examination, the secretome did not have any anti-inflammatory effect on adjuvant arthritis. A cream with a secretome from multipotent mesenchymal stem cells processed with dexamethasone, was the most effective on the model of contact-allergic dermatitis: the clinical improvement occurred on the 2nd day. The secretome from native multipotent mesenchymal stem cells and fluocinolone had a therapeutic effect on the 3rd day of application, the negative control – on the 4th day. The lymphocytic infiltration coefficient was significantly lower ($p<0.05$) in all the cases compared to the negative control (2.8±0.1). However, the lowest infiltration was observed when the cream with secretome from native (1.75±0.1) and dexamethasone-stimulated (1.76±0.1) multipotent mesenchymal stem cells was being used.

Conclusion. The cream with the secretome of multipotent mesenchymal stem cells suppresses lymphocytic infiltration more strongly than the highly active topical glucocorticosteroid – fluocinolone – on the model of contact-allergic dermatitis, which is a classic local delayed-type hypersensitivity reaction. However, a further study of the therapeutic effect of the secretome on models of systemic inflammatory diseases is required after its preliminary purification from large-molecular proteins.

Keywords: stem cells; inflammation; secretion; adjuvant arthritis; allergic dermatitis

Abbreviations: AA – adjuvant arthritis; DTHS – delayed-type hypersensitivity; GCS – glucocorticosteroids; DNFB – dinitrofluorobenzene; IL – interleukin; KAD – contact-allergic dermatitis; kDa – kilodaltons; MMSC – multipotent mesenchymal stem cells; mRNA – micro-ribonuleic acid; RNA – ribonucleic acid; CD – cluster of differentiation; FoxP3 – FOXP3, Forkhead Box Protein P; IFNγ – interferon gamma; NF-κB – nuclear factor "kappa-bi"; NK cells – Natural killer cells; STAT5 – Signal transducer and activator of transcription 5; Th1 – T-helper 1; Th17 – T-helper 17; TNFα – Tumor necrosis factor α.

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ПРИМЕНЕНИЕ СЕКРЕТОМА МУЛЬТИПОТЕНТНЫХ МЕЗЕНХИМАЛЬНЫХ СТВОЛОВЫХ КЛЕТОК В ЛЕЧЕНИИ АДЪЮВАНТНОГО АРТРИТА И КОНТАКТНО-АЛЛЕРГИЧЕСКОГО ДЕРМАТИТА НА ЖИВОТНЫХ МОДЕЛЯХ

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Терапевтическое действие мультипотентных мезенхимальных стволовых клеток доказано на различных моделях заболеваний. Одним из механизмов является паракринное воздействие клеток на окружающие ткани.

Цель. Изучение эффективности секретома мультипотентных мезенхимальных стволовых клеток при лечении адъювантного артрита и контактно-аллергического дерматита у крыс линии Wistar.

Материалы и методы. На 26 самках крыс введением полного адъюванта Фрейнда моделировали адъювантный артрит, лечили введением 100 мкл секретома мультипотентных мезенхимальных стволовых клеток или физиологического раствора. На 30 самках крыс моделировали контактно-аллергический дерматит путём нанесения на кожу 200 мкл масляного раствора динитрофторбензола на 1, 5 и 6-е сутки, затем лечили мазью с флуоцинолоном (положительный контроль), детским кремом (отрицательный контроль), детским кремом с секретомом от нативных мультипотентных мезенхимальных стволовых клеток или от клеток, обработанных дексаметазоном.

Результаты. Секретом не оказал противовоспалительного эффекта при адъювантном артрите, судя по показателям продольных и поперечных размеров лап у крыс и гистологического исследования. Наиболее эффективным на модели контактно-аллергического дерматита оказался крем с секретомом от мультипотентных мезенхимальных стволовых клеток, обработанных дексаметазоном, – клиническое улучшение наступило на 2 сутки. Секретом от нативных мультипотентных мезенхимальных стволовых клеток и флуоцинолон оказали терапевтический эффект на 3 сутки применения, отрицательный контроль – на 4 сутки. Коэффициент лимфоцитарной инфильтрации был достоверно ниже ($p<0,05$) во всех случаях по сравнению с отрицательным контролем (2,8±0,1), однако самая низкая инфильтрация наблюдалась при использовании крема с секретомами от нативных (1,75±0,1) и стимулированных дексаметазоном (1,76±0,1) мультипотентных мезенхимальных стволовых клеток.

Заключение. Крем с секретомом мультипотентных мезенхимальных стволовых клеток на модели контактно-аллергического дерматита, который является классической местной реакцией гиперчувствительности замедленного типа, сильнее подавляет лимфоцитарную инфильтрацию, чем высокоактивный топический глюкокортикостероид – флуоцинолон. Однако требуется дальнейшее изучение терапевтического действия секретома на моделях системных воспалительных заболеваний после его предварительной очистки от крупномолекулярных белков.

Ключевые слова: стволовые клетки; воспаление; секретом; адъювантный артрит; аллергический дерматит

Список сокращений: АА – адъювантный артрит; ГЗТ – гиперчувствительность замедленного типа; ГКС – глюкокортикостероиды; ДНФБ – динитрофторбензол; ИЛ – интерлейкин; КАД – контактно-аллергический дерматит; кДа – килодальтоны; ММСК – мультипотентные мезенхимальные стволовые клетки; мРНК – микро-рибонулеиновая кислота; РНК – рибонуклеиновая кислота; CD – кластер дифференцировки; FoxP3 – белок Forkhead box P3; IFNγ – интерферон гамма; NF-κB – ядерный фактор «каппа-би»; NK-клетки – натуральные киллеры; STAT5 – сигнальный преобразователь и активатор транскрипции 5; Th1, Th17 – Т-хелперы 1, Т-хелперы-17; TNFα – фактор некроза опухоли альфа.

INTRODUCTION

Nowadays, the study of the therapeutic effect of the secretome of multipotent mesenchymal stem cells (MMSC) is relevant in the field of regenerative medicine. Preclinical and clinical studies have proven the efficacy and safety of MMSCs in the treatment of atopic dermatitis, osteoarthritis and other inflammatory diseases [1–4]. The last decade has shown an increase in the research related to the study of the action mechanisms of MMSCs associated with tissue regeneration. The main

directions of the MMSC actions can be considered as follows: secretion of biologically active substances, microvesicles, exosomes [5, 6]. The secret of MMSCs contains a wide range of biologically active substances, possesses immunomodulatory properties, and therefore is potentially applicable in therapy without any use of the cells themselves [7].

Unlike the secretomes of MMSCs, topical glucocorticosteroids (GCSs), which are widely used in therapy, have a number of disadvantages. First, GCSs can lead to local and systemic side effects, the development of which is unlikely when using MMSC secretomes [8]. In addition, contraindications to the use of GCSs are burns and wounds, which, on the contrary, can be indications for the use of MMSC secretomes.

Dexamethasone is a powerful synthetic glucocorticoid that is widely used in the treatment of inflammatory diseases, as well as in cell technologies to enhance the MMSC differentiation in osteo-, chondro- and adipogenic directions *in vitro* [9]. It has been shown that the effect of dexamethasone on apoptosis, cell cycle, proliferation, and the MMSC differentiation depends on the exposure time and the drug concentration [9]. Dexamethasone also affects the profile of RNA and mRNA expressed by MMSCs [10], the migration ability and a cell shape [11]. Dexamethasone-enhanced MMSCs inhibit CD69 expression and IFNγ production, as well as STAT5 phosphorylation in NK cells [12]. It may be possible to use GCSs to suppress the synthesis of proinflammatory cytokines indirectly, using dexamethasone-treated MMSCs and their secretomes, avoiding the increased risk of the complications characteristic of GCSs.

It has been established that the MMSC secretion reduces the production of proinflammatory cytokines and the cytokines by blood mononuclear cells involved in the cell-mediated immune inflammation [13]. In the literature, it has been shown that the galectin network mediates the immunomodulatory effects of MMSCs [14, 15]. The stimulating effect of the adipose tissue MMSC secretome, which contains more than 100 proteins [16], on the proliferative and migratory capacity of various types of skin cells, has been shown [17]. In rheumatoid arthritis, the ability of human MMSCs from the adipose tissue to regulate a wide range of inflammatory mediators together with suppression of Th1 and Th17 responses, has been shown [18]. Earlier, a pilot study of the effect of the secretome of native and dexamethasone-stimulated MMSCs on the course of experimental diseases, had been conducted [19]. In this regard, the appropriate disease models – adjuvant arthritis (AA) and contact-allergic dermatitis (CAD) have been selected. They reproduce an autoimmune reaction similar to the pathogenesis of rheumatoid arthritis [20] and a delayed-type hypersensitivity reaction (DTHS), respectively.

THE AIM of the study was to investigate the secretome of the multipotent mesenchymal stem cells effectiveness in the treatment of inflammatory diseases – adjuvant arthritis and contact-allergic dermatitis – in Wistar rats.

MATERIALS AND METHODS

Obtaining MMSC secretome

MMSC secretome from human adipose tissue was obtained by culturing cells at passage 4 in a gas incubator (37°C, 5% CO_2) for 48 hours in a serum-free nutrient medium without phenol red DMEM/F12 (Paneko, Russia). Some of the cells were treated with 10 µmol/ml dexamethasone. Then, the preparation was washed free from dexamethasone with phosphate-buffered saline and cultured for 48 hours a in serum-free DMEM/F12 nutrient medium without phenol red (Paneko, Russia). The supernatant was collected and concentrated using a Vivaflow 200 ultrafiltration unit (Sartorius, Germany) on membranes with MWCO 3 kDa. Galectin-1 was chosen as a protein for standardization, since its presence in the MMSC secretome have been proven and its anti-inflammatory properties are known [20, 22]. The content of galectin-1 in the concentrated secretome was determined with the help of enzyme-linked immunosorbent assay using CloudClone Corp. kits. (USA).

Then the concentration of galectin-1 was adjusted to 6 pg/ml using phosphate-buffered saline, since galectin-1 had been chosen as an identifiable factor in order to standardize the drug. Calculated using 1 g of total protein, in the MMSCs secretome treated with dexamethasone there was, on average, 1.5 times more galectin-1 than in the secretome of native MMSCs. For the subsequent calculation of the protein concentration, the optical density was measured using a Nano Photometer No 60 (Implen, Germany). The obtained secretome was sterilized using filters with a pore diameter of 0.22 µm (Merk, Millipore, USA) and stored at –20°C until use. The methods for preparing the secretome is described in detail in patent RU 2747024 "Composition with anti-inflammatory and immunosuppressive activity based on the secretome of multipotent mesenchymal stromal cells, and method of preparation".

Animals

Mature rats of both sexes of the Wistar line (weight 200-260 g) were obtained from the nursery of the Research Center for Biomedical Technologies of the Federal Medical and Biological Agency, the Stolbovaya branch (Stolbovay settlement, Moscow region). The animals were kept under controlled conditions: individual ventilated cages with the temperature of 21–22°C and the air humidity of 55–60%. The light conditions were: 16 hours of light and 8 hours of darkness. The animals were fed with all-in-one feed. Wood sawdust was used as bedding. To anesthetize the animals, Zoletil 100 (Virbac, France) was used at the dose of 1 mg / 100 g of animal weight. The work was carried out in compliance with all bioethical standards in accordance with the "European Convention for the Protection of Vertebrate Animals

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used for Experimental or Other Scientific Purposes" [Directive 2010/63 / EU].

Study design

To model AA, 26 female rats were injected with 0.1 ml of Freund's complete adjuvant (Sigma, United States) into the plantar surface of the hind paws [21]. The choice of female animals for the study was due to the increased prevalence of the simulated disease among women [3]. The introduction was repeated a week later. Two weeks after the first injection, the edema of the hind legs and lameness were observed. Then, 100 µL of MMSC secretome was injected subcutaneously into the right hind paw of the experimental animals (10 rats). 10 rats of another experimental group were injected with 100 µl of the secretome from MMSCs pretreated with dexamethasone. 6 rats of the control group were injected with 100 µl of saline in the right hind paw. The left hind paws remained untreated. The edema of the paws was measured using an electronic caliper (Enkor 10740, Russia).

As a CAD inducer, a 3% solution of dinitrofluorobenzene (DNFB) was used on the 1st day. Then, on the 5th and 6th days, a 1% solution of DNFB in olive oil was applied in the volume of 200 µL to the depilated skin of the upper half of the back of 30 female rats of the Wistar line [23]. On the 6th day, inflammation with hyperemia, skin edema and peeling developed. The inflammation was controlled by a single daily application of baby cream (Avanta, Russia) (the negative control), the ointment with topical GCS fluocinolone at the concentration of 0.025% (Flucinar, Jelfa, Poland) (the positive control), baby cream with the addition of a secretome from the dexamethasone treated MMSCs or a secretome from untreated MMSCs. In this respect, in the animals in all the 3 groups, the studied drugs (secretomes), or the positive control (fluocinolone), were applied to the area of the right scapula, and the negative control (baby cream) – to the area of the left scapula. That made it possible to avoid the need to create a separate control group and the associated variability of the individual response. The baby cream was chosen as the base, to which the MMSC secretome was added, due to its availability and the already known, clinically investigated properties of the cream.

The animals were withdrawn from the experiment by cervical dislocation under anesthesia. After a visual assessment and photoprotocol of the body parts exposed to experimental effects, the pieces of the skin or hind paws were excised and fixed in a 10% formalin solution. Histological preparations stained with hematoxylin and eosin, were prepared in a standard manner. The study of micropreparations was carried out under a Lomo microscope equipped with a DV1000 video camera and the McrA-View 7.3.1.7 program (LOMO-microsystems, Russia). For a quantitative assessment of the lymphocytic infiltration degree of the dermis, a semi-quantitative assessment of the of infiltration degree was performed: the weak one (1+) – up to 5 lymphocytes in the area of the papillary layer; the moderate / medium one (2+) – up to 5–10 lymphocytes; the strong / pronounced one (3+) – up to more than 10 lymphocytes. The data were statistically processed using an MS Excel 2016 spreadsheet, with the calculation of the average infiltration coefficient. Complementarily, a frequency analysis of the pronounced infiltration degree was made. This infiltration was accompanied by epidermal lesions, and it was dependent on the drugs used for its treatment.

Statistical processing of results

For statistical analysis, the Pearson Chi-square test with Yates correction, the Fisher test, and the Mann-Whitney U-test were used. The significance level was assumed to be $p<0.05$. The calculations were performed using SPSSStatistics 17.0 software (IBM, USA).

RESULTS

In terms of the longitudinal and transversalis dimensions of the paws in rats with AA, there were no statistically significant differences between the groups. Histological study showed that the use of the MMSC secretome led to the development of chronic arthritis and periathritis with hyperplastic synovitis of an immunoinflammatory nature without involvement of osteochondral structures (Fig. 1, Fig. 2). In the control group, with the introduction of saline, there was a gradual decrease in inflammation in the joints of the paws, the same on both sides. Histologically, single plethoric capillaries without signs of inflammation were found out in the synovial membrane of the joints.

A macroscopic assessment of the skin areas with experimental CAD in the negative control group and their histological examination showed that the used model of the disease adequately reproduces the changes characteristic of a delayed-type hypersensitivity reaction: hyperemia and infiltrative-inflammatory skin changes, mainly around the hair funnels, abundant scaly keratic masses were observed. In some cases, they took place before the formation of large hyperkeratotic plaques. Histological examination (Fig. 3) revealed inflammatory changes due to hyperkeratosis, lymphocytic-macrophage infiltration with single eosinophils, edema and vascular reaction.

When assessing the degree of lymphocytic infiltration using a gradation by the number of lymphocytes in the field of view, it was found out that low (1+) and medium (2+) degrees prevailed, being observed, in total, in 67.2% of the evaluated dermis areas (Table 1). A high degree of lymphocytic infiltration prevailed in the deep areas of the dermis. Lymphocytic and lymphocytic-macrophage infiltrates were often located directly around the venules, creating a picture of lymphocytic venular vasculitis characteristic of CAD. Separate accumulations of mononuclear elements looked like granulomas.

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Table 1 – Index of lymphocytic infiltration in different CAD treatment groups

Group	Lymphocytic infiltration index, mean ± standard deviation
Treatment with baby cream K (–)	2.8±0,1
Fluocinolone K (+) treatment	2.18±0.08*
Treatment with baby cream with MMSC secretome	1.75±0.1*#
Treatment with baby cream with secretome from MMSCs treated with dexamethasone	1.76±0.1*#

Note: * – there are statistically significant differences in comparison with the K (–), p <0.05; # – there are statistically significant differences in comparison with the K (+) group, p<0.05

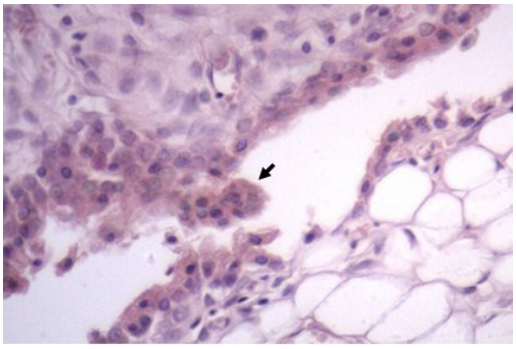

Figure 1 – General aspect of joint with adjuvant arthritis

Note: Stained with hematoxylin and eosin, Magnification ×100.

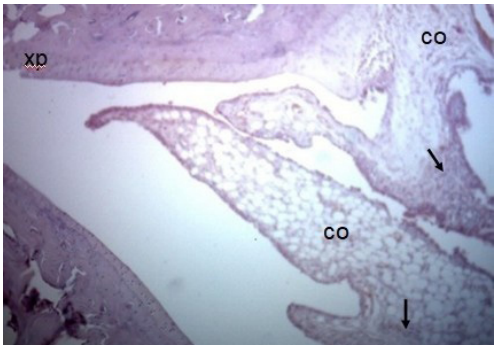

Figure 2 – Hyperplasia of synoviocytes with the formation of pseudopapillary structures (arrow)

Note: Stained with hematoxylin and eosin, Magnification ×100. XP – an articular cartilage bone without visible morphological changes; CO – folds of the synovial membrane with diffuse focal inflammatory infiltration (arrows)

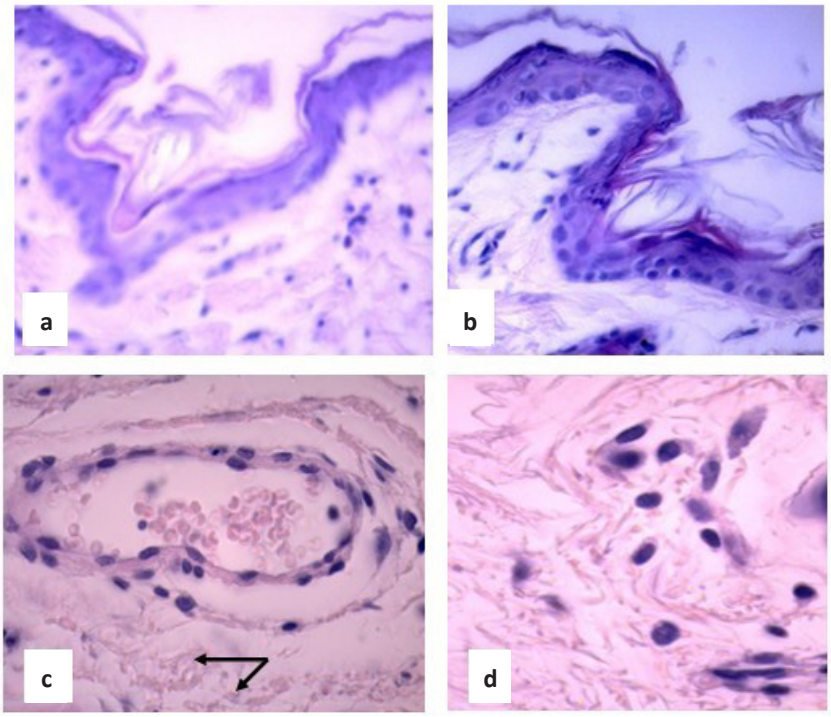

Figure 3 – Skin changes characteristic of contact-allergic dermatitis

Note: Stained with Hematoxylin and eosin, Magnification X400; a, b – epidermis with foci of hyperkertosis and exfoliating of the stratum corneum, edema of the papillary layer, low density of lymphocytic infiltrate; c – sanguine venule on the border with hypodermis, perivascular edema; d – accumulation of immature fibroblasts in the papillary layer; no inflammatory elements

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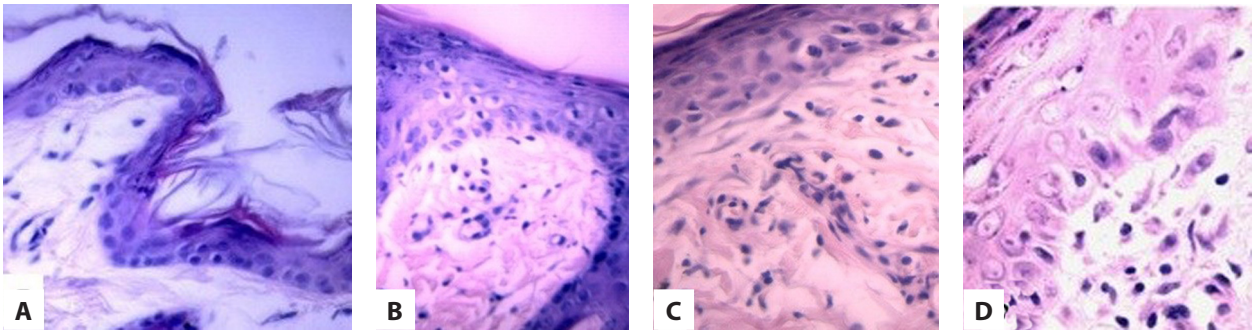

Figure 4 – Lymphocytic infiltrate

Note: Stained with Hematoxylin and eosin, Magnification X400. A – negative control group (baby cream); B — positive control group (treatment with fluocinolone); C – treatment group with baby cream enriched with MMSC secretome; D – treatment group with baby cream enriched with MMSC secretome, treated with dexamethasone

In the animals with simulated CAD and treatment with 0.025% fluocinolone ointment, clinical improvement occurred on the 3rd day of the drug administration. The most significant remaining symptom was diffuse skin erythema. At the same time, the epidermis was without destructive changes, had changes in the form of preservation of foci of hyperkeratosis, desquamation of the stratum corneum. Microscopically, these changes corresponded to plethora of dermal microvessels, edema of the papillary layer, perivascular edema in the deeper tissues. In the epidermis, there was thickening of the stratum corneum with areas of its stratification and desquamation, both as separate keratic scales and as the whole layers of keratic masses. The assessment of the composition and severity of cellular infiltrates in the dermis revealed the predominance of low and medium representation of the lymphocytic component in the whole dermis, with a lesser degree in the papillary layer, in contrast to the deep layers. Quantitatively (Table 1), this was reflected in the values of the lymphocytic infiltration index, which turned out to be significantly less ($p < 0.05$) in comparison with the negative control group. Separately, for the papillary layer, the index was significantly less ($p = 0.004$) than in the negative control group. In addition, in a nonparametric statistical analysis of the effect of fluocinolone on the frequency of a high degree of lymphocytic infiltration, it was found out that in general, for the dermis it was insignificant, and separately for the papillary layer it was significantly mean ($p = 0.03$). However, at the qualitative level and in the quantitative assessment of lymphocytic infiltrates, the effect is unequal in the superficial portions of the dermis, where it is more pronounced, and in its depth. In the papillary layer, both accumulations of immature fibroblasts and a noticeable number of fibroblasts with morphological signs of a functional activity were often found out.

Externally, the state of the skin treated with the baby cream with a MMSC secretome was similar to that observed when the skin was treated with fluocinolone: diffuse erythema, small scaly keratic deposits, foci of the epidermis destruction were absent. An improvement in the condition of the skin was also observed 3 days after the beginning of treatment, as in the fluocinolone group. Histological examination also revealed similar changes in the form of thickening and desquamation of the stratum corneum, edema of the papillary layer, plethora of dermal microvessels. In the papillary layer of the dermis, accumulations of mature fibroblasts with signs of a functional activity were noticeable. A high degree of lymphocytic infiltration was detected only in 10% of the examined skin areas. Accordingly, the infiltration index turned out to be significantly lower ($p=0.05$) in comparison with the negative control and the positive control (fluocinolone treatment) for the dermis as a whole ($p < 0.05$), but did not statistically differ from the papillary layer indicator in this group (Fig. 4).

According to nonparametric criteria, the degree of influence of the secretome on the frequency of a high degree of lymphocytic infiltration was moderate but significant ($p=0.016$). Thus, the MMSC secretome has a positive effect comparable to that of fluocinolone. There is a certain superiority of the secretome, which is due to the presence of an anti-inflammatory effect in the entire thickness of the dermis, as evidenced by the given statistical indicators.

In the experimental group, after the use of the MMSCs secretome cream pre-treated with dexamethasone, the macro- and microscopic patterns of skin changes were similar to those in the previous group. However, a clinical improvement was observed earlier – 2 days after the start of this cream therapy. The skin was erythematous, with dusty and finely scaly keratic overlays, but without destructive foci in the epidermis. Histologically, the foci of thickening of the epidermis were revealed, mainly due to the stratum corneum, which was characterized by stratification and sloughing of stratum corneum layers. The papillary layer of the dermis was with varying degrees of edema. Cellular infiltration was qualitatively and quantitatively different in different parts of the dermis in dependence to the depth. In the papillary layer, with a predominance of low and medium degrees of lymphocytic infiltration (a total of 71% of the assessed areas), there were a few foci with dense lymphocytic infiltrate penetrating into the growth layer of

the epidermis with the formation of peripolysis patterns. In the deep layers of the dermis and in the adjacent hypodermis, the infiltrate was more polymorphic due to the presence of a few eosinophils and partially degranulated mast cells. A quantitative assessment of lymphocytic infiltration gave the results similar to the previous group, including the negative and positive control groups in comparison. In comparison with the negative control group, the lymphocytic infiltration index was significantly lower ($p = 0.001$), but this difference is also significant only in comparison with the dermis on the whole. The groups with the secretome and the baby cream did not differ in this indicator. According to nonparametric estimates, the effect of this drug on the incidence of high lymphocytic infiltration was weak.

According to the main morphological criteria, there are reliable anti-inflammatory effects of the cream with the secretome from dexamethasone-treated MMSCs and untreated MMSCs, which are not inferior to the reference drug fluocinolone, even exceeding its prevalence throughout the entire thickness of the dermis. Thus, the drugs worked equally, but according to nonparametric statistical criteria for the effect on the frequency of a high degree of lymphocytic infiltration, a definite advantage of the cream with MMSC secretion has been revealed.

DISCUSSION

Currently, clinical studies are focused on the therapeutic effects of MMSCs and their exosomes in osteoarthritis [24, 25]. The ability of human MMSCs from adipose tissue has been shown to regulate a wide range of inflammatory mediators together with the suppression of Th1 and Th17 responses in rheumatoid arthritis [26]. The use of cells or their exosomes containing a spectrum of different biologically active substances can provide a therapeutic advantage along with the usual treatment protocols using immunosuppressants. In the literature, there are also data on the study of the effect of stimulated with IFNγ and TNFα *in vitro* MMSCs or their secretome, on the model of osteoarthritis. In this case, the injection of the MMSCs secretome, which had been isolated from the bone marrow of the elderly, as well as the injection of the cells themselves, led to an early reduction in pain and had a protective effect on the development of cartilage damage, without any effect on the subchondral bone in mice [27]. Another scientific source describes an experiment with the induction of osteoarthritis in mice, which was then treated with the injection of the MMSC secretome [28]. In this case, the therapeutic effect of the MMSC secretome led to a significant decrease in synovial infiltrate and hyperplasi-synovial intima and cartilage compared to the control group (the introduction of a nutrient medium). The effect of the MMSC secretome observed in our experiment, may be due to the presence of large-molecular xenogenic proteins in the secretion of human MMSCs, which are also able of causing an immune response – collagen arthritis. The anti-inflammatory and immunosuppressive factors present in the secretome, were unable to prevent its development in this model. The results described in the literature, do not agree with the ones obtained in this investigation, since the authors used a secretome from allogeneic MMSCs. The effectiveness of the cream with the MMSCs secretome on the CAD model, shown by the authors of the present article, is a demonstration of the classic local reaction of delayed-type hypersensitivity. It is not inferior to the topical GCS of high activity – fluocinolone, and even surpasses it in terms of the decrease in lymphocytic infiltration ($p<0.05$). That being the case, since large-molecular proteins are not able to penetrate through the epidermis into the deep layers of the skin, in the CAD model, the effect of increasing inflammation was not observed as in the AA model. The difference in the efficiency of secretomes in the two models of diseases, can also be due to the following. To be used in systemic models, it is necessary to purify the secretome from large-molecular compounds and / or utilize allogeneic cells to obtain it.

It has been shown that the MMSC extract from the human umbilical cord suppresses the T-cell response and NF-κB-dependent activation of transcription in keratinocytes, thereby reducing inflammation in atopic dermatitis [29]. As therapy for skin diseases, MMSCs exosomes of adipose tissue are also studied [30, 31]. They reduce the expression of proinflammatory cytokines. From reviews of preclinical studies, it can be seen that the use of a conditioned medium from MMSCs can be cheaper, faster, and can give an equal or more powerful effect than a drug based on extracellular MMSC vesicles for the treatment of many diseases. Up to date, there have been no reports that culture medium concentrates from MMSCs are not safe compared to extracellular vesicles [32]. However, it is not yet known which secretome molecules are responsible for the therapeutic effect. It has been shown that both proteins and micro RNAs and lipids that do not encode long RNAs, play a role in the treatment of various organs and systems [32]. The cells themselves have been also shown effective in the treatment of atopic dermatitis in dogs [33] and mice [34], as well as in humans [35]. At the same time, a histological examination of the skin of mice with experimental atopic dermatitis revealed that epidermal hyperplasia and lymphocyte infiltration caused by the induction of the disease were weakened by the intravenous administration of cells in a dose-dependent manner. This article shows that after a systemic administration, cells leave the focus of inflammation within 2 hours and exert their therapeutic effect paracrinally [34]. Taking these facts into account, a cell secretion cream for the local treatment of CAD has been chosen.

It has been shown that extracellular vesicles from MMSCs suppress inflammation in the mouse model of CAD by inhibiting cytotoxic T-lymphocytes, T-helper cells

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of type 1, as well as reducing the level of TNF-α and IFN-γ. In this case, the vesicles induced CD4 + CD25 + Foxp3 + regulatory T cells and increased the level of the anti-inflammatory cytokine IL-10 [36]. Other authors showed that in mice with CAD, the simultaneous treatment with MMSC and dexamethasone did not affect the anti-inflammatory effect of the cells. In addition, a co-administration of MMSCs with dexamethasone reduced the local expression of IFN-γ and TNF-α in the ear tissue of mice with CAD [37]. Possibly, the mechanisms of the action listed above, also took place in the present study when the cream with the MMSC secretome was used.

The action of the cream with the MMSC secretome had a visible effect on the skin a day earlier than the topical GCS. The decrease in the degree of lymphocytic infiltration was significantly higher when using a cream with secretome ($p < 0.05$) than with fluocinolone. Unlike the MMSC secretomes, topical corticosteroids have a number of disadvantages. First, GCSs can lead to local and systemic side effects, such as epidermal atrophy, infectious complications, the effect on all types of metabolism during resorption from large surfaces. In a variety of studies it has been shown, that MMSC secretomes are unlikely to be used due to the presence of regenerative properties and the absence of a significant effect on metabolic processes. Second, burns and wounds are contraindications for the use, which, on the contrary, may be indications for the use of MMSC secretomes also due to their regenerative properties [8].

CONCLUSION

Thus, the anti-inflammatory activity of the complex of humoral MMSC secretome factors, both native and dexamethasone-induced *in vitro*, when applied topically, contributes to a significant reduction in skin inflammation. It takes place in the model of contact-allergic dermatitis, with almost a daily advantage in the secretome-induced MMSCs. In this case, the index of lymphocytic infiltration is significantly lower in the use of secretomes than after using topical corticosteroids. This is the basis for a further study of the therapeutic effects of various fractions of the MMSC secretome and the disclosure of its mechanism of the action. However, to study the systemic effect of the secretome, it is necessary to purify it from large molecules or use allogeneic MMSC to obtain it.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS’ CONTRIBUTION

P.A. Golubinskaya – text writing, carrying out laboratory works, conducting experiments with animals, data analysis; M.V. Sarycheva – carrying out laboratory works and experiments with animals, data analysis; A.A. Dolzhikov – production of histological preparations, data analysis; V.P. Bondarev – production of histological preparations, data analysis; M.S. Stefanova – data analysis; V.O. Soldatov – conducting experiments with animals; S.V. Nadezhdin – carrying out laboratory works; M.V. Korokin – validation of critically important content; M.V. Pokrovsky – development of the research concept; Yu.E. Burda – analysis and interpretation of data, review of critically important content, final approval for publication of the manuscript.

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RESEARCH ARTICLE

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CORRECTION OF PSYCHONEUROLOGICAL SIGNS OF ACUTE ALCOHOL INTOXICATION IN RATS WITH A NEW ACETYLCYSTEINE-BASED COMPOSITION

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The aim of the study is an experimental confirmation of the use of a new combination of biologically active substances with tonic and antioxidant effects. This combination contains acetylcysteine in its composition to reduce the severity of psychoneurological consequences of alcohol intoxication.

Materials and methods. The study was conducted on male Wistar rats. The post-intoxication state was simulated by a single injection of ethanol (3 g/kg, intraperitoneally). Half an hour after awakening, the rats were divided into groups, which were injected with saline, acetylcysteine (1 g/kg), taurine (20 mg/kg), caffeine (20 mg/kg), succinic acid (100 mg/kg), lipoic acid (100 mg/kg), pyridoxine (400 mg/kg), or a combination of acetylcysteine with all these substances taken in a twice lower dose (except taurine). Before the treatment and 3 hours after it, the degree of neurological disorders was fixed according to the Combs and D'Alecy scale, in the Open Field test and the Adhesion test. Then the animals were euthanized to assess the level of glutathione, triglycerides and malondialdehyde (MDA) in liver homogenates, to determine the activity of enzymatic antioxidant systems and serum aminotransferases.

Results. In the animals injected with alcohol, there were evident signs of neuropsychiatric disorders, manifested in a low motor activity and a decrease in fine motor skills. This state did not change after an oral administration of saline. After the administration of acetylcysteine, taurine, caffeine, succinic and lipoic acids, pyridoxine and, to a greater extent, their compositions, the compensation of neuropsychiatric disorders and improvement of fine motor skills were notified. In the liver of these animals, the levels of glutathione, MDA, triglycerides, and the activity of antioxidant defense enzymes corresponded to the physiological norm.

Conclusion. The introduction of a combination of acetylcysteine with taurine, caffeine, pyridoxine, lipoic and succinic acids after an acute alcohol intoxication, to a greater extent than each of the substances separately, contributes to the function retention of the antioxidant system of hepatocytes. Besides, it reduces the level of their dystrophic changes and leads to a decrease in the severity of psychoneurological disturbances in the experimental animals.

Keywords: ethanol; acetylcysteine; taurine; caffeine; pyridoxine; lipoic acid; succinic acid; composition; preclinical studies

Abbreviations: AST – aspartate aminotransferase; ALT – alanine transaminase; MDA – malonic dialdehyde; SOD – superoxide dismutase; TNF-α – tumor necrosis factor-alpha; GPR91 – G protein-coupled receptor; GSH – glutathione.

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КОРРЕКЦИЯ ПСИХОНЕВРОЛОГИЧЕСКИХ ПОСЛЕДСТВИЙ ОСТРОЙ ИНТОКСИКАЦИИ АЛКОГОЛЕМ У КРЫС НОВОЙ КОМПОЗИЦИЕЙ НА ОСНОВЕ АЦЕТИЛЦИСТЕИНА

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Цель. Экспериментальное обоснование применения новой комбинации биологически активных веществ с тонизирующим и антиоксидантным действием, содержащей в составе ацетилцистеин для снижения выраженности психоневрологических последствий интоксикации алкоголем.

Материалы и методы. Исследование проведено на крысах-самцах линии Вистар. Постинтоксикационное состояние моделировали однократным введением этанола (3 г/кг, внутрибрюшинно). Через полчаса после пробуждения крыс разделяли на группы, которым вводили: физиологический раствор, ацетилцистеин (1 г/кг), таурин (20 мг/кг), кофеин (20 мг/кг), янтарную (100 мг/кг), липоевую кислоту (100 мг/кг), пиридоксин (400 мг/кг), или комбинацию ацетилцистеина со всеми данными веществами, взятыми в меньшей (в 2 раза) дозе (кроме таурина). До лечения и спустя 3 часа фиксировали степень неврологических нарушений по шкале «Combs и D'Alecy», в тесте «Открытое поле» и «Адгезивный тест». Далее животных подвергали эвтаназии для оценки уровня глутатиона, триглицеридов и малонового диальдегида в гомогенатах печени, определения активности ферментативных антиоксидантных систем и сывороточных аминотрансфераз.

Результаты. У животных, которым вводили алкоголь, отмечались выраженные признаки психоневрологических нарушений, проявляющихся в низкой двигательной активности и снижением мелкой моторики. Данное состояние не изменялось после перорального введения физиологического раствора. После введения ацетилцистеина, таурина, кофеина, янтарной кислоты, липоевой кислоты, пиридоксина и, в большей степени, их композиции, отмечали компенсацию психоневрологических нарушений, улучшение мелкой моторики. Уровни глутатиона, малонового диальдегида, триглицеридов и активность ферментов антиоксидантной защиты в печени этих животных соответствовали физиологической норме.

Заключение. Введение комбинации ацетилцистеина с таурином, кофеином, пиридоксином, липоевой и янтарной кислотами после острой алкогольной интоксикации, в большей степени способствует сохранению функций антиоксидантной системы гепатоцитов, снижает уровень дистрофических изменений в них и приводит к снижению тяжести психоневрологических нарушений у экспериментальных животных.

Ключевые слова: этанол; ацетилцистеин; таурин; кофеин; пиридоксин; липоевая кислота; янтарная кислота; композиция; доклинические исследования

Список сокращений: АСТ – аспартатаминотрансфераза; АЛТ – аланинаминотрансфераза; МДА – малоновый диальдегид; СОД – супероксиддисмутаза; TNF-α – tumor necrosis factor-alpha/факто некроза опухоли-альфа; GPR91 – G protein-coupled receptor/рецептор, связанный с G-белком; GSH – glutathione/глутатион.

INTRODUCTION

Alcohol consumption is one of the leading risk factors for death and disability. Social and economic consequences of large quantities consumption of alcohol-containing beverages negatively affect many social institutions (industry, trade, education, health care). Globally, alcohol use was the seventh leading risk factor for premature death and disability in 2016, accounting for 2.8 million deaths [1]. A post-intoxication state is a primary medical problem for people who drink alcohol in small and moderate amounts (up to 100 g of pure alcohol per day), and a common cause of professional and home injuries due to impaired motor skills and attention [2].

The most commonly used medical products or food supplements do not significantly reduce the overall severity of the post-intoxication state. Although some of them relieve certain symptoms (vomiting and headache), they do not affect tremors or drowsiness The most promising drugs in terms of the effective reduction of the alcohol consumption consequences are sorbents (effective in pre- or co-intake with alcohol), which inhibit the synthesis of prostaglandins and accelerate the metabolism of ethanol [3].

It should be notified that such drugs have a significant drawback. Alcohol metabolism and, what is more important in terms of the relief of post-intoxication consequences, acetaldehyde biotransformation mainly depends on the hepatocyte antioxidant system activity. The use of antioxidants, such as acetylcysteine, in order to correct the complex of negative consequences of a high dose alcohol consumption, is justified and confirmed by the results of preclinical studies [4]. The development of fixed combinations of known medications is a well-known and promising strategy for working out and implementing new drugs in practice, which makes it possible not only to increase the effectiveness of treatment, but also to make the therapy more accessible and/or more convenient for the patient.

THE AIM. To justify experimentally the use of a new combination of biologically active substances containing acetylcysteine, with tonic and antioxidant effects, to reduce the severity of the psychoneurological consequences of the acute alcohol intoxication.

MATERIALS AND METHODS

Laboratory animals

The study was conducted in accordance with the legislation of the Russian Federation and the technical standards of the Eurasian Economic Union on good laboratory practice (GOST R 53434-2009, GOST R 51000.4-2011). The protocol was approved by the Regional Independent Ethics Committee at Volgograd State Medical University (IRB 00005839 IORG 0004900 (OHRP), protocol No. 132 dated 20.05.2019).

In the study, male Wistar rats (300–350 g, Rappolovo animal house) were used. The animals were kept in a 12/12 hour alternating light-dark cycle, the temperature of 20 ± 2°C and the humidity of 40–60%.

Design

48 hours before the alcohol intoxication, all the rats were trained in the conditioned passive avoidance reaction (CPAR) test, and the training success was confirmed during 24 hours.

The doses of the study drugs were selected according to the literature data [4- 8], and the following 9 groups (n = 10) were formed:

1. Intact group – normal saline (15 mL/kg i.p. and 5 mL/kg p.o.);
2. Ethanol (3 g/kg) + normal saline (5 mL/kg; placebo);
3. Ethanol (3 g/kg) + acetylcysteine (1 g/kg);
4. Ethanol (3 g/kg) + taurine (20 mg/kg);
5. Ethanol (3 g/kg) + caffeine (20 mg/kg);
6. Ethanol (3 g/kg) + succinic acid (100 mg/kg);
7. Ethanol (3 g/kg) + lipoic acid (100 mg/kg);
8. Ethanol (3 g/kg) + pyridoxine (400 mg/kg);
9. Ethanol (3 g/kg) + combination of substances.

The composition of the substances combination was as follows: acetylcysteine (500 mg/kg), taurine (20 mg/kg), caffeine (10 mg/kg), succinic acid (50 mg/kg), lipoic acid (50 mg/kg), pyridoxine (200 mg/kg).

The animals from the intact group were administrated with normal saline (i.p. and p.o.). The rats of the other groups were administrated with a 20% aqueous solution of ethyl alcohol (3 g/kg) (i. p.) and after awakening and assessing the level of neurological deficits, they were administrated with normal saline, the substances or their combination given as prescribed single doses. The volume of the injected fluid was 15 mL/kg for the intraperitoneal administration and 5 mL/kg for the administration p. o.

The average sleep duration in the rats was comparable between the groups (8 h ± 30 min) [4]. After waking up, the level of neurological deficits was determined using the Combs & D'Alecy scale, and a few tests. They were: the Adhesive test (the time of detection and removal of the 5x5 mm patch on the palmar surface of the forepaws for 180 seconds), the Open field test (the motor activity was recorded as the number of the sectors crossed for 3 minutes), and the exploratory activity – as a sum of the number of examined holes and the number of standing on the hind paws) [9]. After that, the animals were administrated with normal saline, one of the substances tested, or their combination in a twice lower dose (with an exception of taurine, which was administered as a part of the combination in a similar dose to maintain its potential cardioprotective effect notified in a number of studies) [10]. A two-fold dose reduction in the composition of the combination was performed to assess the drug-drug potentiation effects of the individual components in the combination.

The behavioral tests were repeated 3 hours later. Euthanasia (cardiac puncture) was performed under anesthesia with zoletil 20 mg/kg (Zoletil®100, Valdepharm, France) + xylazine 8 mg/kg (Xyla, Interchemie, Netherlands). After euthanasia, the liver tissue samples were taken for the subsequent biochemical analysis. The concentration of reduced glutathione was measured in the reduction reaction of 5.5-dithiobis-(2-nitrobenzoic acid).

The analysis was carried out in triplicates. The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma was determined using appropriate reagents (manufactured by Diacon-DS, Russia). The content of triglycerides in homogenates of the liver tissue was determined after the extraction with heptane and isopropanol followed by photometrical fractionation with sodium alcoholate (after the incubation with 2,4-pentanedione at the wavelength of 410 nm). The concentration of malonic dialdehyde (MDA) in homogenates was determined by the reaction with thiobarbituric acid; the concentration of reduced glutathione was determined in the reduction reaction of 5,5-dithiobis-(2-nitrobenzoic acid). A superoxide dismutase (SOD) activity was determined by a photometric method based on assessing the degree of inhibition of the epinephrine oxidation reaction.

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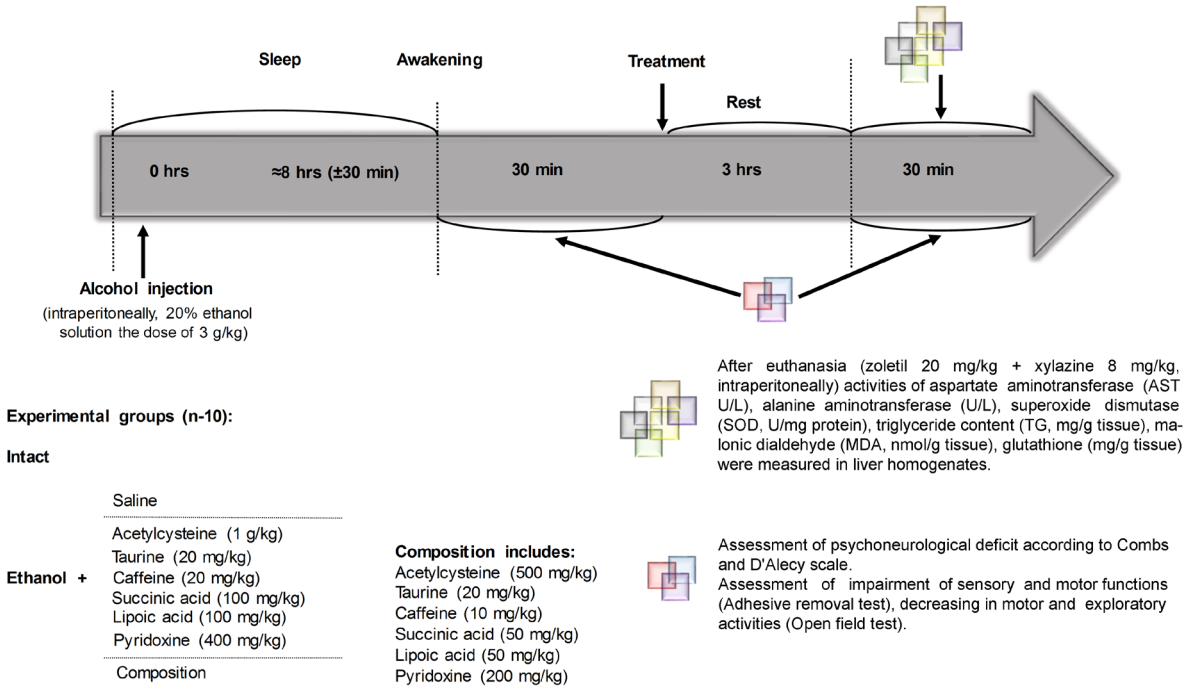

Figure 1 – Study design

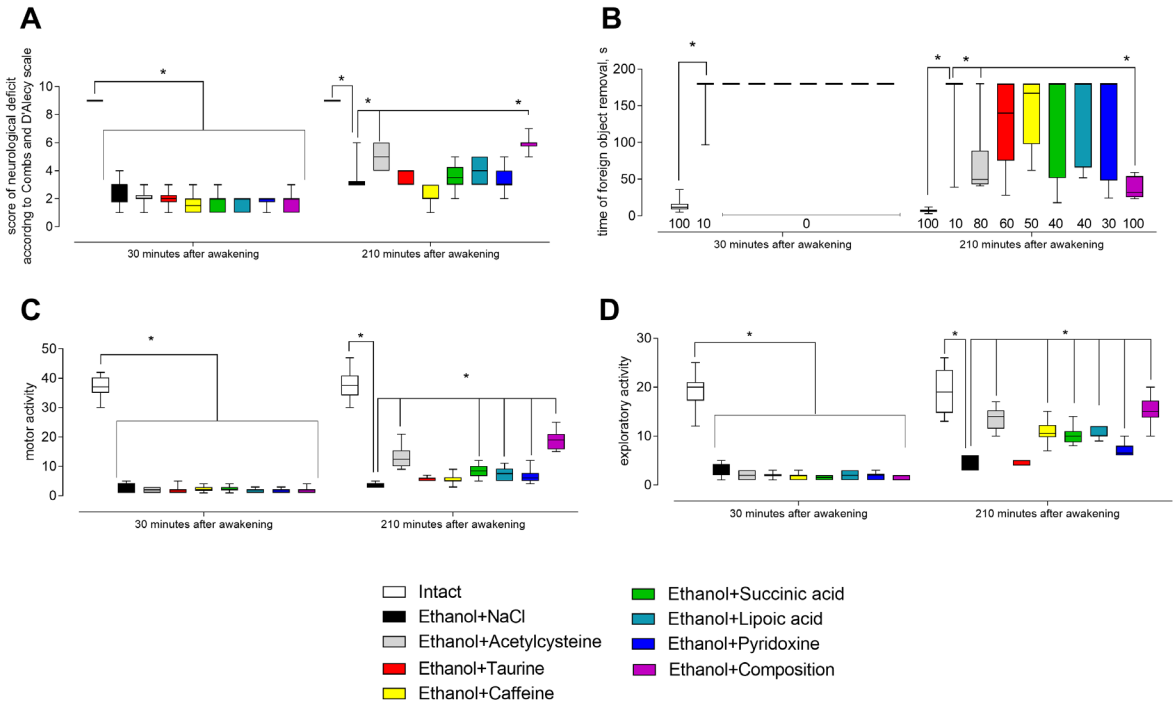

Figure 2 – The levels of neurological deficits assessed by the Combs & D'Alecy scale (A) and the time of removing a foreign object from the volar surface of the rats' fore paws (B), indicators of locomotor (C) and exploratory (D) activities in the open field test in the rats after acute alcohol intoxication

Note: * – $p < 0.05$, one-way variance analysis with Newman-Keuls post-hoc test; the compared samplings are indicated by lines; exploratory activities are the sum of the number of pointing acts and the number of holes examined; locomotor activity is the number of crossed sectors of the installation; numbers represent the numbers of the animals that found and got rid of foreign objects fixed on the volar surface of their forepaws (%)

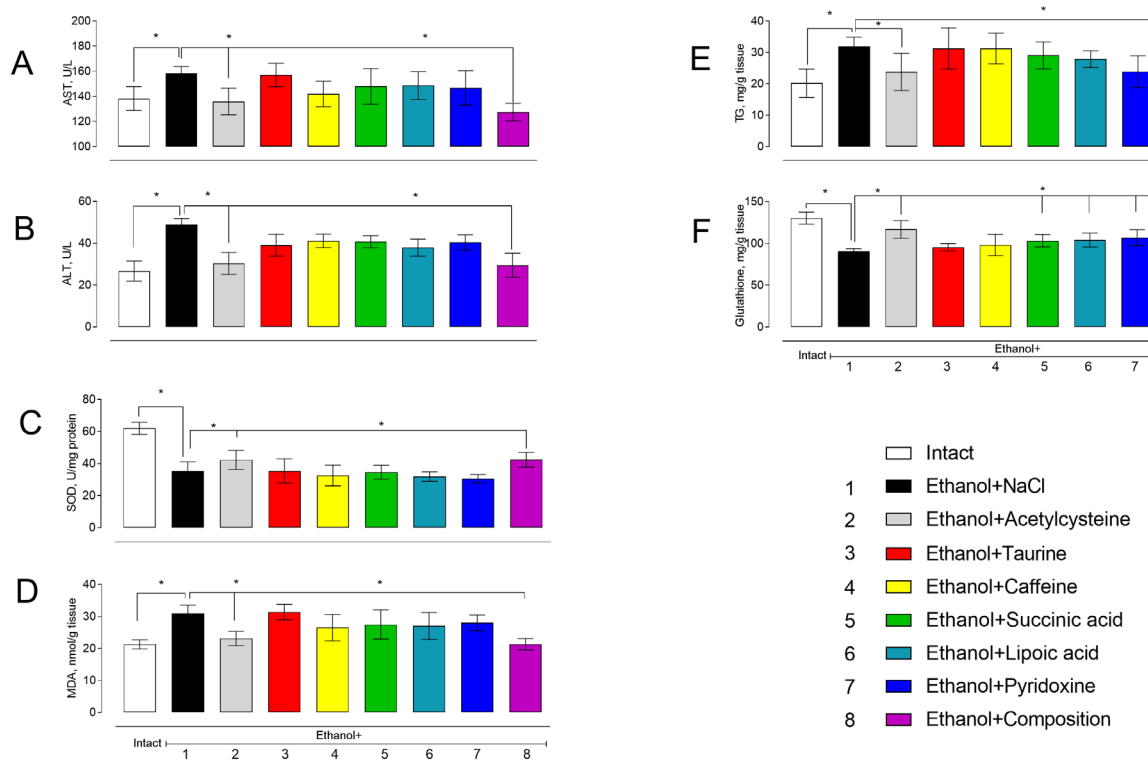

Figure 3 – Activity of superoxide dismutase (SOD), aspartate aminotransferase (AST) and alanine aminotransferase (ALT); the content of triglycerides, malonic dialdehyde (MDA), glutathione in rat liver homogenates, obtained after acute alcohol intoxication

Note: AST – aspartate aminotransferase (U/L, A), ALT – alanine aminotransferase (U/L, B), SOD – superoxide dismutase (U/mg protein, C), TG – triglyceride content (mg/g tissue, D), MDA – malonic dialdehyde (nmol/g tissue, E), glutathione (mg/g tissue, F); the data are presented as averaged individual values (n = 3 at each of the points), the arithmetic mean and standard error of the arithmetic mean; * – $p < 0.05$ (one-way variance analysis with Newman-Keuls post-hoc test); the compared groups are indicated by horizontal lines.

The results were statistically processed using descriptive and analytical statistics. The distribution of quantitative variables was assessed using the Shapiro-Wilk test. Intergroup differences were assessed using a one-way variance analysis with the Newman-Keuls post-hoc test, and the numerical values were presented as the arithmetic mean and standard error of the mean. The chi-square test was used to assess the differences in categorical data.

RESULTS

Ethyl alcohol injection (3 g/kg, i. p.) caused a progressive sedative effect. The animals fell asleep within 2–3 minutes and were asleep for 8 ± 0.5 hours. The animals that fell asleep after a longer period of time or slept longer than the designated period, were excluded from the experiment.

After awakening, the animals did not demonstrate active actions, all the observed behavioral acts were at the minimum level: the animals tended not to move, staying in one place (if they moved, they did it very slowly), they practically did not react to irritating stimuli (touching vibrissae, lateral pushes). After awakening, the rats, compared to the intact rats in the open field test, showed low motor and exploratory activities.

Three hours after the treatment in the control group (Ethanol + 0.9% sodium chloride solution) in the Open field test, the assessed variables were significantly lower than in the intact group. In the groups administrated with acetylcysteine, succinic acid, lipoic acid, pyridoxine, or a composition of the listed substances, motor and exploratory activities were significantly higher (Fig. 2, C), which indicates an accelerated recovery after the ethanol intoxication.

After awakening, the rats showed signs of pronounced neurological deficits (by the Combs & D'Alecy scale), which slightly decreased in the control group 180 minutes later (Ethanol + 0.9% sodium chloride solution) and significantly decreased after the administration of acetylcysteine, succinic and lipoic acids, pyridoxine, or their composition (Fig. 2, A).

During the Adhesive test, after awakening, the animals did not react to foreign objects fixed on the volar surface of their paws, i.e. their sensory and motor functions were significantly reduced, and this was not corrected by the oral administration of normal saline and having a rest for 3 hours. The oral administration of

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acetylcysteine, succinic and lipoic acids, pyridoxine, or their composition (more pronounced) led to a significant improvement in the sensory and motor functions of the animals (Fig. 2, B). Thus, 80% and 100% of the animals, which had been orally administered with acetylcysteine or the test composition, respectively, when tested in the Adhesive test, removed foreign objects from the palmar surface of the forepaws ($p < 0.05$, chi-square test).

After a biochemical analysis of liver homogenates obtained from the intact animals or rats, the animals were first injected with ethanol, and then with one of the above-mentioned substances or their composition. It was found out that the administration of acetylcysteine and, which was more pronounced, its composition with taurine, caffeine, succinic acid, lipoic acid, pyridoxine, reduced the expression of hepatic metabolism disorders to a minimum. In the control group (Ethanol + 0.9% NaCl solution), the activities of AST and ALT in liver homogenates reached 158.3 ± 5.4 U/L and 48.8 ± 3 U/L, respectively (versus 138.2 ± 9.6 U/L and 26.7 ± 4.8 U/L, respectively, in intact animals; $p < 0.05$), the triglyceride content was higher (31.7 ± 2.9 mg/g tissue versus 20.2 ± 4.5 mg/g tissue; $p < 0.05$), and glutathione was lower (90.5 ± 3.4 mg/g tissue versus 130.5 ± 7.2 mg/g tissue; $p < 0.05$). In the placebo group animals, the disturbances in the hepatocyte antioxidant system functioning were also manifested to a greater extent, as evidenced by the low activity of SOD (35.4 ± 5.6 U/mg versus 62 ± 3.8 U/mg in the intact group, $p < 0.05$) and a high content of MDA (30.9 ± 2.6 nmol/g tissue versus 21.3 ± 1.4 nmol/g tissue in the intact group, $p <0.05$).

In liver homogenates of the animals that had been orally administered with acetylcysteine or its combination with the above-mentioned substances after the alcohol injection, the activities of AST and ALT were 135.9 ± 106 U/L, 30.3 ± 5.2 U/L and 127.5 ± 7.0 U/L, 29.4 ± 5.8 U/L, respectively ($p < 0.05$, relative to the animals in the placebo group), triglyceride content 23.8 ± 5.9 mg/g tissue and 22.5 ± 4.5 mg/g tissue ($p < 0.05$, relative to the animals in the placebo group), glutathione 117 ± 10.6 mg/g tissue and 133.3 ± 7.6 mg/g tissue, respectively ($p < 0.05$). Functioning of the antioxidant system of these animals' hepatocytes was close to normal. The SOD activity in the animals' liver of these groups was 42.3 ± 5.9 U/mg and 42.4 ± 4.6 U/mg, respectively ($p < 0.05$), and the MDA content was 23.1 ± 2.2 and 21.3 ± 1.8 nmol/g tissue, respectively ($p < 0.05$). The indicated biochemical parameters in the animals of the other groups were of the intermediate values, and the differences between the intact group, placebo group, acetylcysteine or combination group did not reach statistical significance (with the exception of the positive effect of pyridoxine, succinic and lipoic acids, on the level of glutathione, which is obviously insufficient to affect other variables).

A single administration of acetylcysteine and, to a greater extent, its combination with taurine, caffeine, pyridoxine, succinic and lipoic acids obviously restored or increased the glutathione content in the liver, prevented the development of the oxidative stress and dystrophic changes, which helped to reduce the severity of the alcohol intoxication and, accordingly, all its neuropsychiatric manifestations.

DISCUSSION

The increased stressogenic conditions, combined with the growing variety and availability of alcoholic beverages, contribute to an increase in their consumption. As mentioned above, the main medical problem of the alcohol consumption is post-intoxication neuropsychiatric and somatic symptoms (a post-intoxication state), and not the consequences of chronic ethanol effects on the body, such as cardiomyopathy, cirrhosis, or mental illnesses. It is the pathogenetic symptom complex, interpreted as "an alcohol withdrawal syndrome" that causes significant economic and medical consequences for a modern society. Available and effective treatments (infusion therapy, despite the effectiveness and safety, is available to a minimum number of people) for the relief of this condition are currently limited and require significant expansion. The key factor in the pathogenetic action of ethanol is a violation of metabolism of its main derivative, acetaldehyde, which occurs as a result of depletion of substrates involved in oxidoreductions occurring in hepatocytes and largely dependent on functioning of their antioxidant system. The main antioxidant of hepatocytes is glutathione, which does not only protects the cell from free radicals, but also determines the redox characteristics of the intracellular environment, and the intake of acetylcysteine leads to the replenishment of glutathione reserves. In previous studies, the authors have established that both preliminary and therapeutic administration of acetylcysteine prevents or promotes the recovery of the experimental animals after acute alcohol poisoning. In these studies, acetylcysteine was administered at the dose of 1 g/kg, which limits its clinical use. The authors tried to reduce the effective dose of acetylcysteine by potentiating its action with other substances that can affect alcohol metabolism or add additional positive effects (psychostimulating).

Caffeine, having an activating and attention-maintaining effect, is found (sometimes in combination with related substances) in energy drinks and is often consumed with alcohol. The animal studies have shown the ability of methylxanthines, including caffeine, to modulate the psychopharmacological effects of certain psychoactive substances, such as amphetamine [11], nicotine [12], cocaine [13] and ethanol [6].

There is a widespread belief that caffeine can counteract the intoxicating effects of alcohol. Caffeine indirectly modulates the activity of many neurotransmitters and neuromodulators, including dopamine, acetylcho-

line, or glutamate, in various areas of the brain. The main effect of caffeine is associated with an antagonistic activity against adenosine receptors (A_1 and A_{2A}) in the central nervous system [6, 14]. Ethanol increases the extracellular levels of adenosine by increasing its synthesis (which is facilitated by acetate formed during metabolism of ethanol), secretion, and a decrease in absorption, as a result of dysfunction of the nucleoside transporter [15]. Thus, caffeine can have a stimulating effect in post-alcoholic intoxication conditions.

In the experimental studies, ethanol and caffeine affected a locomotor activity with a bell-curve dependence of the observed effect from the following doses: low doses are stimulating, while high doses are depressive [16]. Caffeine can affect a locomotor activity in a two-phase manner [17]. At low doses, an acute administration of caffeine can enhance the stimulatory effect of ethanol. However, when the dose of caffeine or ethanol is higher, there is a pronounced suppressive effect of the both substances. Low doses of caffeine reduce the discoordination effects of ethanol, while high doses enhance them.

Adenosine mediates the intoxicating effects of ethanol such as ataxia and sedation [15]. Adenosine agonists prolong the duration of sleep induced by high doses of ethanol, while its antagonists shorten it.

A combined administration of ethanol and caffeine has a neuroprotective effect on various models of brain damage [18, 19]. A single oral dose of caffeinol (a combination of 10 mg/kg caffeine and 0.65 g/kg alcohol) 15 minutes after a traumatic brain injury improved the performance of animals in the Morris water maze. These data are important because it is known that the risk of cardiovascular complications increases with the intake of large doses of alcohol and in a post-intoxication state. Caffeine prevents memory loss caused by high doses of ethanol. Given the potentially beneficial effects of caffeine, it was included in the study composition.

A taurine pre-administration slows down the ethanol-induced increase in acetaldehyde in rats and humans without affecting blood ethanol levels. In a clinical study, taurine was used at the dose of 20 mg/kg (1 hour before and 1 hour after ethanol), which led to a decrease in the level of acetaldehyde in the blood by one third [20].

In alcohol intoxication, taurine has a hepatoprotective effect: it reduces oxidative stress, TNF-α levels, and steatosis. Taurine had a positive effect on the adipocytes damaged by ethanol, it is anti-inflammatory and maintains the normal secretion of adiponectin [21]. Taurine supplementation (1% in drinking water) prevents the induction of hypertension in rats chronically administrated with ethanol (15% in drinking water) [22].

Taurine decreases the concentration of acetaldehyde in blood after the oral administration of ethanol [23]. In some models, taurine has anti-atherosclerotic and antihypertensive effects; limited clinical data indicate that it also exhibits antihypertensive activity [23], which is important given the damaging effect of high doses of ethanol on the cardiovascular system. Taurine has a stabilizing effect on platelets, which is expected to reduce the risk of myocardial infarction or stroke. Taurine has a positive inotropic effect in congestive heart failure [10]. In the central nervous system, taurine prevents the development of excitotoxicity, and a long-term intake of taurine reduces age-related memory decline in mice [24]. Thus, taurine was chosen as the third component of the combination.

Lipoic acid supports the optimal activity of aldehyde dehydrogenase 2 (ALDH-2), which has a protective effect against an oxidative stress, negatively affecting the efficiency of hepatic ALDH-2 and, accordingly, the metabolism of alcohol and acetaldehyde. Several studies in rats have shown that the administration of lipoic acid protects the stomach lining, liver and developing brain from the side effects of alcohol [8].

ALDG-2 provides the metabolism of acetaldehyde and physiologically acts as an antioxidant, it also participates in metabolism and, therefore, detoxifies certain toxic aldehydes such as 4-hydroxynonenal (4-HNE), decomposition products of oxidized membrane lipids. It is important for protecting mitochondria from the oxidative stress [25, 26]. Due to its antioxidant effect, a prophylactic course of the oral administration of lipoic acid at the dose of 100 mg/kg effectively prevents ethanol-induced damage to the gastric mucosa [8].

Lipoic acid and taurine have protective effect in ischemia-reperfusion models [8, 27]. Lipoic acid increases the activity of ALDH-2 in the heart, which has a protective effect in postischemic reperfusion [27] and may be a promising agent for the prevention of alcoholic cardiomyopathy [26].

As a metabolic intermediate, succinic acid is a dicarboxylic acid that plays several biological roles: it is involved in the production of ATP and, as a signaling molecule, reflects the state of cellular metabolism [28]. Succinate is produced in mitochondria through the tricarboxylic acid (TCA) cycle and functions in the cytoplasm as well as in the extracellular space, altering gene expression patterns, modulating epigenetic metabolism or hormone-like signaling [28], linking cellular metabolism, especially in terms of ATP production.

Succinate signaling often occurs in response to a state of hypoxia. In the liver, succinate serves as a paracrine signal secreted by anoxic hepatocytes, and acts on stellate cells via GPR91 [29]. This leads to the activation of stellate cells and fibrogenesis. Succinate plays a significant role in liver homeostasis [29] and alcohol metabolism. Thus, lipoic and succinic acids were included in the composition as the substances similar to acetylcysteine, they have a pronounced antioxidant action and some unique effects.

Pyridoxine and some of its derivatives (pyrithioxine and metadoxine) showed hepatoprotective and neuroprotective properties under intoxication conditions. Intramuscular administration of pyridoxine to rats

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(187 mg/kg) significantly reduced the lethality from ethanol and increased the LD_{50} of ethanol from 4.46 to 5.19 g/kg ($p < 0.005$) [30]. Metadoxine (pyridoxine pyrrolidone carboxylate) has a hepatoprotective effect at the doses of 200 and 400 mg/kg reducing oxidative stress and preventing depletion of reduced glutathione levels, when rats are intoxicated with alcohol, CCl_4 and paracetamol. [31]. Pyritinol (pyrithioxine) is a combination of two molecules of vitamin B_6 (pyridoxine) with a disulfide bond. Its pharmacokinetic profile affects the profile of the parent compound, which easily penetrates the blood-brain barrier and regulates the signaling pathways of various neurotransmitters, including acetylcholine, γ-aminobutyric acid, NMDA. It also acts as an antioxidant and anti-inflammatory agent and reduces plasma viscosity. For pyrithioxine (pyritinol), its ability to reduce the severity of post-intoxication state symptoms in people aged 21–40 years, was noted after taking alcohol [32]. In experimental studies, pyridoxine was assessed in a wide range of doses: while high doses can be dangerous with a chronic administration, a dose of 400 mg/kg was chosen for monotherapy, and 200 mg/kg in the combination was given when taking into consideration the acute nature of the reproducible pathology.

Thus, a combined administration of the substances described above, taken in twice lower doses than administered separately, effectively improved the restoration of psychoneurological functions, impaired by the acute administration of alcohol. It should be notified that the isolated intragastric administration of each of the substances, with the exception of acetylcysteine, did not contribute to a significant reduction in the symptoms of psychoneurological deficit after acute ethanol intoxication. This is obviously due to their insufficient dose, but when combining these substances, a significant potentiation of the protective effect was observed.

The main active ingredient of the composition, in the authors' opinion, is acetylcysteine, the antioxidant effects of which are potentiated by pyridoxine, lipoic and succinic acids, taurine, and the introduction of caffeine has a psychoactivating and stimulating effect. The developed combination can be used not only for the prevention and/or correction of post-intoxication neuropsychiatric disorders, but also in the conditions accompanied by antioxidant system disorders.

CONCLUSION

Under the conditions of acute alcohol intoxication, an oral administration of a combination of acetylcysteine with taurine, caffeine, pyridoxine, lipoic and succinic acids, improves the functional state of the antioxidant system, reduces the severity of destructive changes in the liver, and helps to reduce the severity of neurological deficits in the laboratory animals in a more pronounced way than the substances, administered separately. The obtained results indicate that the use of the studied combination is promising in order to eliminate the severity of the post-intoxication state (hangover syndrome).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR'S CONTRIBUTION

D.V. Kurkin – research idea and planning, article writing; E.I. Morkovin – participation in the development of the research design, correction of the article, translation; N.A. Osadchenko – carrying out biochemical research; D.A. Bakulin – modeling of pathology, assessment of neurological deficit, preparation of the final version of the article; E.E. Abrosimova – article translation, conduct of behavioral tests; M.A. Dubrovina – conduct of behavioral tests; N.S. Kovalev – introduction of compounds, conduct of behavioral tests; Yu.V. Gorbunova – conduct of behavioral tests; I.N. Tyurenkov – general project management.

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RESEARCH ARTICLE

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PHARMACOECONOMIC EVALUATION OF ANTI-PNEUMOCOCCAL VACCINATION IN RISK GROUPS FOR THE PREVENTION OF COMMUNITY-ACQUIRED PNEUMONIA AMONG ADULTS IN THE ASTRAKHAN REGION

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The aim. To evaluate the economic efficiency and the choice of the vaccination strategy in the respiratory pneumococcal infection risk groups among the adult population of the Astrakhan region.

Materials and methods. The data for the period of 2015–2018 were analyzed on the number of registered diseases in the patients living in the service area of the medical organizations (Form No.12, Federal State Statistics Service Orders No. 591, dated 27 November, 2015; No. 679, dated 22 November, 2019). The following working directives were studied: the base medical examination documentation submitted by medical institutions (Form No. 030/y "Dispensary Monitoring Checklist"; lists of the persons subjected to medical observation in the reporting year; Orders of the Ministry of Health of the Russian Federation: No. 1344, dated 12 December, 2012; No. 173n, dated 29 March, 2019). Statistical materials of the territorial fund for compulsory medical insurance of the Astrakhan region on the payment of medical care to 12,970 patients who had pneumonia in 2015–2018, were analyzed. The financial support of vaccination based on the results of tenders for the procurement of pneumococcal vaccines organized by the regional Ministry of Health, was considered. The calculations were carried out in accordance with the guidelines of "Cost-effectiveness of vaccine prophylaxis" (Methodological guidelines 3.3.1878-04, dated 04.03.2004).

Results. The prospective calculation of the vaccination cost showed that the benefits of vaccination with pneumococcal conjugate vaccine Prevenar13 (PCV13) and pneumococcal polyvalent vaccine Pneumovax 23 (PPV23) with a 95% vaccination coverage, are recorded after 2 years. The economic benefit of vaccination by reducing the possible number of pneumonias at the end of 2028 will be 968.2 million rubles.

Conclusion. The economic feasibility of vaccine prophylaxis of the adult contingent with an increased risk of developing pneumococcal infection has been established. The sequential strategy of PCV13 and PPV23 application provides the most effective localization of pneumococcal infection. The research results should be widely introduced into the long-term plans for vaccination and healthcare practice in the Astrakhan region.

Keywords: vaccine prophylaxis; vaccination, pneumococcal infection; community-acquired pneumonia; PCV13; PPV23; risk groups; pharmacoeconomic analysis; Astrakhan region

Abbreviations: PN – pneumonia; PCV13 – pneumococcal conjugate vaccine "Prevenar 13"; PPV23 – pneumococcal polyvalent vaccine "Pneumovax 23"; CAP – community-acquired pneumonia; PI – pneumococcal infection; COPD – chronic obstructive pulmonary disease; CHD – coronary heart disease; CHF – chronic heart failure; DM – diabetes mellitus; HIV – human immunodeficiency virus.

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ФАРМАКОЭКОНОМИЧЕСКОЕ ОБОСНОВАНИЕ ПРОВЕДЕНИЯ АНТИПНЕВМОКОККОВОЙ ВАКЦИНАЦИИ В ГРУППАХ РИСКА ДЛЯ ПРОФИЛАКТИКИ ВНЕБОЛЬНИЧНЫХ ПНЕВМОНИЙ СРЕДИ ВЗРОСЛОГО НАСЕЛЕНИЯ АСТРАХАНСКОЙ ОБЛАСТИ

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Цель. Обосновать экономическую эффективность и выбор стратегии вакцинопрофилактики в контингентах повышенного риска развития респираторной пневмококковой инфекции среди взрослого населения Астраханской области.

Материалы и методы. За период с 2015 по 2018 гг. проанализированы данные о числе зарегистрированных заболеваний у пациентов, проживающих в районе обслуживания медицинских организаций (форма № 12, Приказы Росстата № 591 от 27.11.2015; № 679 от 22.11.2019). Изучена основная документация по диспансеризации, представленная медицинскими учреждениями (форма № 030/у «Контрольная карта диспансерного наблюдения», списки лиц, подлежащих диспансерному наблюдению в отчетном году, Приказы МЗ РФ № 1344 от 21.12.2012; № 173н от 29.03.2019). Проанализированы отчетно-статистические материалы территориального фонда обязательного медицинского страхования Астраханской области (ТФОМС АО) по оплате медицинской помощи 12970 пациентам, перенесшим пневмонию в 2015–2018 гг. Для финансового обеспечения вакцинопрофилактики учитывались результаты конкурсных торгов по закупкам пневмококковых вакцин, организованных региональным министерством здравоохранения. Расчеты выполнялись в соответствии с методическими указаниями «Экономическая эффективность вакцинопрофилактики». МУ 3.3.1878-04 от 04.03.2004.

Результаты. При проспективном расчете затрат на реализацию вакцинопрофилактики установлено, что окупаемость вакцинации с применением ПКВ 13(«Превенар 13») и ППВ 23 («Пневмовакс 23») в условиях 95% охвата прививками регистрируется через 2 года. Экономическая выгода вакцинопрофилактики за счет снижения возможного числа пневмоний (ПН) по окончании 2028 года будет составлять 968,2 млн руб.

Заключение. Установлена экономическая целесообразность проведения вакцинопрофилактики взрослого контингента с повышенным риском развития пневмококковой инфекции. Наибольшей эффективностью для ограничения распространения пневмококковой инфекции характеризуется стратегия последовательного применения ПКВ13 и ППВ23. Результаты исследования подлежат широкому внедрению в перспективные планы вакцинопрофилактики и практику здравоохранения Астраханской области.

Ключевые слова: вакцинопрофилактика; вакцинация; пневмококковая инфекция; внебольничная пневмония; ПКВ 13; ППВ 23; группы риска; фармакоэкономический анализ; Астраханская область

Список сокращений: ПН – пневмония; ПКВ13 – пневмококковая конъюгированная вакцина «Превенар 13»; ППВ23 – пневмококковая поливалентная вакцина «Пневмовакс 23»; ВП – внебольничная пневмония; ПИ – пневмококковая инфекция; ХОБЛ – хроническая обструктивная болезнь легких; ИБС – ишемическая болезнь сердца; ХСН – хроническая сердечная недостаточность; СД – сахарный диабет; ВИЧ – вирус иммунодефицита человека

INTRODUCTION

Community-acquired pneumonia (CAP) has a leading position in the structure of morbidity and mortality from infectious diseases in the world. In terms of treatment costs, community-acquired pneumonia leads to significant economic losses suffered by the state. There are 3.9 cases of this disease in 1000 people per year among people over 18 years of age in Russia [1, 2]. However, these indicators do not reflect the true prevalence of CAP in Russia. According to various sources, it reaches 14–15% and comprises about 1.5 million people [3]. The most vulnerable categories for the pneumococcal infection (PI) development are young children and adults with chronic diseases of the lungs, cardiovascular system, liver, kidneys, diabetes mellitus (DM) and immunodeficiency diseases [4, 5]. The studies show that the risk of PI developing is significantly increased in patients with background diseases, aged 50–64 years. In comparison with somatically healthy individuals, the risk of PI developing is 13.3 times higher in oncohematological patients, 9.8 times higher in chronic obstructive pulmonary disease (COPD), 6.5 times higher in HIV infection, and 5.8 times higher in immunosuppressive conditions and chronic liver diseases. The risk of PI developing is

also increased by smoking by 4.4 times, in cardiovascular diseases by 4.2 times, in chronic kidney diseases and diabetes, respectively, by 4.2 and 3 times [6, 7].

In Russia, out of 500 thousand cases registered per year, 76% of adults and 90% of children under 5 years of age have pneumococcal etiology [8]. The current situation already calls for specific vaccination in risk groups. In cases of COPD, vaccination reduces the number of exacerbations, stabilizes the performance of functional tests, it also helps slow down the progression of respiratory failure [9, 10]. The relevance of vaccination increases with comorbid pathology. Thus, in 1/3 of cases, COPD is comorbid with an ischemic heart disease (IHD). The total mortality of patients with a chronic heart failure (CHF) with pneumonia development increases by 1.8-4.6 times, and the risk of death increases by 49.5%. It was established that in this category of patients, pneumonia contributes to the progression of circulatory failure by 17.7%, increases the risk of acute coronary syndrome by 14.1% and any rhythm disturbance by 5.3% [11–13]. A significant increase in economic losses due to direct and indirect costs up to 61.6 billion rubles is a negative contribution due to progressive COPD [14]. PI contributes to the sensitization and exacerbation of chronic non-specific inflammation in the bronchi, increases their hyperreactivity in the patients with bronchial asthma [15, 16]. In the presence of kidney diseases, PI is more common than generally in the population, and it is more severe, with possible fatal outcomes. Pneumonia and sepsis frequently develop in the patients with chronic renal failure, especially in those receiving long-term hemodialysis. It should be pointed out that the risk of death from pneumonia in this situation increases by 14–16 times [17, 18].

The need for vaccination against PI in DM and metabolic syndrome has been proved. These categories of patients are known to have defects in immunological protection against all types of microbial infection, including infections of pneumococcal etiology. Their risk of developing invasive forms of PI is 6 times higher in comparison with healthy individuals [19]. Furthermore, Streptococcus pneumoniae is one of the most frequent pathogens of recurrent pneumonia in patients with diabetes mellitus, which often develops with oropharynx or stomach content microaspiration due to esophageal paresis [20]. Unfortunately, researches show that immunization against PI in the already mentioned category of patients still does not correspond to the sufficient volume [21, 22].

Among the general range of problems related to the prevention of PI, the priority has been given to specific vaccination, which prevents the spread of antibiotic-resistant strains and reduces the development of the most severe clinical forms of pneumonia. Vaccination is not only effective but also costly. It is especially true in the countries with limited material resources that are not able to provide a sufficient vaccination coverage of the population. However, the damage from pneumococcal diseases that can be prevented by widespread immunization of the population is higher than expected. This statement specifically determines the high cost-effectiveness of vaccination. Thus, the solution of the problem lies in the prospective regional vaccination programs development in PI risk groups. These programs have a convincing pharmacoeconomical justification.

THE AIM of the study was to evaluate the economic efficiency and the choice of the vaccination strategy in the respiratory pneumococcal infection risk groups among the adult population of the Astrakhan region.

MATERIALS AND METHODS

The study was conducted in the Astrakhan region (according to Federal State Statistics Service, the population is 1005,967 people). Astrakhan has a population of 504,501. He overall population at risk of PI, is represented by adult patients of 18 years and older with chronic forms of cardiovascular (CHD, cardiomyopathy) and bronchopulmonary diseases (COPD, chronic bronchitis, bronchial asthma), patients with DM and obesity, convalescents after acute otitis media, as well as meningitis. Smaller groups included patients with chronic liver, kidney diseases and hemoblastoses (Table 1).

In total, the number of people in risk groups for the period of 2015–2018, amounted to an average of 99,228. If there were chronic diseases that required monitoring and treatment, all of them were assigned to the 3-rd group of dispensary registration.

To select patients for risk groups, verify their clinical diagnoses and plan vaccinations, normative legal documents were used. The data were analyzed for the period of 2015–2018 on the number of registered diseases in the patients living in the service area of the medical organizations (Form No. 12)[1,2]. The basic prophylactic medical examination documentation submitted by medical institutions, was studied (Form No. 030 / "Dispensary Monitoring Checklist", lists of persons subjected to medical observation in the reporting year)[3,4]. Statistical

[1] Order of Rosstat No. 591, dated 27.11.2015 (as amended on 31 December 31, 2020) "On approval of statistical tools for the organization of the Ministry of Health of the Russian Federation of federal statistical observation in the field of health". Available at: http://www.consultant.ru/document/cons_doc_LAW_190056 / (in Russian)

[2] Order of Rosstat No. 679, dated 22.11.2019 "On approval of the federal statistical observation form with instructions for filling it out for the organization by the Ministry of Health of the Russian Federation of federal statistical observation in the field of health protection". Available at: http://www.consultant.ru/document/cons_doc_LAW_338995/ (in Russian)

[3] Order of the Ministry of Health No. 1344, dated 21.12.2012 "On approval of the dispensary observation". Available at: http://www.consultant.ru/document/cons_doc_LAW_142423/. (in Russian)

[4] Order of the Ministry of Health No. 173, dated 29.03.2019 "On approval of the procedure for conducting dispensary observation of adults". Available at: http://www.consultant.ru/document/cons_doc_LAW_323527/ (in Russian)

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materials of the territorial fund for compulsory medical insurance of the Astrakhan region on the payment of medical care to 12,970 patients who had had pneumonia in 2015–2018, were analyzed.

Implementation of prophylactic measures in medical organizations was evaluated in accordance with the national calendar of preventive vaccinations[5].

In vaccination costs calculating the results of competitive tenders for the purchase of pneumococcal vaccines carried out by the regional Ministry of health in 2015–2018, were taken into consideration. In 2015, the cost of PPV23 ("Pneumovax 23") amounted to 2,500 rubles; in 2016, it decreased to 1,800 rubles. The cost of PCV13 ("Preventar 13") remained unchanged in 2017–2018 and corresponded to 1,700 rubles.

Pharmacoeconomic analyses of the already carried out (retrospective) and future (prospective) preventive measures, were used in the interpretations of the research results gained. The calculation algorithm was based on the guidelines "Cost-effectiveness of vaccine prophylaxis"[6]. To determine the components of vaccination economic evaluations, formulas 1–18 were sequentially applied. For obtaining the results of initial regional indicators changing in the calculation algorithm, the MS Excel software base was used.

The sequential calculation of the costs associated with the implementation of the selected vaccination strategies and prevention of damage, was carried out in stages. The main stages of the computational algorithm were:

– A retrospective analysis of the incidence and vaccination coverage of adult patients at risk for PI in the Astrakhan region for 2015–2018;

– A retrospective analysis of the incidence of pneumonia in adult patients and calculation of actual financial costs associated with the treatment in dynamics for 4 years;

– A prospective calculation of costs for the pneumonia treatment, depending on the economic feasibility of applying vaccination in the alternative variations – "without vaccination" and "with vaccination";

– A comparison of "costs" and "benefits" based on the final results of the divariant analysis of vaccination and determining its effectiveness in the prospect of 10 years.

RESULTS AND DISCUSSION

At the first stage of the study, the analysis of incidence for the development of PI in risk groups among the adult population of the Astrakhan region for the period of 2015–2018, was carried out. The annual growth trend in the total number of patients was revealed. Special attention was paid to cardiovascular diseases. The increase in its indicators after 4 years amounted to 17,117 registered cases, and in relation to the initial 2015 year, it reached 46.5%.

The second place was taken by the patients with DM and obesity who made up 30.8% of the total number of the patients in risk groups in 2018. The trends in the growth of chronic diseases of lungs, ENT organs and liver were also revealed. In comparison with 2015, their indicators at the end of 4 years had increased by 18.3%, 6.9% and 17.8%, respectively. Thus, the annual increase of incidence in the above-mentioned risk groups is a determining condition for the optimization of preventive measures and mandatory use of modern technologies for immunoprophylaxis.

The annual average growth rate (Agr_a) of the patients' number in risk groups were calculated using the formula [23]:

$$Agr_a = (N_f - N_i)^{\frac{1}{S}} - 1,$$

where: N_f – the number of people in risk groups in the last year of observation; N_i – the number of people in risk groups in the previous year of observation; S – the number of years of observation.

The calculations performed showed that the average rate of the PI development in the risk groups (the quantitative growth) for the period of 2015–2018, was 5,981 people (6.3%). The obtained result, based on the analysis of initial indicators for 4 years, demonstrates the need for urgent solutions to the issues of vaccination.

The second stage of the study retrospectively analyzed the incidence of pneumonia (PN) among the adult population of the Astrakhan region, and calculated the cost of the treatment implemented in 2015–2018. The total number of the patients, who had had PN, was 12,970 people during this period. The cost of the treatment they had receied was calculated using the formula [24]:

$$Cost_{PN} = Cost_{1PN} \times N,$$

where: $Cost_{PN}$ – the cost of PN treatment; $Cost_{1PN}$ – the cost of one treatment case; N – the number of cases.

According to Table 2, the average cost of one pneumonia treatment case was 20,156.23 rubles. Therefore, the total financial costs reached a significant amount of 265,234,598.53 rubles.

Thus, the ongoing trends towards an increase in the incidence of PN for 4 years and rise in the financial costs for the treatment, characterize the current region's situation, which clearly indicates the requirement to optimize measures for the prevention and treatment of this disease. According to clinical practice, immunoprophylaxis with the use of modern pneumococcal vaccines is one of the ways to increase the effectiveness of treat-

[5] Order of the Ministry of Health No. 125n, dated 21 March 2014 "On the approval of the national calendar of preventive vaccinations and the calendar of preventive vaccinations for epidemic indications "with amendments to Appendices No. 1 and No. 2, dated 2016, and federal clinical recommendations" Vaccine prevention of pneumococcal infections in adults", dated 2018. Available at: http://www.consultant.ru/document/cons_doc_LAW_162756/. (in Russian)

[6] Methodological guidelines 3.3.1878-04 "Cost-effectiveness of vaccine prophylaxis", dated 04.03.2

ment, preventive measures and influence on the PN incidence.

Based on this position, the possibility of the annual vaccination coverage of the adult population from the PI risk groups, and the average annual increase in the PN incidence in the region's population over 4 years (Table 3), was analyzed. 2015 is considered the beginning of vaccination with PPV23 against PI among the adult population of the Astrakhan region. Due to the risk of developing PI, 2,185 people were vaccinated during this year. In 2016, this indicator was the lowest and represented by 176 people. In 2017, the strategy of preventive vaccination changed due to the use of two vaccines. In total, 2289 people were vaccinated with both vaccines: 635 with PPV23 and 1654 with PCV13. In 2018, a group of 2,197 people were only vaccinated with PCV13.

The results on the vaccination status obtained in risk groups indicate the required prospective planning of vaccination measures with the most efficiency.

On the one hand, the PI preventive vaccination has started to implement into the real regional health practice, and on the other hand, vaccination coverage in the risk groups remains low. Considering the average annual increase in incidence, it should be assumed that the amount of newly registered PN cases among the adult population is just increasing. The PN is considered a human disease with reduced immunological reactivity of the body; therefore the role of vaccination in the situation of the Astrakhan region becomes even more relevant.

At the next stage of the study, the estimated number of people in risk groups for the next 10 years has been calculated. The calculation was based on the average growth rate of patients amount (Tgr_a) in the PI risk groups from 2015 to 2018. This approach made it possible for us to calculate comparable indicators representing the number of individuals in risk groups in the period of 2019–2028, prospectively (Table 4).

The role of alternative strategies "without vaccination" and "with vaccination" in the prevention of probable PN cases should be also evaluated in a comparative aspect. Considering the low level of vaccination in 2015–2018 (an average of 1.7%), we chose this temporary episode to be a period without vaccination. Based on the strategy of non-interference ("no vaccination"), to calculate an annual incidence PN rate (with newly registered cases during the year), the following formula was used [9]:

$$K_{avrg} = \frac{k_{avrg}}{n_{avrg}} \times 100000,$$

where: K_{avrg} – an average incidence rate; k_{avrg} – a average number of diseases for the period; n_{avrg} – an average number of vaccinated, expressed in absolute terms.

Here of it follows that for the period of 2015–2018 K_{avrg} of PN incidence in the Astrakhan region amounted to 3,268 people. The resulting indicator was also relevant for calculating the probable number of PN cases that would have occurred without preventive vaccination after 2018 for 10 years (Table 4). Based on the aforementioned, the calculation was carried out according to the following formula [10]:

$$m_i = K_{avrg} \times n_i / 100000,$$

where: m_i – an average number of cases during the period without vaccination; K_{avrg} – an average incidence rate; n_i – n number of people expressed in absolute values.

Thus, the data obtained can witness that there are interrelated trends towards an increase in the number of people in risk groups and probable PN cases, which are generally caused by a low coverage and a low effectiveness of vaccination. There is no doubt that strategies with the absence of vaccination are better to remain as versions of go-ahead events, but the predicted increase in the PN incidence during the next 10 years, is already a wake-up call on the present day. An increase in the financial costs for providing medical care to patients, always causes aggravation of the economic aspects of the regional PN problem.

The data for the prospective calculation of PN cases treatment costs with a research horizon of 10 years providing a non-intervention strategy "without vaccination", are presented in Fig. 1.

It should be pointed out that the cost of PN treatment among insured individuals in the Astrakhan region will significantly increase throughout the 10 years of the study. According to the results of a financial costs prospective calculation on the background of an alternative strategy "without vaccination", the medical care provided to this category of patients, can be estimated at the amount of 1.31 billion rubles.

In order to predict the delayed outcomes of preventive measures in the "with vaccination" option for 10 years ahead, the costs of vaccination and the damage prevented by it were calculated. The cost of vaccination calculation in this alternative version was carried out using the following formula [24]:

$$Cost_v = Cost_d \times N,$$

where: $Cost_v$ – the cost of the preventive vaccination; $Cost_d$ – the cost of the drugs; N – the number of vaccinated.

The cost of vaccination was calculated in accordance with the current vaccination scheme in the country. Patients are vaccinated with PCV13 once-in-a-lifetime according to the national calendar. The individuals, who join the list of risk groups each year, will also need to be vaccinated with PCV13. At the end of the first year, the primary vaccination with PPV23 takes place, and the patients are subjected to the booster vaccination with the same vaccine five years later.

Planned for the next 10 years, vaccination seems a

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Table 1 – Nosological features and the number of patients at risk for pneumococcal infection

Risk groups (people), years	2015	2016	2017	2018
Cardiovascular diseases	36839	43898	49782	53956
Chronic lung diseases	10680	11899	12008	13076
Diabetes and obesity	30357	32394	32117	33874
Acute otitis media, meningitis	6610	7417	7554	7064
Liver disease, including liver cirrhosis	2247	2452	2467	2648
Kidney diseases, chronic kidney failure	238	266	285	273
Hemoblastoses	678	698	8428	809
Total number of patients in risk groups:	86118	97433	103318	110042

Table 2 – Number of reported pneumonia cases and treatment costs

Period, years	2015	2016	2017	2018	Final result
Cases of pneumonia	2,766	3,234	3,193	3,777	12,970
Cost of 1 case, RUB.	15,420.93	22,638.17	19,525.00	23,040.81	20,156.23
The total cost of treatment, RUB.	42,654.292.38	73,211,841.78	62,343,325	87,025,139.37	265,234,598.53

Table 3 – Results of vaccination in risk groups and the average annual increase in the pneumonia incidence in adults of the Astrakhan region

Period, years	2015	2016	2017	2018
Total number of vaccinated people	2.185	176	2.297	2.197
Vaccination coverage, %	2.5	0.2	2.2	2
Increase in the pneumonia incidence, %	Initially: 2,766 of pneumonia cases	16.9	15.4	36.5

Table 4 – Dynamics of quantitative indicators in risk groups and pneumonia incidence in the Astrakhan region, in a 10-year prospective calculation

Years	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028
Risk groups, people	11,6997	12,4392	13,2253	14,0612	14,9499	15,8948	16,8994	17,9675	19,1031	20,3105
Probable PN cases	3,823	4,065	4,322	4,595	4,885	5,194	5,522	5,871	6,242	6,637

Table 5 – The number of prevented pneumonia cases and the cost of prevented damage within a 10-year prospective

Period, years	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028
Probable cases of pneumonia without vaccination (m)	3,823	4,065	4,322	4,595	4,885	5,194	5,522	5,871	6,242	6,637
Probable cases of pneumonia against the background of vaccination (including the vaccinated and unvaccinated) (L)	118	95	47	86	91	98	77	71	75	93
Prevented pneumonia cases (a)	3,705	3,970	4,275	4,509	4,794	5,096	5,445	5,800	6,167	6,544
The cost of prevented damage, million rubles	74.6	80.0	86.2	90.9	96.6	102.7	109.7	116.9	124.3	131.9

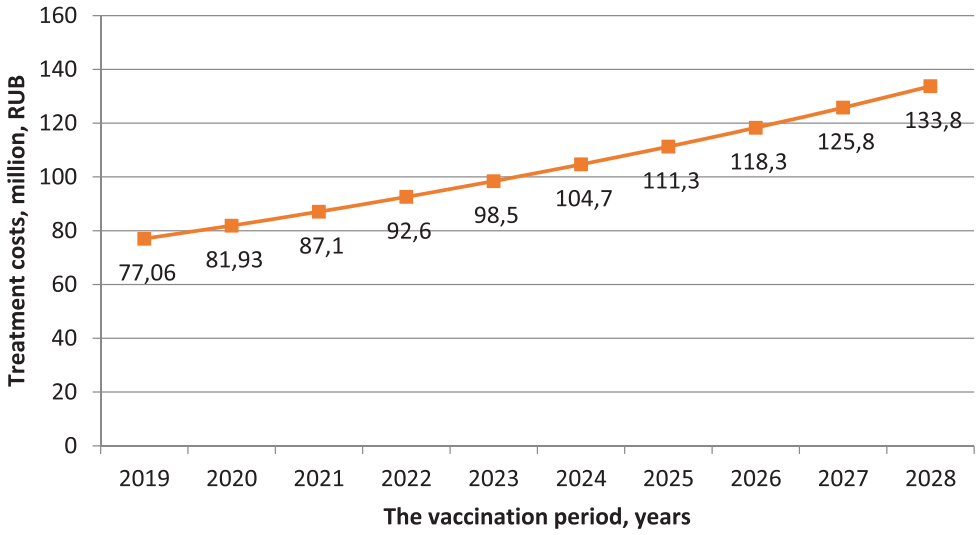

Figure 1 – Prospective calculation of insured pneumonia cases treatment costs in the Astrakhan region with the use of «without vaccination» strategy

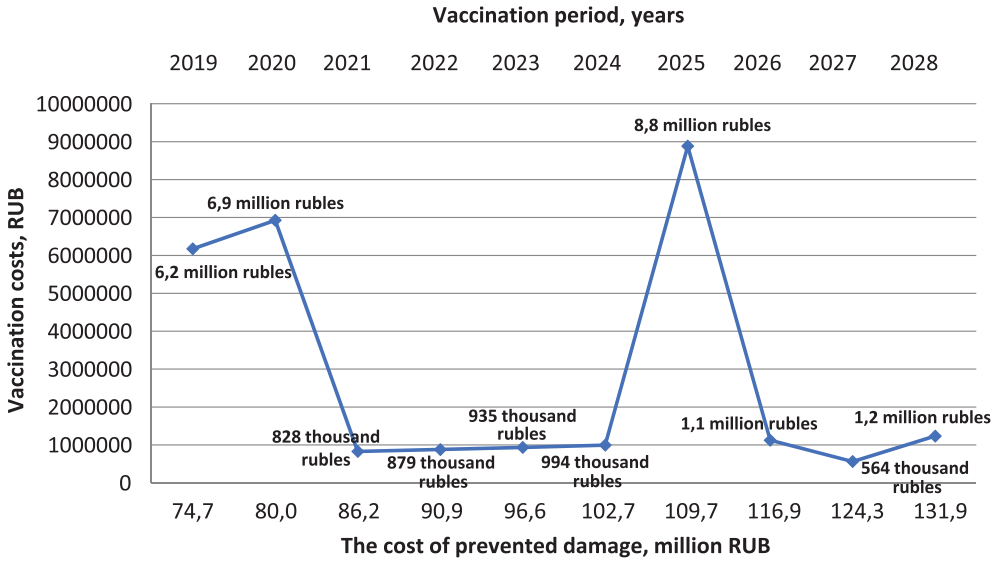

Figure 2 – Ratio of planned vaccination costs to prevented damage in the period of 2019–2028

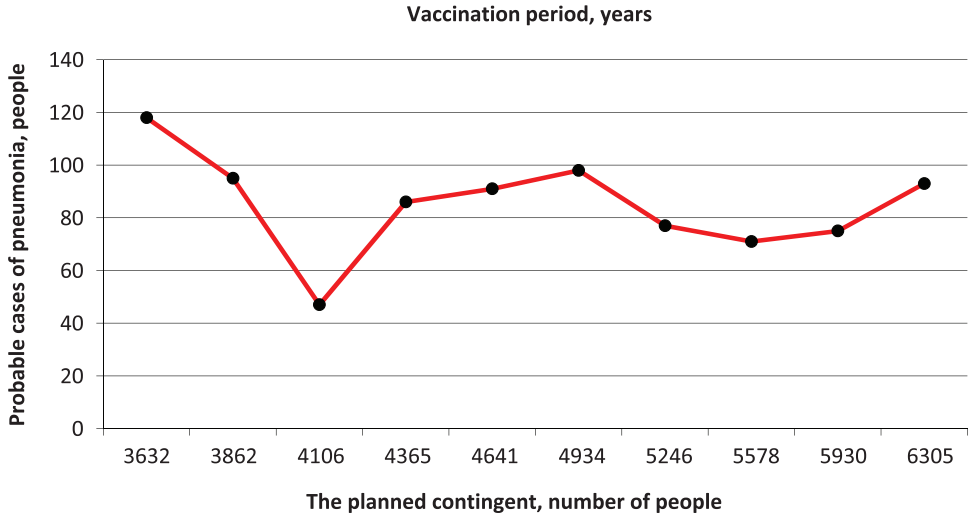

Figure 3 – Estimated indicators of probable PN cases among the vaccinated population

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highly economical and accessible preventive measure that can have a significant impact on reducing the number of PN cases. The use of two non-replaceable polysaccharide vaccines, PPV23 and PCV13, was the pacing factor for improving the effectiveness of PI vaccination. According to the estimated data, in 2019, only the results of primary PCV13 vaccination, conducted in 3,632 people, should have been expected. In 2020, the same individuals were vaccinated with PPV23, and the newly presenting 230 patients were primarily vaccinated with PPV13. Thus, the potentiated effects of the two vaccines began to be realized that year when the biological effect of PPV23 developed against the background of the PCV13 vaccine inoculated in 2019. An overall trend of preventive vaccination during the entire 10-year period will be a regularly repeated event in which the first use of PCV13 in the individuals who annually join the risk groups, is followed by the primary vaccination with PPV23 in another calendar year. Considering the calculations carried out, the number of individuals enrolled into the risk group for the suspected PN each year, will range from 230 to 375 people. It should be notified that the planned number of the vaccinated population will increase in 2028 about twice in comparison with the initial parameter of 2019, and it will amount to 6,305 people. An important feature of the planned vaccination will be the booster vaccination of PPV23 in 2025, covering 4,641 people. For this reason, beginning from 2026, vaccination with PCV13 and PPV23 will proceed in accordance with the established procedure for newly presenting patients. This change in the vaccination schedule also involves a decrease in the financial cost of purchasing drugs within 5 years, i.e. till the next booster vaccination of PPV23. A prospective calculation showed that for PI vaccination based on the requirements of the national calendar of preventive vaccinations, an amount of 28.5 million rubles will be required within the space of 10 years (Fig. 2).

A significant component of the economic evaluation of the preventive vaccination was the calculation of the prevented damage. The number of PN cases prevented during the year, with a 95% coverage of pneumococcal vaccination, was calculated according to the formula (18):

$$a_i = m_i - L_i,$$

where: a_i – the number of prevented PN cases within a year; m_i – the probable number of PN cases that would have occurred each year without preventive vaccination; L_i – the number of people with PN (including the vaccinated and unvaccinated).

Table 5 shows that probable PN cases against the background of preventive vaccination have acquired a one-way downward trend at the end of 2020. However, the probability of developing PN in 2028 will remain increased in the newly presenting patients in the risk groups and receiving only the primary PCV13 vaccination.

This category includes 375 people who are scheduled for the primary PPV23 vaccination in 2029, which is out of time proportion of this study, Nevertheless, in 2028, 352 more people who will join the risk groups in 2027, will be able to be vaccinated with PPV23.

Thus, the evaluation of the prevention strategy benefits “with vaccination” comes from the prevented costs, presented as the economic damage associated with the spread of PN cases that had been prevented as a result of vaccination with PCV13 and PPV23. In order to specify this financial aspect, the prevented costs were determined, calculated as multiplying one case average cost of disease by the number of prevented cases. As a result of the calculation, the total prevented economic damage was 1.14 billion rubles

As Fig. 3 shows, the number of probable PN cases in vaccinated and unvaccinated individuals in the presence of “with vaccination” strategy, has generally decreased positively, and along with them, the cost of treatment has decreased.

At the final stage of this study, the difference between the cost of vaccination in the selected PI risk group and the damage prevented during its implementation within the space of 10 years was calculated. The planned vaccination coverage was 95%. The comparison of the “vaccination costs” and the “benefits” in terms of costs, was the main approach to evaluating the economic parameters of vaccination.

An important aspect of the planned process of preventive vaccination modeling was taking into account all vaccinated persons, including those who are joining the risk groups, adjusting for the annual average growth rate (Tgr_a). As mentioned above, the financial cost of vaccination increased up to 8.8 million rubles due to the booster vaccination with PPV23 in 2025. At the same time, the prevented damage amounted to 109.7 million rubles in financial terms and continued to increase, reaching 131.9 million rubles in 2028. An early index for the efficacy of preventive work should be taken as the fact of vaccination self-sufficiency in 2 years, when the cost of performing it for the first time decreased to 828 thousand rubles. (Fig. 2).

The total economic benefit indicator was calculated as the difference between the prevented damage (1.14 billion rubles), the amount of costs for vaccination (28.5 million rubles) and the cost of PN cases treatment in vaccinated and unvaccinated patients (17.1 million rubles). Based on the obtained result, it is possible that at the end of 2028, the net economic benefit will amount to 968.2 million rubles.

CONCLUSION

In this study, the possibilities of improving the PI vaccination efficiency in the adult population of the Astrakhan region and approaches to the pharmacoeconomical analysis of vaccinoprophylaxis, have been examined. In order to objectify the current situation, the dynamics

of morbidity in risk groups for the PI development in the period of 2015–2018, was analyzed. It has been established that there are no positive trends in the diseases of the cardiovascular system. For the past 4 years, their total increase has reached 46.5%. The next most important indicators were diabetes and obesity, which made up 30.8% of the total structure of pathology at the end of 2018. A special focus is on increasing chronic bronchopulmonary pathology. After 4 years, the indicator has increased by 22.4%. The number of patients with hemoblastosis, chronic liver and kidney diseases increased slightly during the above period. In total, the number of people in risk groups for PI developing was an average of 99,228 people, which is quite a significant indicator in the Astrakhan region.

CAP is a widespread disease of adults that develops against the background of reduced immunological reactivity of the body. The prognosis of CAP in patients with COPD is especially unfavorable. In adult patients, the risk of developing fatal outcomes from CAP is much higher in comorbid pathology: CHD, DM, and CHF. All these factors are particularly relevant in risk groups, which create a "threat situation" for the CAP development against the background of each abovementioned disease.

At the end of the period of 2015–2018, the study of the pneumonia disease dynamics among the population of the Astrakhan region, revealed an increase of PN cases by 36.5%. The absolute indicator of the total number of patients, who had PN, was 12,970 people. A retrospective calculation of the treatment costs for all reported cases during 4 years showed a quite impressive value, which amounted to 265.23 million rubles. An increase in PN incidence and the financial costs of its treatment required a reconsideration of approaches to the prophylaxis and treatment of this disease. Extremely low efficiency of preventive measures in 2015–2018 was demonstrated by a vaccination coverage in risk groups that did not exceed 2.5%.

In the study, this period was considered a "without vaccination" period. To get an answer to the question about the amount of possible damage when there is no vaccination, a prospective calculation of PN treatment costs in such conditions for the next 10 years (2019–2028), was conducted. According to the final results of calculating financial costs, the PN treatment against the background of an alternative strategy "without vaccination", can be estimated at the amount of 1,31 billion rubles.

To prove the economic feasibility of the strategy "with vaccination", the prevented costs were calculated as the equivalent of the prevented economic damage. As a result of the sequential calculations, the total amount of prevented damage against the background of vaccination with a duration of 10 years can reach 1.14 billion rubles. The estimated cost of vaccination is 28.5 million rubles, and the PN cases treatment costs among vaccinated and unvaccinated individuals, is 17.1 million rubles. Thus, 968.2 million rubles are returned to the healthcare budget.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

E.A. Orlova – data collection, text writing and editing; I.P. Dorfman – data collection and editing;
M.A. Orlov – text writing and editing; M.A. Abdullaev – text writing and editing.
All authors made a significant contribution to the search and analytical work and preparation of the article, read and approved the final version before the publication.

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RESEARCH ARTICLE

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USE OF QUANTUM-CHEMICAL PARAMETERS FOR FORECASTING ANTIRADICAL (HO·) ACTIVITY OF RELATED STRUCTURES CONTAINING A CINNAMIC MOLD FRAGMENT. III. CHALCONES, FLAVANONES AND FLAVONES WITH PHLOROGLUCINIC TYPE OF RING "A"

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42 derivatives of chalcone, flavanone and flavone having a phloroglucinic type of ring "A" and containing the same electron-donating substituents on ring "B", have been studied. Flavonoids with the phloroglucinic type of ring "A" are the most common in nature, which is due to the peculiarities of biogenetic formation with the participation of malonyl and acetyl fragments.

The aim. To determine the effect of the hydroxy group in position 6' of chalcones and in position 5 of flavanones and flavones on bond numbers (Nμ), free valence indices (Fμ), Mulliken charges (a.e), electron density, unsaturation indices (IUA) of the carbon atoms C-1 → C-6 → C-7 → C-8.

Materials and methods. The calculations of the listed above parameters with the use of the semi-empirical method PM7 (WinMopac 2016 program) have been carried out on a workstation with an Intel Xeon E5-1620 3.5 GHz processor, 20 GB of RAM.

Results. The quantum-chemical characteristics of the considered derivatives having a phloroglucinic type of the "A" ring, indicate that the OH group in position 6' of chalcones (in the corresponding flavanones and flavones in position 5) has different effects: a slight increase occurs in chalcones negative charge (a.e.) and electron density, the bond numbers take different values, which depends on the position and number of substituents on the ring "B". In flavanones, Nμ practically remains at the same level of 3.822–3.829. For flavones, the binding numbers Nμ for C-8 are in the range of 3.700–3.706, and the Mulliken charges are in the range from –0.4120 to –0.4356. For position-substituted C-3 (6anone and 7anone), the charges are –0.4436 and –0.4479, respectively. The charge on C-7 of chalcones is negative for compounds 4x, 5x, 10x and 13x from –0.0204 to –0.0470. The remaining derivatives of the chalcone, as well as the corresponding flavanones and flavones, are characterized by a positive value of a.e. on C-7. Based on the bond numbers (Nμ), free valency indices (Fμ) have been found for the carbon atoms of the cinnamoyl fragment C-1 → C-6 → C-7 → C-8. When comparing the obtained data, it was found out that for chalcones on C-1 → C-8 atoms, the values of the free valence indices are in the range of 0.900–0.980 for compounds 12x, 13x, where Fμ>1. For flavanones on C-1, C-3, and C-5 atoms (compounds 12anone and 13anone), the free valence indices are in the range of 0.984-1.024, and for the remaining atoms the value of Fμ is approximately the same as that of chalcones. On the C-8 atoms of all the derivatives, as well as on C-1, C-3 and C-5 (compounds 12one, 13one), Fμ≥1.0. It can be assumed that at values of Fμ=0.850–0.955 for all the analyzed compounds, coupling reactions on the double bond are possible, and if Fμ≥1, the coupling will take place according to the free radical mechanism. The data obtained indicate that the OH group in position 6' for the chalcone and 5 for the flavanones, does not significantly effect the Mulliken charge (a.e) and the electron density on C-8 atoms.

Conclusion. It has been established that the OH group in position 6' of the "A" ring of chalcones (in position 5 of the "A" ring in flavones and flavanones) has a conflicting effect on the bond numbers: when passing from chalcone to flavanone, Nμ increases, and then, in flavone, sharply decreases. For C-8 of all flavone derivatives, Fμ≥1. The following conclusion has been confirmed: at the initial stage of the reaction the electrophilic hydroxyl radical is attached at the C-8 position of the cinnamoyl fragment

Keywords: 2',4',6'-threehydroxychalcones, 5,7-dihydroxyflavanones, 5,7-dihydroxyflavones, bond numbers, free valence indices

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ИСПОЛЬЗОВАНИЕ КВАНТОВО-ХИМИЧЕСКИХ ПАРАМЕТРОВ ДЛЯ ПРОГНОЗИРОВАНИЯ АНТИРАДИКАЛЬНОЙ (HO·) АКТИВНОСТИ РОДСТВЕННЫХ СТРУКТУР, СОДЕРЖАЩИХ ЦИННАМОИЛЬНЫЙ ФРАГМЕНТ. III. ХАЛКОНЫ, ФЛАВАНОНЫ И ФЛАВОНЫ С ФЛОРОГЛЮЦИНОВЫМ ТИПОМ КОЛЬЦА «А»

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Изучено 42 производных халкона, флаванона и флавона, имеющих флороглюциновый тип кольца «А» и содержащих одинаковые электронодонорные заместители в кольце «В». Флавоноиды с флороглюциновым типом кольца «А» наиболее распространены в природе, что обусловлено особенностями биогенетического формирования при участии малонильных и ацетильных фрагментов.

Цель. Выявление влияния гидроксигруппы в положении 6’ у халконов и в положении 5 у флаванонов и флавонов на связевые числа (Nμ); индексы свободной валентности (Fμ); Малликеновские заряды (а.е); электронную плотность; индексы ненасыщенности (IUA) атомов углерода С-1→С-6→С-7→С-8.

Материалы и методы. Перечисленные выше квантово-химические параметры анализируемых соединений рассчитаны полуэмпирическим методом PM7 (программа WinMopac 2016) на рабочей станции с процессором Intel Xeon E5-1620 3,5 ГГц, 20 Гб оперативной памяти.

Результаты. Квантово-химические характеристики рассматриваемых производных, имеющих флороглюциновый тип кольца «А», свидетельствуют о том, что ОН-группа в положении 6’ у халконов, (у соответствующих флаванонов и флавонов в положении 5) влияет по-разному: у халконов имеет место незначительное повышение отрицательного заряда (а.е) и электронной плотности, связевые числа принимают разные значения, что зависит от положения и числа заместителей в кольце «В». У флаванонов Nμ практически остается на одном уровне 3,822–3,829. У флавонов значения связевых чисел Nμ у С-8 находятся в интервале 3,700–3,706, а Малликеновские заряды находятся в пределах от –0,4120 до –0,4356. У замещенных по положению С-3 (6-флаванон и 7-флаванон) заряд равен –0,4436 и –0,4479 соответственно. У халконов заряд на С-7 имеет отрицательное значение у соединений 4х, 5х, 10х и 13х от –0,0204 до –0,0470. Остальные производные халкона, а также соответствующие флаваноны и флавоны, характеризуются положительным значением а.е на С-7. Исходя из связевых чисел (Nμ), найдены индексы свободной валентности (Fμ) для атомов углерода циннамоильного фрагмента С-1→С-6→С-7→С-8. При сопоставлении полученных данных установлено, что у халконов на атомах С-1→С-8 значения индексов свободной валентности находятся в пределах 0,900–0,980 у соединений 12х, 13х, где Fμ>1. У флаванонов на атомах С-1, С-3 и С-5 (соединения 12анон и 13анон) индексы свободной валентности находятся в интервале 0,984–1,024, а у остальных атомов величина Fμ примерно такая же, как у халконов. На атомах С-8 всех производных, а также С-1, С-3 и С-5 (соединения 12он, 13он) Fμ≥1,0. Можно предположить, что при значениях Fμ=0,850–0,955 для всех анализируемых соединений возможны реакции присоединения по двойной связи, а если Fμ≥1, то присоединение будет проходить по свободнорадикальному механизму. Полученные данные свидетельствуют о том, что ОН-группа в положении 6’ у халкона и 5 у флаванонов – не оказывает существенного влияния на Малликеновский заряд (а.е) и электронную плотность на атомах С-8.

Заключение. На основании полученных данных установлено, что ОН-группа в положении 6’ кольца «А» халконов (в положении 5 у флавонов и флаванонов) разнонаправлено влияет на величину связевых чисел: при переходе от халкона к флаванону Nμ возрастает, а затем у флавона резко уменьшается. Для атомов С-8 всех производных флавона Fμ≥1. Это вновь доказывает высказанный ранее нами вывод, что на начальном этапе электрофильный гидроксильный радикал присоединяется по положению С-8 циннамоильного фрагмента.

Ключевые слова: 2,’4’,6’-тригидроксихалконы; 5,7-дигидроксифлаваноны; 5,7-дигидроксифлавоны; связевые числа; индексы свободной валентности

INTRODUCTION

In nature, flavonoids with the phloroglucinic type of ring “A” [1–4] are the most common.

With reference to the previous reports [5, 6], this paper presents an interpretation of the quantum-chemical characteristics of chalcone, flavanone and flavone derivatives, characterized by a phloroglucinic type of ring (42 compounds in total) and having electron-donating substituents on ring “B”.

THE AIM of the article is to determine the effect of the hydroxy group in position 6’ of chalcone derivatives, which upon heterocyclization react to form the corresponding flavanones and flavones, on the quantum-chemical parameters; as well as the calculation of

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the free valence indices (Fμ) of the carbon atoms of the main conjugation chain to explain the reactivity of the compounds under study relative to the hydroxyl radical OH.

MATERIALS AND METHODS

The calculations of the listed above parameters with the use of the semi-empirical method PM7 (WinMopac 2016 program), have been carried out on a workstation with an Intel Xeon E5-1620 3.5 GHz processor, 20 GB of RAM.

RESULTS AND DISCUSSION

This report presents the data on the analysis of the quantum chemical characteristics of the structures presented in Table 1.

Bond numbers (Nμ), free valence indices (Fμ)

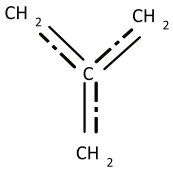
The reactivity of organic molecules is conveniently explained taking into account the free valence indices of the corresponding regions. There is a relationship between this parameter and the bond number [7]:

$$F\mu = N_{max} - N\mu$$

It is appropriate here to describe the physical meaning of the bond number in more details, because it is actually the sum of the orders of all the bonds adjacent to this atom [7].

The value of Nμ reflects the degree of saturation of a particular atom: the larger Nμ, the more saturated a particular atom is and, conversely, the lower the value of Nμ, the more pronounced is the tendency of a given atom to form new bonds.

The theoretical maximum of the sum of the bond orders should be 4.732, which is considered a constant value. It corresponds to the sum of the bond orders of a carbon atom bound by three σ-bonds and three π-bonds with neighboring atoms in the threemethylmethane biradical [8, 9].



It should be notified that by the value of Fμ one can judge the possibility of an addition reaction involving the compounds containing π-bonds: the higher Fμ, the more pronounced the activity of the addition of neutral particles. For values of Fμ>1, a radical mechanism will prevail in these reactions.

Tables 2–4 shows the numerical values of the free valence indices of carbon atoms of the main conjugation chain of the analyzed groups of compounds.

Derivatives of 2',4', 6'-threehydroxychalcones

In the absence of substituents on the B ring (compound 1x), the C-8 atom is characterized by the smallest value of bond number 3.799 (Fμ = 0.953); therefore, the attachment of the hydroxyl radical is most likely due to this position.

The bond numbers for C-1 and C-5 (3.813) with a free valence index of 0.919 for both atoms, are slightly larger. For the rest of the atoms C-2, C-4, C-6 and C-7, the values of Nμ are in the range of 3.850, with a value of Fμ from 0.881 to 0.884.

The introduction of a hydroxy group into the C-1 position of the "B" ring (compound 2x) helps to reduce the bond number of this atom to 3.745 (Fμ = 0.987 at a.e. +0.3426) compared to the same position for the unsubstituted ring B, where Nμ = 3.813 (Fμ = 0.919 with a.e. = –0.1183). In the same compound, C-8 has the smallest bond number (Nμ = 3.732, Fμ = 1.000, a.e = –0.3721), and at the C-6 atom Nμ = 3.765 (Fμ = 0.967, a.e = –0.2414).

Relatively low values of the bond numbers and free valence indices are characterized by positions C-2 and C-5, where Nμ are equal to 3.788 (Fμ = 0.944) and 3.806 (Fμ = 0.926). Replacing the OH group in C-1 by OCH_3 retains the indicated patterns at the C-1, C-2, C-5, C-6, and C-8 atoms (Table 2).

There is OH group in the C-2 position of compound 4x, and this contributes to a decrease in the bond number at the C-1 atoms (Nμ = 3.752, Fμ = 0.980, a.e = -0.2504), C-3 (Nμ = 3.769, Fμ = 0.963, a.e = –0.3068). At C-8, the value of Nμ is significantly lower (Nμ = 3.822, Fμ = 0.910, a.e = –0.3225) than at C-1 and C-3.

The methoxy group in position C-2 (compound 5x) contributes to a decrease in the bond number by C-1 (Nμ = 3.746, Fμ = 0.986, a.e = –0.2567) and C-3 (Nμ = 3.767, Fμ = 0.965 , a.e = –0.2964). As for the C-8 atom, here Nμ = 3.823 (Fμ = 0.909, a.e = –0.3575).

The hydroxy group in position C-3 (compound 6x) contributes to a significant decrease in the bond number (Nμ = 3.768, Fμ = 0.964, a.e = -0.3475) compared to other carbon atoms in the compound 6x. Rather low bond numbers are recorded for C-2 (Nμ = 3.788, Fμ = 0.944, a.e = –0.2618) and C-4 (Nμ = 3.778, Fμ = 0.954, a.e = –0.3219). In the case of OCH_3 in position C-3 (compound 7x), practically the same features as in 6x, are retained.

Two hydroxy groups or two methoxy groups (compounds 8x and 11x) in positions C-2 and C-3 have a competitive effect on the propenone fragment C-7 → C-8 → C-9. According to the σ-Taft constants of the substituents for the C-3-OH group, σ = –0.370, for OCH_3 σ = –0.268; for C-2-OH groups, σ = +0.127, for OCH_3 σ = +0.115 [10]. This type of substitution contributes to a decrease in Nμ to 3.763 and 3.765 (C-2 and C-3 di-OH), 3.765 and 3.777 (C-2 and C-3 di-OCH_3) at C-4 and C-5 atoms, respectively, ring "B").

For compounds 9x and 10x, where 2-OH and 3-OCH_3 (2-OCH_3 and 3-OH) groups are presented, almost all Nμ and Fμ values are the same on all carbon atoms of the propenone fragment.

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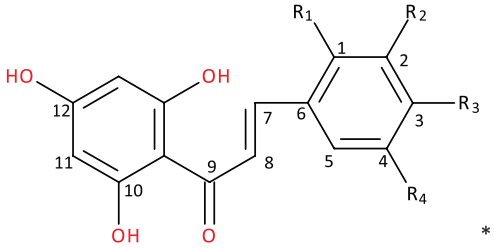
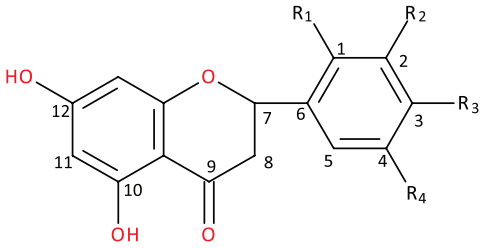
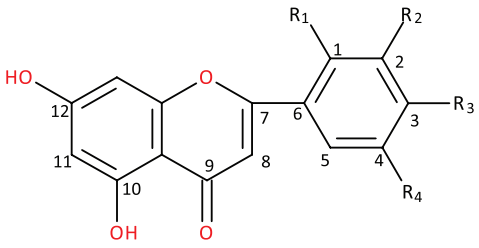
Table 1 – Chalcone, flavanone and flavone derivatives with a phloroglucinic type of ring A*

Com-pounds, Ser. No.	 **		
	Chalcones	Flavanones	Flavones
	X	anone	one
	The nature of the substituents on the ring "B"		

	1	2	3	4	1	2	3	4	1	2	3	4
1	H	H	H	H	H	H	H	H	H	H	H	H
2	OH	H	H	H	OH	H	H	H	OH	H	H	H
3	OCH_3	H	H	H	OCH_3	H	H	H	OCH_3	H	H	H
4	H	OH	H	H	H	OH	H	H	H	OH	H	H
5	H	OCH_3	H	H	H	OCH_3	H	H	H	OCH_3	H	H
6	H	H	OH	H	H	H	OH	H	H	H	OH	H
7	H	H	OCH_3	H	H	H	OCH_3	H	H	H	OCH_3	H
8	H	OH	OH	H	H	OH	OH	H	H	OH	OH	H
9	H	OCH_3	OH	H	H	OCH_3	OH	H	H	OCH_3	OH	H
10	H	OH	OCH_3	H	H	OH	OCH_3	H	H	OH	OCH_3	H
11	H	OCH_3	OCH_3	H	H	OCH_3	OCH_3	H	H	OCH_3	OCH_3	H
12	H	OH	OH	OCH_3	H	OH	OH	OCH_3	H	OH	OH	OCH_3
13	H	OCH_3	OCH_3	OCH_3	H	OCH_3	OCH_3	OCH_3	H	OCH_3	OCH_3	OCH_3
14	H	$C(CH_3)_3$	OH	$C(CH_3)_3$	H	$C(CH_3)_3$	OH	$C(CH_3)_3$	H	$C(CH_3)_3$	OH	$C(CH_3)_3$

Note: * – Chalcone, flavanone and flavone derivatives under No. 14 are virtual and, in accordance with the forecast, will be synthesized later; ** – Numbering of carbon atoms corresponds to the numbers generated by the calculation programs

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Table 2 – The values of the bond numbers (Nμ) and free valency indices (Fμ) on the C-1 → C-6 → C-7 → C-8 atoms of the main conjugation chain of the 2',4',6'-threehydroxychalcone derivatives. $F\mu=N_{max}-N\mu$

Ser. No.	Carbons R	C-1		C-2		C-3		C-4		C-5		C-6		C-7		C-8	
		Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ
1	$R_1=R_2=R_3=R_4=H$	3.813	0.919	3.851	0.881	3.835	0.897	3.853	0.879	3.813	0.919	3.858	0.874	3.848	0.884	3.779	0.953
2	$R_1=OH$. $R_2=R_3=R_4=H$	3.745	0.987	3.788	0.944	3.829	0.903	3.842	0.890	3.806	0.926	3.765	0.967	3.829	0.903	3.732	1.000
3	$R_1=OCH_3$. $R_2=R_3=R_4=H$	3.766	0.966	3.780	0.952	3.838	0.894	3.837	0.895	3.820	0.912	3.790	0.942	3.861	0.871	3.828	0.904
4	$R_2=OH$. $R_1=R_3=R_4=H$	3.752	0.980	3.793	0.939	3.769	0.963	3.846	0.886	3.806	0.926	3.856	0.876	3.877	0.855	3.822	0.910
5	$R_2=OCH_3$. $R_1=R_3=R_4=H$	3.746	0.986	3.796	0.936	3.767	0.965	3.847	0.885	3.804	0.928	3.857	0.875	3.875	0.857	3.823	0.909
6	$R_3=OH$. $R_1=R_2=R_4=H$	3.813	0.919	3.788	0.944	3.771	0.961	3.778	0.954	3.809	0.923	3.853	0.879	3.842	0.890	3.768	0.964
7	$R_3=OCH_3$. $R_1=R_2=R_4=H$	3.815	0.917	3.782	0.950	3.772	0.960	3.775	0.957	3.809	0.923	3.829	0.903	3.839	0.893	3.765	0.967
8	$R_2=R_3=OH$. $R_1=R_4=H$	3.805	0.927	3.786	0.946	3.762	0.970	3.763	0.963	3.765	0.967	3.837	0.895	3.845	0.887	3.773	0.953
9	$R_2=OCH_3$. $R_3=OH$. $R_1=R_4=H$	3.808	0.924	3.779	0.953	3.758	0.974	3.761	0.971	3.762	0.970	3.835	0.897	3.842	0.890	3.771	0.961
10	$R_2=OH$. $R_3=OCH_3$. $R_1=R_4=H$	3.809	0.923	3.788	0.944	3.760	0.972	3.763	0.969	3.764	0.968	3.840	0.892	3.873	0.859	3.810	0.922
11	$R_2=R_3=OCH_3$. $R_1=R_4=H$	3.805	0.927	3.779	0.953	3.750	0.982	3.765	0.967	3.757	0.975	3.835	0.897	3.840	0.892	3.768	0.964
12	$R_2=R_3=R_4=OH$. $R_1=H$	3.798	0.994	3.776	0.956	3.708	1.024	3.770	0.962	3.736	0.996	3.850	0.882	3.852	0.880	3.784	0.948
13	$R_2=R_3=R_4=OCH_3$. $R_1=H$	3.729	1.003	3.776	0.956	3.692	1.040	3.768	0.964	3.721	1.011	3.854	0.878	3.874	0.858	3.820	0.912
14	$R_2=R_4=C(CH_3)_3$. $R_3=OH$. $R_4=H$	3.794	0.938	3.794	0.938	3.748	0.984	3.790	0.942	3.798	0.934	3.834	0.898	3.858	0.874	3.788	0.944

Note: * – Numbering of carbon atoms of the main conjugation chain is given in accordance with the numbers generated by the calculation programs

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Table 3 – The values of the bond numbers (Nμ) and free valence indices (Fμ) on the C-1 → C-6 → C-7 → C-8 atoms of the main conjugation chain of 5,7-dihydroxyflavanone derivatives

No.	Carbon atoms	C-1		C-2		C-3		C-4		C-5		C-6		C-7		C-8	
	R	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ
1	R_1=R_2=R_3=R_4=H	3.829	0.903	3.849	0.883	3.844	0.888	3.85	0.882	3.828	0.904	3.840	0.892	3.838	0.894	3.827	0.905
2	R_1=OH, R_2=R_3=R_4=H	3.776	0.956	3.795	0.937	3.842	0.89	3.843	0.889	3.822	0.91	3.770	0.962	3.832	0.900	3.819	0.913
3	R_1=OCH_3, R_2=R_3=R_4=H	3.780	0.952	3.801	0.931	3.842	0.89	3.842	0.89	3.828	0.94	3.792	0.940	3.839	0.893	3.817	0.915
4	R_2=OH, R_1=R_3=R_4=H	3.762	0.970	3.792	0.94	3.773	0.959	3.845	0.887	3.814	0.918	3.841	0.891	3.839	0.893	3.826	0.906
5	R_2=OCH_3, R_1=R_3=R_4=H	3.757	0.975	3.795	0.937	3.77	0.962	3.846	0.886	3.813	0.919	3.842	0.890	3.837	0.895	3.827	0.905
6	R_3=OH, R_1=R_2=R_4=H	3.828	0.904	3.785	0.947	3.784	0.948	3.777	0.955	3.823	0.909	3.820	0.912	3.835	0.897	3.826	0.906
7	R_3=OCH_3, R_1=R_2=R_4=H	3.830	0.902	3.779	0.953	3.785	0.947	3.774	0.958	3.824	0.908	3.817	0.915	3.835	0.897	3.828	0.904
8	R_2=R_3=OH, R_1=R_4=H	3.764	0.968	3.778	0.954	3.749	0.983	3.798	0.934	3.832	0.919	3.832	0.900	3.837	0.895	3.827	0.905
9	R_2=OCH_3, R_3=OH, R_1=R_4=H	3.759	0.973	3.776	0.956	3.746	0.986	3.796	0.936	3.814	0.918	3.833	0.899	3.836	0.896	3.829	0.903
10	R_2=OH, R_3=OCH_3, R_1=R_4=H	3.767	0.965	3.771	0.961	3.754	0.978	3.792	0.94	3.814	0.918	3.831	0.901	3.835	0.897	3.826	0.906
11	R_2=R_3=OCH_3, R_1=R_4=H	3.782	0.950	3.767	0.965	3.76	0.972	3.779	0.953	3.82	0.912	3.824	0.908	3.835	0.897	3.829	0.903
12	R_2=R_3=R_4=OH, R_1=H	3.748	0.984	3.772	0.96	3.712	1.02	3.776	0.966	3.748	0.984	3.833	0.899	3.838	0.894	3.822	0.910
13	R_2=R_3=R_4=OCH_3, R_1=H	3.739	0.993	3.767	0.965	3.69	1.042	3.769	0.963	3.722	1.01	3.841	0.891	3.837	0.895	3.831	0.901
14	R_2=R_4=$C(CH_3)_3$, R_3=OH, R_4=H	3.804	0.928	3.793	0.939	3.755	0.977	3.785	0.947	3.811	0.921	3.818	0.914	3.838	0.894	3.829	0.903

Note: * – Numbering of carbon atoms of the main conjugation chain is given in accordance with the numbers generated by the calculation programs

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Table 4 – The values of the bond numbers (Nμ) and free valency indices (Fμ) on the C-1 → C-6 → C-7 → C-8 atoms of the main conjugation chain of 5,7-dihydroxyflavone derivatives

No.	Carbon atoms R	C-1		C-2		C-3		C-4		C-5		C-6		C-7		C-8	
		Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ	Nμ	Fμ
1	R_1=R_2=R_3=R_4=H	3.808	0.924	3.851	0.881	3.835	0.897	3.852	0.88	3.809	0.923	3.829	0.903	3.793	0.939	3.701	1.031
2	R_1=OH, R_2=R_3=R_4=H	3.759	0.973	3.785	0.947	3.832	0.9	3.837	0.895	3.807	0.925	3.724	1.008	3.782	0.950	3.712	1.02
3	R_1=OCH_3, R_2=R_3=R_4=H	3.769	0.963	3.77	0.962	3.834	0.898	3.834	0.898	3.81	0.922	3.739	0.993	3.793	0.939	3.712	1.02
4	R_2=OH, R_1=R_3=R_4=H	3.745	0.987	3.798	0.934	3.771	0.961	3.848	0.884	3.797	0.935	3.837	0.895	3.798	0.934	3.707	1.25
5	R_2=OCH_3, R_1=R_3=R_4=H	3.738	0.994	3.8	0.932	3.767	0.965	3.848	0.884	3.795	0.937	3.838	0.894	3.796	0.936	3.705	1.027
6	R_3=OH, R_1=R_2=R_4=H	3.805	0.927	3.789	0.943	3.769	0.963	3.774	0.958	3.805	0.927	3.798	0.934	3.787	0.945	3.694	1.038
7	R_3=OCH_3, R_1=R_2=R_4=H	3.808	0.924	3.783	0.949	3.771	0.961	3.771	0.961	3.805	0.927	3.794	0.938	3.783	0.949	3.691	1.041
8	R_2=R_3=OH, R_1=R_4=H	3.746	0.986	3.784	0.948	3.742	0.99	3.794	0.938	3.796	0.936	3.820	0.912	3.793	0.939	3.700	1.032
9	R_2=OCH_3, R_3=OH, R_1=R_4=H	3.738	0.994	3.781	0.951	3.738	0.994	3.793	0.939	3.796	0.936	3.819	0.913	3.790	0.942	3.698	1.034
10	R_2=OH, R_3=OCH_3, R_1=R_4=H	3.749	0.983	3.788	0.944	3.746	0.986	3.79	0.942	3.796	0.936	3.816	0.916	3.789	0.943	3.697	1.035
11	R_2=R_3=OCH_3, R_1=R_4=H	3.741	0.991	3.775	0.957	3.743	0.989	3.788	0.944	3.796	0.936	3.816	0.916	3.787	0.945	3.697	1.035
12	R_2=R_3=R_4=OH, R_1=H	3.737	0.995	3.776	0.956	3.709	1.023	3.771	0.961	3.732	1.0	3.825	0.907	3.797	0.935	3.705	1.027
13	R_2=R_3=R_4=OCH_3, R_1=H	3.723	1.009	3.771	0.961	3.689	1.043	3.773	0.959	3.71	1.022	3.833	0.899	3.791	0.941	3.704	1.028
14	R_2=R_4=$C(CH_3)_3$, R_3=OH, R_4=H	3.785	0.947	3.794	0.938	3.742	0.99	3.787	0.945	3.79	0.942	3.795	0.937	3.777	0.955	3.689	1.043

Note: * – Numbering of positions in accordance with figures generated by settlement programs

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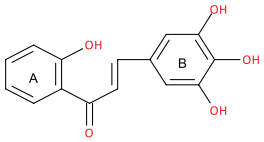
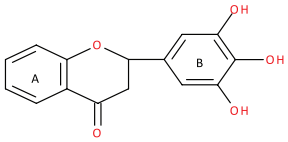
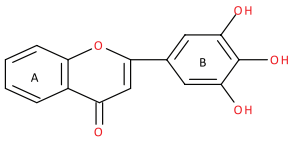
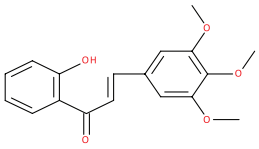
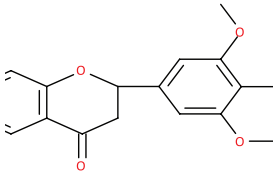
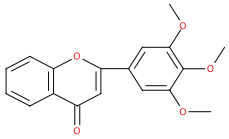
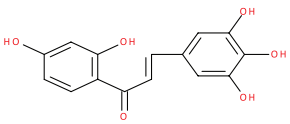
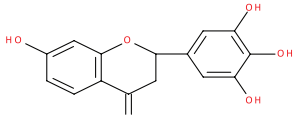
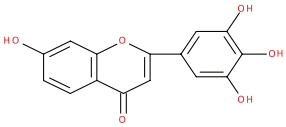
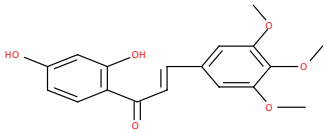
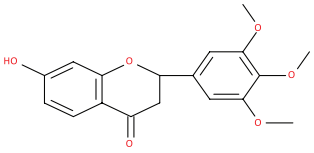
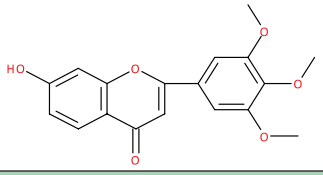
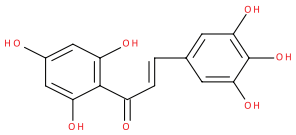
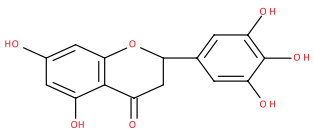
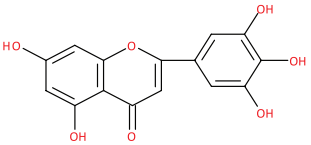
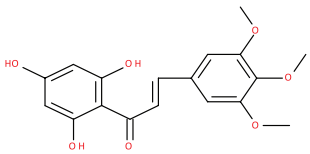
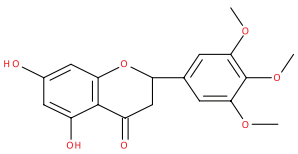
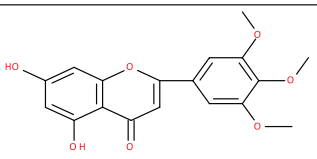
Table 5 – Comparative quantum-chemical characteristics of the C-8 atom of 2'-hydroxy-, 2', 4'-dihydroxy- and 2',4',6'-threehydroxychalcones derivatives containing substituents in positions 3, 4, 5 of the ring "*B*"*

			
a.e.	–0.3016	–0.4337	–0.4337
Nμ	3.817	3.815	3.815
Fμ	0.915	0.917	0.917
El.d.	4.3195	4.3737	4.4337
			
a.e.	–0.3136	–0.4370	–0.4370
Nμ	3.813	3.821	3.821
Fμ	0.919	0.911	0.911
El.d.	4.3136	4.4370	4.437
			
a.e.	–0.3013	–0.4342	–0.4158
Nμ	3.818	3.816	3.699
Fμ	0.914	0.916	1.033
El.d.	4.3013	4.4343	4.4158
			
a.e.	0.3130	–0.4378	–0.4495
Nμ	3.815	3.821	3.686
Fμ	0.917	0.911	1.046
El.d.	4.3113	4.4378	4.4495
			
a.e.	–0.3115	–0.4252	–0.4170
Nμ	3.784	3.822	3.705
Fμ	0.948	0.91	1.027
El.d.	4.3115	4.4253	4.4170
			
a.e.	–0.3265	–0.4291	–0.4190
Nμ	3.820	3.831	3.704
Fμ	0.912	0.901	1.028
El.d	4.3266	4.4292	4.4190

Note: * – Numbering of positions in accordance with figures generated by settlement programs

The most characteristic features are recorded in compounds 12x and 13x, where ring "B" is represented by the remainder of pyrogallol or its fully methylated derivative. This arrangement of hydroxy groups is characteristic of natural flavonoids – myricetin, tricetin and tricin [1,2,3], which are the most active antioxidants [10] with respect to OH radical. Atoms C-1, C-2, C-5, C-8 are characterized by approximately the same values of Nμ and Fμ for both 12x and 13x (Table 2).

There is a very important feature: the carbon atoms on the «B» ring, to which the electron-donating OH and OCH_3 groups are bound with, are characterized by a positive charge (C-1 for 2x and 3x; C-2 for 4x and 5x; C-3 for 6x, 7x, 8x, 9x, 10x, 11x, 12x and 14x; C-4 at 8x, 9x, 10x, 11x, 12x, 13x).Table 2 shows that the carbon atoms listed above, are characterized by rather high values of the free valence indices, however, the presence of a positive charge on these atoms precludes the addition of an electrophilic hydroxyl radical to them at the initial stage.

5,7-dihydroxyflavanone derivatives

Table 3 shows the values of Nμ and Fμ for the analyzed flavanones. Comparing them with similar characteristics for chalcones (Table 2), it can be notified that flavanones have bond numbers and, accordingly, free valence indices, not fundamentally different from those of chalcones.

In flavanones 12anone and 13anone on C-1, C-3, C-5 atoms, the values of Fμ are very close or equal to 1.0, which indicates the predisposition of the indicated positions of the ring "B" to the attachment, possibly, by the radical mechanism.

The values of the free valence indices of C-6, C-7 and C-8 atoms also indicate the ability of these atoms to addition reactions.

5,7-dihydroxyflavone derivatives

It should be notified that in this group of derivatives, the C-7 carbon atom is characterized by a positive Mulliken charge in all compounds, without exception.

There is a similar pattern for atoms C-2 (compounds 4, 5, 9, 10–13one), C-3 (compounds 6–12one and 14one) and C-4 (compounds 12one and 13one).

The presence of a positive charge on carbon atoms in these positions indicates that at the initial stage, the addition of an electrophilic hydroxyl radical to the listed carbon atoms is excluded.

At the same time, the C-8 position is characterized by such features as low bond numbers (Nμ = 3.689–3.712) in comparison with other carbon atoms in combination with rather high free valence indices (Fμ = 1.02–1.25) (Table 4). At the same carbon atom, the negative Mulliken charge is in the range from –0.4122 to –0.4508.

Thus, the C-8 position is the most probable center where the radical OH should join.

It is clear that the interaction of an attacking particle with a given carbon atom of the vinylene group will entail a redistribution of the electron plane, bond numbers, Mulliken charge and free valence indices. This, in turn, will lead to the formation of new nucleophilic centers, where the subsequent ·OH radical will join, and so on until the carbon atoms in the flavone transformation products become extremely saturated. Every time, this can be judged by the changing values of the bond numbers, free valence indices, as well as the charges of carbon atoms and their electron density.

Such an approach, in our opinion, predetermines a quantitative assessment of the ratio of the moles number of the OH radical to 1 mole of the initial flavone during their interaction.

Table 5 shows the comparative quantum chemical characteristics of C-8 atoms in the analyzed groups of chalcones, flavanones and flavones containing electron-donating substituents in the rings "A" and "B". The choice of the compounds containing three -OH and $-OCH_3$ groups (pyrogallol type of substitution) is determined, first of all, by their greatest antioxidant and anti-inflammatory kinds of activity, which was experimentally proved [10–12].

It follows from the table that, when passing from chalcones to flavones, the bond numbers (Nμ) decrease, which entails an increase in the values of the free valence indices (Fμ). That indicates an increase in the reactivity of the C-8 atom with respect to the electrophilic hydroxyl radical.

CONCLUSION

The article is devoted to the analysis of the quantum-chemical parameters of chalcones, flavanones and flavones, in which the "A" ring is represented by the phloroglucinic type, and the "B" ring of all the three groups of compounds in the same positions, contains electron-donating –OH and $–OCH_3$ groups.

In all the three compounds –14x, 14anone and 14one – the "B" ring contains two *tert*-butyl substituents and a phenolic hydroxyl between them (see Table 1), i.e. the "B" ring is a spatially hindered phenol which, due to the homolytic cleavage of the OH bond, is able to form a stable phenoxyl radical that actively interacts with the hydroxyl radical.

For the carbon atoms of the main conjugation chain C-1 → C-6 → C-7 → C-8, free valence indices (Fμ) have been found.

It has been established that the OH group in position 6′ of the "A" ring of chalcones, and in position 5 of the "A" ring of flavanones and flavones, has conflicting effects on the bond numbers: when passing from chalcone to flavanone, Nμ increases, and then, in flavone, sharply decreases. Using the dependence Fμ = Nmax – Nμ, the corresponding free valency indices–have been found out; for the C-8 atoms of all flavone derivatives, they correspond to the expression Fμ≥1.

With reference to the previous reports and taking into consideration both the Mulliken charge (a.u.) and the electron density on this atom, the following conclusion has been confirmed: at the initial stage of the reaction the electrophilic hydroxyl radical is attached at the C-8 position of the cinnamoyl fragment.

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The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION
E.T. Oganesyan – search and analysis of literature, interpretation of results, writing the text of the manuscript;
S.S. Shatokhin – search and analysis of literature, performance of quantum-chemical calculations, interpretation of the results

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RESEARCH ARTICLE

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STUDY OF PHARMACEUTICAL SPECIALISTS' INFORMATION AWARENESS ON THE MATTERS OF DRUG ABUSE

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Prevention of non-medical use of drugs is the most important task of the state anti-drug policy of the Russian Federation, the effectiveness of which can largely be due to the professional awareness of pharmaceutical specialists, their compliance with the established regulations for the dispensing of drugs, and proper pharmaceutical advice.

The aim. The research of pharmaceutical specialists' information awareness on the matters of drug abuse.

Material and methods. The study was based on the analysis of the regulatory legal acts of the Russian Federation governing the procedure for prescribing and dispensing drugs, instructions for the medical use of the drugs used for the purpose of abuse. In the course of the study, a systematic approach has been applied. It includes methods of structural-logical, cluster and content analyses, methods of generalization and grouping. The study of pharmaceutical specialists' information awareness on the matters of drug abuse was carried out using a random sample survey using a specially developed questionnaire: 396 employees of pharmacy organizations of various forms of property from the Perm Territory, the Chelyabinsk and Kirov regions, the Udmurt and Chuvash Republics, the Komi Republic, were questioned in the period from 2017 to 2019. The questionnaire included 35 questions, structured in 4 blocks. The first block included questions on education, position, work experience of the respondents, the second – questions on identifying knowledge on the range of drugs used for abuse, and categories of consumers of such drugs. The third block contained questions on the regulation and compliance with the procedure for dispensing drugs. The fourth block of the questionnaire was intended to establish the methods and sources of obtaining information on the abuse of the drugs sold by specialists of pharmacy organizations.

Results. On the basis of the analysis carried out, an insufficient level of knowledge by specialists of the regulatory legal acts governing the trade of drugs, the range of drugs used for the purpose of abuse, and the categories of their consumers have been established. The violation of the rules for dispensing drugs has been revealed, as well as the lack of systematic sources of information on drug abuse.

Conclusion. The need to develop a training program for pharmaceutical specialists on the prophylaxis and prevention of drug abuse has been established. In order to work out additional competencies in the prevention of non-medical use of drugs and improve the quality of pharmaceutical consulting, it is necessary to conduct appropriate educational activities.

Keywords: pharmaceutical specialists' information awareness; drug abuse

ИЗУЧЕНИЕ ИНФОРМИРОВАННОСТИ ФАРМАЦЕВТИЧЕСКИХ РАБОТНИКОВ ПО ВОПРОСАМ ЗЛОУПОТРЕБЛЕНИЯ ЛЕКАРСТВЕННЫМИ ПРЕПАРАТАМИ

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Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ
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Профилактика немедицинского использования лекарственных препаратов является важнейшей задачей государственной антинаркотической политики Российской Федерации, эффективность которой в значительной степени может быть обусловлена профессиональной информированностью фармацевтических работников, соблюдением ими установленных регламентов отпуска лекарственных препаратов и надлежащего фармацевтического консультирования.

Цель. Изучение информированность фармацевтических специалистов по вопросам злоупотребления лекарственными препаратами.

Материал и методы. Исследование базировалось на анализе нормативных правовых актов Российской Федерации, регламентирующих порядок назначения и отпуска лекарственных препаратов, инструкций по медицинскому применению лекарственных препаратов, используемых с целью злоупотребления. В процессе исследования использовался системный подход, включающий в себя методы структурно-логического, кластерного и контент-анализов, методы обобщения и группировки. Изучение информированности фармацевтических работников по вопросам злоупотребления лекарственными препаратами проводилось методом случайного выборочного опроса по специально разработанной анкете: 396 работников аптечных организаций различных форм собственности из Пермского края, Челябинской и Кировской областей, Удмуртской и Чувашской Республик, Республики Коми в период с 2017 по 2019 годы. В анкету были включены 35 вопросов, структурированных в 4 блока. В первом блоке были включены вопросы по образованию, должности, стажу работы респондентов, во втором – вопросы по выявлению знаний по ассортименту лекарственных препаратов, используемых с целью злоупотребления и категориям потребителей таких лекарственных препаратов. В третьем блоке содержались вопросы по регламентации и соблюдению порядка отпуска препаратов. Четвертый блок анкеты предназначался для установления способов и источников получения информации по вопросам злоупотребления препаратов, используемых специалистами аптечных организаций.

Результаты. На основе проведенного анализа установлен недостаточный уровень знаний специалистами нормативных правовых актов, регламентирующих обращение лекарственных препаратов, ассортимента лекарственных препаратов, используемых с целью злоупотребления и категорий их потребителей. Выявлено нарушение правил отпуска лекарственных препаратов, отсутствие систематизированных источников информации по вопросам злоупотребления лекарственными препаратами.

Заключение. Установлена необходимость разработки программы повышения квалификации фармацевтических работников по вопросам профилактики и предотвращения злоупотребления лекарственными препаратами. С целью формирования дополнительных компетенций по профилактике немедицинского употребления лекарственных препаратов и повышения качества фармацевтического консультировании необходимо проводить соответствующие образовательные мероприятий.

Ключевые слова: информированность фармацевтических специалистов; злоупотребление лекарственными препаратами

INTRODUCTION

Over a period of several years, drug abuse has remained a serious public health problem, both in Russia and abroad. A review of literature sources made it possible to establish that the unfavorable situation associated with the facts of non-medical use of drugs continues to persist [1–7].

Most often, drugs with psychoactive effects are used for non-medical purposes. These drugs are used to achieve a state of doping (temulence), which occurs after taking a drug, and consists in disorders of consciousness, cognitive functions, perception of reality, emotions, behavior, reactions, statics, motion coordination, vegetative and other functions [1, 6–11].

Popular medicinal preparations (MPs) containing codeine or dextromethorphan [1, 4, 12–14], m-cholinoblockers [6, 8, 15–17], benzodiazepine derivatives and somnifacients of the non-benzodiazepine series [2, 18–20], baclofen [21, 22] , pregabalin [5, 7, 11] and others, are used to enhance the narcotic effect of opioids, relieve abstinence symptoms (in the formed drug abuse), potentiate the effect of alcohol, and to individually achieve a state of doping, which can lead to negative consequences, including overdose or drug addiction.

The problem of non-medical use of drugs is of great social importance, since the main category of consumers is the young generation. The authors notify that the phenomenon of intoxication with the use of available drugs is a serious problem among adolescents, however, there have been suicide cases caused by the use of available drugs in adolescents without an established fact of abuse, and it is described in the literature data [8].

It has been also emphasized that this kind of drugs are used in suicide attempts, especially among women. When analyzing intoxication cases, their seasonal prevalence was also notified [4, 20, 23].

According to “Strategy of the State Anti-Drug Policy of the Russian Federation until 2020” [1], the abuse of drugs with psychoactive effects poses a serious threat to the security of the state and the healths of its population. Within the framework of this Strategy, the intensified work is being carried out to stabilize the drug

[1] Decree of the President of the Russian Federation of 09.06.2010 No. 690 “On Approval of the Strategy of the State Anti-Drug Policy of the Russian Federation until 2020”. Available from: http://www.consultant.ru/document/cons_doc_LAW_101259/ (Date of access 19 Aug 2019).

situation and non-medical use of drugs, including the implementation of preventive measures among the population.

The main source of purchase of the above-mentioned drugs is pharmacy organizations (PhOs), whose specialists must have updated information about the range of drugs, categories of consumers, the consequences of abuse and regulation of drug dispensing from pharmacies in order to prevent their subsequent non-medical use. Herewith, the role of a pharmaceutical specialist in the prevention of drug addiction and the prevalence of drug abuse is increasing. This is ensured by compliance with the rules for dispensing drugs from PhOs and providing appropriate pharmaceutical advice.

THE AIM of our study is the research of pharmaceutical specialists' information awareness on the matters of drug abuse.

MATERIAL AND METHODS

The study is based on the regulatory legal acts governing the procedure for prescribing and dispensing drugs, instructions for the medical use of drugs used for the purpose of abuse. In the course of the study, a systematic approach has been applied. It includes methods of structural-logical, cluster and content analyses, methods of generalization and grouping. The study was carried out using a random sample survey of the pharmaceutical specialists from the Perm Territory, the Chelyabinsk and Kirov regions, the Udmurt and Chuvash Republics, the Komi Republic, in the period from 2017 to 2019. The number of the specialists required for the study, had been determined by the formula for calculating a representative sample size for sociological researches [24]. 396 employees of the PhOs took part in the survey. To assess the professional competence of specialists in this area, a questionnaire had been made up. It included 35 questions, structured in 4 blocks. The first block included questions on education, position, work experience of the respondents, the second – questions on identifying knowledge on the range of the drugs used for abuse, and categories of consumers of such drugs. The third block contained questions on the regulation and compliance with the procedure for dispensing drugs. The fourth block of the questionnaire was intended to establish the methods and sources of obtaining information on the abuse of the drugs used by specialists of pharmacy organizations.

RESULTS

The processing of the questionnaires showed that the sample included specialists with higher pharmaceutical education (142 specialists -35.8%); secondary pharmaceutical education (234 people – 59.1%); 20 specialists (5.1%) with secondary and higher kinds of pharmaceutical education.

Grouping of the respondents by the positions occupied, showed the following: 59.1% of the respondents hold the position of a pharmacist; 39.9% were pharmaceutical sales representatives; 1.0% worked as trading floor administrators.

The results of the pharmaceutical specialists' distribution by work experience in PhOs gave grounds to conclude that the majority of the respondents have a work experience from 5 to 20 years or more (91.9%), the rest have the experience from 1 to 5 years (8.1%).

Almost half of the respondents (40.9% – 162 people) are specialists with a pharmaceutical experience from 5 to 10 years, 83 specialists have the experience from 10 to 15 years (21%), 48 employees (12.1%) have been working in PhOs from 15 to 20 years, and 71 specialists (17.9%) have over 20 years of work experience.

The study of the survey data revealed that, along with the regulated mandatory advanced training in educational programs of additional vocational education, followed by obtaining a specialist certificate at least once every 5 years (100% of respondents), pharmaceutical specialists regularly improve their professional qualifications in various ways, i.e.:

– participate in educational conferences and seminars with the involvement of specialists – 61.4% of the interviewed employees;

– receive information from professional printed issues – 60.1% of PhO employees;

– use reference materials in their work – 55.6% of the respondents;

– attend trainings and classes conducted by pharmacy organizations – 34.8% of employees (Fig. 1).

One of the main tasks of the survey was to study the professional awareness of pharmacies' employees about the drug groups that can be used for other than medical purposes, the order of their dispensing, as well as the completeness of the specialists' knowledge of the drugs most often misused.

In the questionnaire, there were "Combined medicinal preparations containing codeine", "Combined medicinal preparations containing dextrometrophan", and specific names of drugs – tropicamide, cyclopentolate, etc., as well as proposed prescription forms for their dispensing from PhOs.

When analyzing the questionnaires, it was found out that the respondents identified 13 groups of drugs (represented by 19 pharmacotherapeutic groups) used (in their opinions) for the purpose of abuse. The largest number of respondents notified such groups of drugs as M-cholinoblockers containing tropicamide and cyclopentolate (92.7%), analgesics and alcohol-containing drugs (89.9%).

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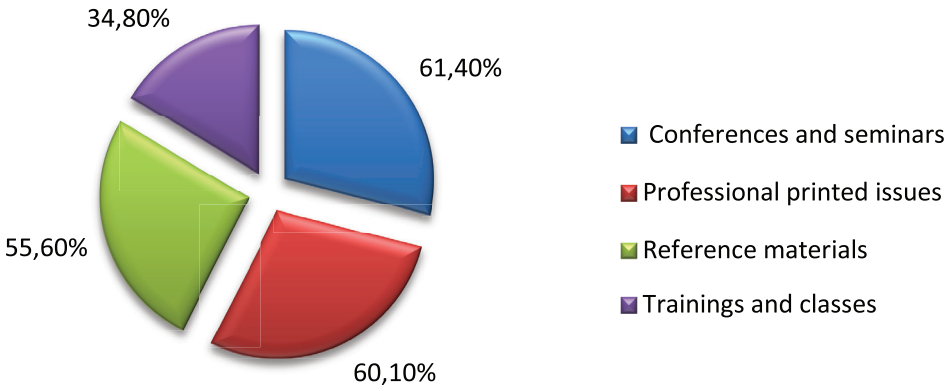

Figure 1 – Ways of advanced training by employees of PhOs

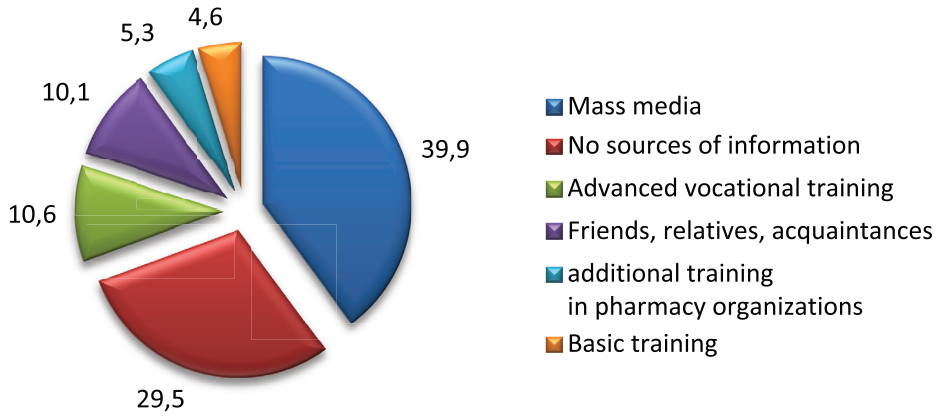

Figure 2 – Methods of advanced training by PhO employees

Almost half of the specialists pointed out at the acquisition of drugs for non-medical purposes. They were as follows: from the muscle relaxant groups (48.9%), antiepileptic drugs (50.0%), as well as combined drugs containing dextromethorphan (46.2%). In the questionnaires, about 40.0% of the respondents notified combined drugs containing codeine (39.4%), and alpha-adrenergic agonists (35.1%). In the questionnaires, fewer than a third of the specialists paid great attention to the drugs used not only for medical purposes: benzodiazepine derivatives (28.2%), combined drugs containing ephedrine (24.7%), antidepressants (14.6%) and somnifacients of the non-benzodiazepine series.

According to the opinions of the pharmaceutical specialists, in the course of the questionnaire survey, some specific medicinal preparations were also identified as requested for misuse.

The majority of the respondents identified a combined analgesic MP containing dicycloverin 20.0 mg + paracetamol 500.0 mg (the "Analgesics" group), alcohol-containing solutions/tinctures (89.9%), as well as tropicamide (the "M-anticholinergics" group) – 75.0%.

Almost half of the specialists focused their attention on purchasing the following drugs for non-medical purposes: 50.0% – pregabalin from the "Antiepileptic drugs" group and 48.9% – baclofen (the "Muscle relaxants" group). According to the respondents, the following medicinal preparations are purchased for misuse: dextromethorphan 15.0 mg + paracetamol 500.0 mg + phenylephrine 10.0 mg + chlorphenamine 2.0 mg from the group of "Combined medicinal preparations containing dextromethorphan" (39.9%); codeine 10.0 mg + caffeine 50.0 mg + metamizole sodium 150.0 mg + paracetamol 300.0 mg + phenobarbital 15.0 mg (30.1%) from the group of "Combined drugs containing codeine"; naphazoline from the group of "Alfa-adrenergic agonists" (35.1%).

Some respondents also identified other drugs, including: bromodihydrochlorophenylbenzodiazepine from the group of "Medicinal preparations, benzodiazepine derivatives" (28.2%); a combined preparation containing ephedrine 4.6 mg + glaucine 5.75 mg in the composition from the group of "Combined drugs containing ephedrine" (24.7%); diphenhydramine from the "Antiallergic agent" group (21.9%); doxylamine from the group of "Somnifacients " (15.9%); amitriptyline from the group of "Antidepressants" (14.6%).

In the course of the survey, it turned out that pharmacies are asked for the medicines of all these groups. Nevertheless, some of the pharmacists do not pay spe-

cial attention to such demands or do not have sufficient knowledge of the possible consequences of non-medical use of the drugs.

The prevailing number of respondents (356 people) notified different drugs purchased (in their opinions) not for medical purposes, while 40 informants generally believe that drugs cannot be purchased for misuse.

Judging by the questionnaire data, the main consumers of drugs for non-medical purposes are adolescents and the young generation. The exception is the acquisition of alcohol-containing drugs (100 ml) by elderly people, mainly men, as well as middle-aged and elderly women, who mainly buy 25 ml of motherwort, hawthorn, peony tinctures – "They come every day or take 30 packs at once" (hereinafter, a quote from a questionnaire). Herewith, in the questionnaires, the respondents indicate that the number of regular consumers of alcohol-containing products is, on average, 10-15 people, and in some pharmacies it reaches 20. As a rule, each of the buyers can come 3 to 6 times a day. Moreover, pharmaceutical spelialists "would be glad not to sell these drugs but there are no legislative grounds for refusal," and the management of pharmacies classifies alcohol-containing drugs (in particular, in the volume of 100 ml) as a high-margin group of goods, therefore, they do not strive to remove them from the pharmacy assortment.

One of the important tasks of the survey was to study the pharmaceutical specialists' information awareness about the regulation and compliance with the rules for dispensing drugs from PhOs, used for the purpose of abuse.

As a result of studying the pharmaceutical specialists' information awareness about the regulation of the procedure for dispensing combined drugs containing codeine, dextromethorphan, ephedrine, it was found out that 60 specialists out of 396 (15.1%) know the normative document governing this procedure.[2]

In accordance with the regulatory standards[3], dispensing of combined medicinal preparations containing codeine, is carried out by 89.9% of specialists (356 people) on the basis of the 148-1/u-88 prescription form; the remaining 10.1% believe that prescribing and dispensing such medicinal preparations should be provided by the 107-1/u prescription form.

The opinions of pharmacy specialists were divided when answering the question: "What is the order for dispensing combined MPs containing dextromethorphan (cough syrups)?" Despite the requirements of the regulatory document governing the procedure for dispensing these drugs on the basis of the 148-1/u-88 prescription form[4], only 237 specialists (59.8%) believe that the listed drugs should be dispensed on the basis of the 148-1/u-88 prescription form; 139 surveyed employees (35.1%) claim that it should be the 107-1/u prescription form; 20 people (5.1%) believe that these drugs are available without a prescription

The survey data showed that dispensing of a combined medicinal product containing 15.0 mg dextromethorphan + paracetamol 500.0 mg + phenylephrine 10.0 mg + chlorphenamine 2.0 mg (which must be drawn using the 107-1/u prescription form), should be carried out (according to the pharmaceutical specialists' opinions) in the following way: 88 specialists (22.2%) believe, it should be sold on the basis of the 148-1/u-88 prescription form, 281 (71.0%) believe – on the basis of the 107-1/u prescription form, and 27 (6.8% of the employees) – without a doctor's prescription.

The majority of PhO employees (324 people, 81.8%) believe that a combined drug containing 4.6 mg ephedrine + 5.75 mg glaucine, is dispensed according to the 107-1/u prescription form, 49 people (12.4%) believe that the prescription form should be 148-1/u-88, and 23 people (5.8%) believe that this drug can be dispensed without a prescription.

More than half of pharmacy specialists (55.8%) know that bromodihydrochlorophenylbenzodiazepine should be dispensed according to the 107-1/u prescription form. A significant part (39.4%) of the interviewed specialists answered that this drug is dispensed according to the 148-1/u-88 prescription form, and 4.8% of PhO specialists believe that this drug can be dispensed without a prescription.

The following answers were received to the question about the procedure for dispensing medicinal preparation baclofen (it is to be dispensed according to the 107-1/u prescription form): according to the 148-1/u-88 prescription form (opinions of 5.3% of employees), according to the 107-1/u prescription form (86.1%), without a doctor's prescription (8.6%).

A study of the procedure for dispensing a combined drug containing dicycloverine 20.0 mg + paracetamol 500.0 mg showed that 84.6% of the surveyed pharmaceutical specialists know about the possibility of its dispensing without a prescription, and the remaining

[2] Order of the Ministry of Health and Social Development of the Russian Federation of 17 May 2012 No. 562n "On Approval of the Procedure for Dispensing Medicines for Medical Use to Individuals Containing Other Pharmacological Active Substances in addition to small amounts of narcotic drugs, psychotropic substances and their precursors". Available from: http: //www.consultant.ru/document/cons_doc_LAW_130675/ (Date of access 26 Aug 2019). Russian

3 http: //www.consultant.ru/document/cons_doc_LAW_130675/

[4] Order of the Ministry of Health and Social Development of the Russian Federation of 17 May 2012 No. 562n "On Approval of the Procedure for Dispensing Medicines for Medical Use to Individuals Containing Other Pharmacological Active Substances in addition to small amounts of narcotic drugs, psychotropic substances and their precursors". Available from: http: //www.consultant.ru/document/cons_doc_LAW_130675/ (Date of access 26 Aug 2019). Russian

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15.4% believe that the drug belongs to the prescription group of medicinal preparations.

All specialists notified that they have difficulty in dispensing a combined medicinal preparation prescribed by a group name, and they do not have any available source of information about group names of medicinal preparations in Latin.

It should be notified that the regulatory document contains requirements for the mandatory prescription of medicinal preparations in Latin by the international non-proprietary name (INN), in case of its absence – by a group or chemical name. However, in the available official sources of information about medicinal preparations ("State Register of Medicinal Remedies" [5], instructions for medical use of drugs, etc.), such information is not available. In some cases (in accordance with the requirements of the narcological dispensary), in the presence of medical grounds, the prescription of medicinal preparations is carried out according to trade names by decision of the medical commission of a medical organization, while a special mark (stamp) is put on the back of the prescription.[6]

To the question "How should a prescription be drawn up in case of prescribing a combined drug by trade name?" the opinions of pharmaceutical specialists were divided: 75.0% believe that a special mark (stamp) of the medical commission should be put on the back of the prescription (according to the rule), and 25.0% believe that the prescription does not require additional registration.

The prescriptions drawn up on forms 148-1/u-88 should be stored in the PhOs after the release of combined codeine-containing drugs, but only 79.5% of specialists know about this. Judging by the questionnaire data, 5.1% of the surveyed pharmacy specialists believe that there is no need to store prescriptions in a pharmacy, and 15.4% of respondents do not know the answer to this question. Despite the regulation of the mandatory storage of prescriptions for combined preparations containing dextromethorphan (cough syrups) in the pharmacy for 3 years, only a little over a half of drug dispensing specialists (55.6% – 220 people) believe that PhOs should keep such recipes; 19 respondents (4.8%) argue that such recipes should not be stored in PhOs; 157 specialists (39.6%) found it difficult to answer.

To the question "How long is the protocol storage period for the prescriptions for combined MPs containing codeine and dextrometrophan (cough syrups) [7], provided by PhOs?" 64.9% of the respondents answered the question correctly (within three years), 13.6% believe that prescriptions should be stored for 5 years and 21.5% of specialists are sure that such recipes should not be stored in a pharmacy.

In accordance with the established requirements, prescriptions for combined MPs containing dextromethorphan 15.0 mg + paracetamol 500.0 mg + phenylephrine 10.0 mg + chlorphenamine 2.0 mg and ephedrine 4.6 mg + glaucine 5.75 mg, issued for 107-1/u forms, must be redeemed with the stamp "The drug has been dispensed". [8] However, the study of the survey results showed that 64 pharmaceutical specialists (16.2%) out of 396 respondents theoretically know about the need to redeem the prescription for these drugs, the remaining 83.8% do not have this information. At the same time, not a single specialist redeems prescriptions for these MPs with a stamp. In some PhOs the stamp "The drug has been released" is missing at all.

The study of compliance with the procedure for the release of medicines showed that all respondents know: the release of prescription medicines without a prescription form is a gross violation of licensing requirements.[9] Despite this, all employees involved in the survey (100.0%) dispense prescription drugs (form 107-1/y) without a doctor's prescription.

To the question "Will you release the drug, assuming in advance that it is used not for medical purposes?" – 68 pharmaceutical specialists (17.2%) gave a negative answer, the rest 328 (82.8%) answered in the affirmative.

According to the pharmaceutical specialists, the main reasons for deliberate drug dispensing without prescriptions, used for abuse, are: the desire to increase the pharmacy's revenue (notified by 317 respondents – 80.1%), lack of information on drug abuse (notified by 198 people – 50.0%) and indifference to the problems of buyers (42 respondents – 10.6%) for a total.

As a result of studying the methods of obtaining in-

[5] State Register of Medicines. Available from: http://grls.rosminzdrav.ru/default.aspx (Date of access 23 Aug 2019). Russian

[6] Order of the Ministry of Health of the Russian Federation 14 of January 2019 No. 4 "On approval of the procedure for prescribing medicinal products, forms of prescription forms for medicinal products, the procedure for issuing these forms, their accounting and storage". Available from: http://www.consultant.ru/document/cons_doc_LAW_321140/ (Date of access 26 Aug 2019)

[7] Order of the Ministry of Health of the Russian Federation of January 14, 2019 No. 4 "On approval of the procedure for prescribing medicinal products, forms of prescription forms for medicinal products, the procedure for issuing these forms, their accounting and storage". Available from: http://www.consultant.ru/document/cons_doc_LAW_321140/ (Date of access 26 Aug 2019)

[8] Order of the Ministry of Health and Social Development of the Russian Federation of 05 May 2012 No.562n "On Approval of the Procedure for Dispensing Medicines for Medical Use to Individuals Containing Other Pharmacological Active Substances in addition to small amounts of narcotic drugs, psychotropic substances and their precursors"http://www.consultant.ru/document/cons_doc_LAW_130675/ (Date of access 26 Aug 2019)

[9] Decree of the Government of the Russian Federation of 22 December 2011 No.1081 "On licensing of pharmaceutical activities"http://www.consultant.ru/document/cons_doc_LAW_124279/ (Date of access 26 Aug 2019)

formation by pharmacists on the matters of non-medical use of MPs, the following was revealed:

– 39.9% of the total number of surveyed pharmaceutical specialists (158 people) acquire knowledge on the matters of non-medical use of drugs independently with the help of the mass media;

– 29.5% of the respondents (117 people) claim that there are no sources of information for them on the topic of the survey;

– 10.6% of specialists (42 employees) acquire information while taking advanced vocational training;

– 10.1% of specialists (40 p employees) acquire knowledge in this area from friends, relatives, acquaintances (cases from life);

– 5.3% of specialists (21 employees) learn about drug abuse during additional training conducted in pharmacy organizations;

– the rest of the specialists (4.6% – 18 employees) acquire information while receiving secondary vocational or higher education (Fig. 2).

The prevailing number of respondents (297 people – 75.0%) are confident in the need for special training on the subject of assortment and regulation of the release of drugs used for abuse. 78.8% of pharmaceutical specialists also believe that the availability and use of systematized information on the range and regulation of the release of drugs used for abuse, will help to improve their professional level.

All the pharmaceutical professionals interviewed confirmed the need for more information on the drugs used for abuse.

As shown by the results of the survey, the opinions of pharmaceutical specialists on drug dispensing, in a number of cases do not coincide with the requirements of regulatory documents, despite the fact that more than half (51.0%) of the respondents are experienced professionals with more than 10 years of work experience. The actions of specialists often contradict the legislation and ethical and deontological norms adopted in pharmacy. It should be notified that at the stage of patient care, specialists do not strive to prevent drug abuse.

DISCUSSION

The results of our study showed that the percentage of pharmaceutical specialists who know the assortment of drugs that can be used for non-medical purposes is low and differs depending on the drug group. For example, combined drugs containing dextromethorphan were identified by 6% of PhO employees; combined drugs containing ephedrine – by 25% of them; the drugs, benzodiazepine derivatives – by 28%; muscle relaxants – by 49%, somnifacient of the non-benzodiazepine series doxylamine – by 16%, etc.

The majority of specialists have not demonstrated the knowledge of all possible groups of drugs used for abuse, due to the lack of awareness on the topic of abuse. The results of the survey revealed the pharmaceutical specialists who had not named any of the listed drug groups.

PhO employees do not in full know the requirements of regulatory documents governing the rules for dispensing MPs, which leads to their violations. Many specialists dispense prescription drugs without a doctor's prescription. Knowing about the possibility of non-medical use of drugs, most of the interviewed specialists still release them, explaining this by the desire to increase the pharmacy's revenue, lack of information on abuse matters and indifference to the problems of buyers.

All specialists experience difficulties in dispensing combined MPs, prescribed in drug orders according to a group name, and they do not have an available source of information on the group names of MPs in Latin. 25.0% of PhO employees who have taken part in the survey, do not know the requirements for drawing up drug orders in case of prescribing a combined MP by trade name.

117 specialists (29.5%) do not have a source of information on the matters of non-medical use of MPs. A rather low percentage of the specialists receive such information in the courses of training under the programs of secondary vocational or higher education (4.6%) and advanced training (10.6%).

The analyses of the survey data of the PhO employees on the level of pharmaceutical education, position held, work experience and training in additional professional training programs, suggests the presence of a fairly high percentage of specialists with professional knowledge and sufficient experience among the sample size of 396 people. However, the results of studying the specialists' information awareness showed that more stable knowledge on drug abuse matters was demonstrated by specialists from 5 to 10 years of work experience, while pharmaceutical specialists with more than 20 years of experience have a low level of knowledge.

CONCLUSION

Thus, the study carried out indicates a lack of awareness on the non-medical use of drugs among pharmaceutical specialists. It gives a possibility to conclude that the attention paid to these matters during training in advanced training programs, is insufficient. This necessitates the development of a special advanced training program for additional professional education on the prophylaxis and prevention of drug abuse. It is necessary to conduct appropriate educational activities in order to form additional competencies among pharmaceutical specialists in the prevention of non-medical use of drugs. It is important to improve the quality of pharmaceutical consulting, as well as to motivate employees to self-education and awareness of responsibility for human health.

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The authors declare no conflict of interest

AUTHORS' CONTRIBUTION
N.Yu. Porseva – study design; review of publications on the topic of the article; data collection; analysis and interpretation of study results; text writing, formulation of conclusions; A.V. Soloninina – study design; formulation of conclusions; editing and revision of the article; O.N. Dvorskaya – a review of publications on the topic of the article; data collection; interpretation of research results; editing of the article

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LIFE QUALITY ASSESSMENT OF PATIENTS WITH STABLE CORONARY ARTERY DISEASE AFTER MYOCARDIAL REVASCULARIZATION

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The aim of this study is to assess the life quality of patients with stable coronary artery disease after angioplasty and stenting of coronary arteries at the post-hospital stage.

Materials and methods. Methods of the sociological analysis (questionnaire surveys) and methods of mathematical statistics (descriptive statistics, time series method, factor and variance analyses) were used at different stages of the prospective observational study. The research materials were as follows:1458 electronic patient records with a stable coronary heart disease (SCHD) after angioplasty and stenting of coronary arteries (ASCA); 620 questionnaires filled in by patients before the surgery, 1, 6, 12 months after discharge. The statistical analysis was performed using the IBM SPSS Statistics software.

Results. The results of a comprehensive survey make it possible for us to assert that during the studied period, stable good healths of cardiac surgery patients with ASCA were maintained. Within the framework of the EQ-5D-5L questionnaire, it was revealed that more than 50% of patients have no physiological problems. The results of the SAQ analysis demonstrate that 58% of the patients feel better, and more than 34% of the patients do not have shortness of breath 1 year after the surgery. A statistically significant improvement in their healths was established according to a visual analogue scale relatively to the annual observation mark (62.82 ±20.95), which corresponds to the high results assessment of the medical technology use. At the same time, 53% of the patients notify that the treatment results meet their own expectations.

Conclusion. The proposed calculation of the integrated index of patients' treatment efficiency demonstrated by the patients with stable coronary heart disease after angioplasty and stenting of the coronary arteries is based on the results of the factor analysis. This calculation can be used to assess the efficiency of pharmacotherapy in the framework of a value-oriented approach to the treatment of a number of other pathologies.

Keywords: life quality assessment; stable coronary heart disease; angioplasty and stenting of coronary arteries

Abbreviations: IHD – ischemic heart disease; SCHD – stable coronary heart disease; ASCA – angioplasty with and stenting of coronary arteries.

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ОЦЕНКА КАЧЕСТВА ЖИЗНИ ПАЦИЕНТОВ СО СТАБИЛЬНОЙ ИШЕМИЧЕСКОЙ БОЛЕЗНЬЮ СЕРДЦА ПОСЛЕ РЕВАСКУЛЯРИЗАЦИИ МИОКАРДА

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Цель. Оценка качества жизни пациентов со стабильной ишемической болезнью сердца после ангиопластики со стентированием коронарных артерий на постгоспитальном этапе.

Материалы и методы. На разных этапах проспективного обсервационного исследования использовались методы социологического анализа (анкетирование) и методы математической статистики (описательная статистика, метод динамических рядов, факторный и дисперсионный анализ). Материалами исследования служили: 165 электронных историй болезней пациентов со стабильной ишемической болезнью сердца после ангиопластики со стентированием коронарных артерий, а также 620 анкет, заполненных больными до операции, через 1, 6 и 12 месяцев после выписки. Статистическая обработка данных проводилась с использованием программного обеспечения IBM SPSS Statistics.

Результаты. Результаты комплексного опросника позволяют утверждать о сохранении стабильно хорошего самочувствия кардиохирургических пациентов после ангиопластики со стентированием коронарных артерий на протяжении исследуемого промежутка времени. В рамках опросника EQ-5D-5L выявлено, что более 50% пациентов не имеют физиологических проблем. Результаты анализа SAQ демонстрируют, что 58% пациентов отмечают улучшение самочувствия, а также более 34% больных не имеют одышки через 1 год после операции. Установлено статистически достоверное улучшение состояния здоровья по визуально-аналоговой шкале годовой отметки наблюдения (62,82±20,95), что соответствует высокой оценке результатов применения медицинской технологии. При этом 53% больных отмечают, что результаты лечения соответствуют личным ожиданиям.

Заключение. Предложенный расчет интегрированного индекса эффективности лечения пациента на примере больных со стабильной ишемической болезнью сердца после ангиопластики со стентированием коронарных артерий, базирующийся на результатах факторного анализа, может быть использован для оценки эффективности фармакотерапии в рамках ценностно-ориентированного подхода к лечению ряда других патологий.

Ключевые слова: оценка качества жизни; стабильная ишемическая болезнь сердца; ангиопластика со стентированием коронарных артерий

Сокращения: ИБС – ишемическая болезнь сердца; СИБС – стабильная ишемическая болезнь сердца; АСКА – ангиопластика со стентированием коронарных артерий.

INTRODUCTION

Despite the impressive progress achieved over the past decades in the prevention and treatment of cardiovascular diseases in general, coronary artery disease in particular, pathology still occupies a leading position in the structure of morbidity and mortality in the population of developed countries. According to the World Health Organization data, coronary artery disease covers up to 15% of the total mortality structure worldwide [1].

In the Russian Federation, in 2018, the cause of the patients' primary disability was the pathology of the circulatory system in 30% of cases. At the same time, the mortality indicator due to coronary heart disease reaches 301.6 per 100 thousand population, which is 24% of

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the total mortality. The losses of the Russian economy from the consequences of cardiovascular diseases were 3.2% of the gross domestic product. It determines the relevance of the search for effective and economical treatment strategies that correspond to the modern model of organizing medical care according to the principle of the 4P Medicine [2–4]. Thus, the implementation of the pilot projects aimed at improving the quality and life expectancy of the population in the rational use conditions of health care resources is the basis of the "Strategy for drug provision of the population of the Russian Federation for the period up to 2025" [5].

Currently, the combination of rational pharmacotherapy with myocardial revascularization is recognized as the most promising treatment of severe, rapidly progressive and drug-resistant coronary artery disease [6, 7]. It is important to emphasize that in this case the potential improvement of the life quality and prognosis of patients takes place in the post-surgery period.

In this regard, prospective studies of the outcomes and life quality of cardiac surgery patients at the stage of rehabilitation are of scientific and practical importance [8, 9].

THE AIM of this study is to assess the life quality of patients with stable coronary artery disease after angioplasty and stenting of coronary arteries at the post-hospital stage.

MATERIALS AND METHODS

Inclusion criteria

The patients aged 18 – 90; the patients who have ICD-10 diagnosis codes: I20.8, I25.0, I25.1, I25.2, I25.6, I25.8, I25.9; the patients who had undergone a planned myocardial revascularization surgery with the use of the ASCA method.

Exclusion criteria

The patients under 18 years of age, the patients who have all other ICD-10 diagnosis codes in the section "Ischemic heart disease" (codes I.20-I.25), with the exception of those mentioned in the inclusion criteria; the patients who underwent emergency ASCA surgery.

Design

An observational prospective study on the assessment of the outcomes of treatment of–patients with stable coronary heart disease (SCHD) after ASCA, was carried out at the Federal State Budgetary Institution "V.A. Almazov National Medical Research Center" of the Ministry of Health of the Russian Federation. The study was conducted in accordance with the provisions of the 1975 Declaration of Helsinki (revised in 2000), as well as with the standards of the local ethics committee. All admitted patients were asked to read the information for the patient and sign an informed consent in duplicate. After signing the informed consent, the patients filled out a questionnaire without a doctor's or third parties' participation. After the discharge from the hospital, the questionnaires were sent to the patients by e-mail (if the patient indicated it) or the information was collected by phone. Additional methods of examination or the use of medication and non-medication methods of treatment, including the correction of the ongoing pharmacotherapy, had not been provided. After performing ASCA, the patients were discharged from the hospital on time and with the recommendations determined by the attending physician. If necessary, the patients were sent to medical organizations at their place of residence. The questionnaires were filled out by patients upon admission to a planned ASCA, as well as 1, 6 and 12 months after the discharge from the hospital.

For the most correct conduct of the study, a comprehensive questionnaire was developed. It includes the Russian-language validated versions of international questionnaires and consists of 6 blocks:

1. General questions necessary to form a patient's profile;
2. Questions about the medication received, the doses and frequency of the drugs administration;
3. Questions characterizing the general self-assessment of the patients' state of health at the moment, in comparison with their peers, as well as satisfaction with the operation;
4. Dyspnea scale of the Medical Research Council (to determine the degree of dyspnea);
5. Non-specific questionnaire of the life quality EQ-5D-5L with a visual analogue scale – VAS (to assess the health status);
6. Seattle Angina Questionnaire (SAQ), a specific questionnaire for patients with stable angina that has a sufficient reliability, validity and sensitivity in relation to the clinical symptoms of coronary artery disease [10–12].

For a polyhedral value-oriented results assessment of the studied medical technology, the patients were also asked to determine the importance degree of each life quality indicator included in the comprehensive questionnaire [13].

Statistical processing of research results

Statistical data processing was carried out using the IBM SPSS Statistics package. Descriptive statistical indicators were calculated on the basis of the data obtained. For continuous values. The mean value, the standard deviation, and the median were calculated for continuous values. The frequency of trait occurrence was calculated for categorical values. The one-sample Kolmogorov-Smirnov test was used to check the normal / abnormal distribution. In the comparative analysis, to test the hypothesis about the difference between two dependent samples, the Wilcoxon test was used, and the Mann-Whitney test was used for independent samples. The selection of generalized factors in the frame-

work of a two-stage factor analysis was carried out using the method of principal components and the method of rotation – varimax with Kaiser normalization. To assess the applicability of the factor analysis for the studied variables, the Kaiser-Mayer-Olkin (KMO) coefficient was used as a measure of the sample adequacy and the Bartlett coefficient, which determines the quality of the analyzed correlation matrix. To substantiate the homogeneity of the sample in the framework of the analysis of variance, the Livigne test and the R-squared value were used. The differences were considered significant at $p<0.05$.

The data set was based on 165 electronic medical records of the patients with stable angina. The observational study included 165 patients (11.3% of the total number of patients with stable angina pectoris who had undergone the ASCA operation from 01.01.2017 to 31.12.2017). All patients signed informed consent to participate in the study. Since fewer than 1% of the patients use e-mail, oral telephone interviews had been the primary means of obtaining the primary data.

RESULTS

It has been established that in the study group, the share of men is 71.50%, of women – it is 28.50%. The age of the patients ranged from 36 to 89 years, the average age was 66.25±9.52 years. The patient baseline characteristics are presented in Table 1.

Painless / newly diagnosed angina pectoris was detected in 26.67% of patients. Functional class (FC) 1 angina was registered in 0.61% of people, FC 2 angina – in 47.88% of patients, FC 3 angina – in 24.85% of patients. At the same time, 67.88% of patients had a history of myocardial infarction, 7.27% of patients had previously undergone coronary artery bypass grafting. The average period from myocardial revascularization to ASCA is 10.67±5.40 years. The main concomitant cardiovascular and cerebrovascular diseases are presented in Table 2.

The questionnaire survey results of the patients on the general health status self-assessment and the assessment of health status in comparison with other people of the same age are presented in Table 3.

It has been established that the significant ($p<0.05$) improvement in health was observed 1, 6, 12 months after the discharge from the hospital compared with the initial state of the patients. At all other times, the self-assessment of their health state did not change.

The analysis of the questionnaire results of a 100-point scale for assessing the patients' healths makes it possible to assert a significant increase in the indicator of the patients' life quality in comparison with the same indicator at the initial observation point (Table 4).

The responses of patients according to the European Quality of life five-dimension five-level questionnaire (EQ-5D-5L) indices indicate the presence of a positive dynamics of the patients' well-being by the one-year observation point, which is confirmed by statistically significant differences at individual points in comparison with the baseline values (Table 5).

The results obtained when processing the data of the SAQ questionnaire makes it possible to state that by the end of the 1-st year, the patients' physical activity, the stability of angina pectoris, the quality of their lives have increased, and there is a decrease in the severity of the underlying pathology (the differences are significant compared to the baseline) (Table 6).

The obtained data on the dyspnea severity assessment in patients demonstrate a decrease in the number of the respiratory system disorders, the pathogenesis of which are due to the cardiovascular pathology (Table 7).

It has been revealed that the majority of patients are satisfied with the operation results and evaluate the performed surgical intervention on a scale from "–10" to "+10" with a fairly high score ("+7" and above). However, at later points, the satisfaction with the operation results decreases slightly (Table 8). In addition, by the annual observation mark, the number of patients whose state of health from the performed surgical intervention does not meet expectations, increases in almost 1.5 times (Table 9).

As a tool for compressing the multidimensionality of the initial characteristics presented in a complex questionnaire, which takes into account the characteristics of each observation point, a two-stage factor analysis to the new generalized variables was used.

The 1st stage results of the factor analysis made it possible to identify 3 generalized components at each observation mark, i. e.: "Physiological status", "Cardiological status", "Rehabilitation status" (Table 10).

The factor scores for the studied variables were determined in a similar way by analyzing each generalized component at each observation point with specified statistical settings (Table 11).

The determination of the factor loading of the indicators in each component, made it possible to identify the most significant parameters, i. e.: usual activities, angina stability, visual analogue scale.

Based on the results obtained, a formula for calculating the integrated indicator of the pharmacotherapy effectiveness in patients after ASCA at the postoperative stage was proposed. Hereby, the value-based approach was taken into account:

$$I = \sum_{i=1}^{n} k_1 * w_i * x_i \qquad (1),$$

where: I – an integrated index of the patient's treatment efficiency; n – the number of performance indicators (generalized indicators); k_i – a weight coefficient of the performance indicator (based on the factor load); w_i – a coefficient of importance / significance of the indicator for a patient; x_i – a value of the patient's efficiency indicator.

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Table 1 – Baseline characteristics of patients with stable angina pectoris who underwent ASCA

Indicatos	Units of measure-ment	Mean value	Minimum	Maximum
Age	Year	66.25±9.52	36	89
Height	cm	169.76±9.41	146	192
Weight	Kg	85.72±16.51	48	135
Body mass index	kg/m^2	29.72±5.13	19.41	47.05
Systolic blood pressure	MmHg	133.36±15.36	90	190
Diastolic blood pressure	MmHg	80.35±8.80	60	110
Heart rate	Beats per minute	69.18±8.69	50	110

Table 2 – The structure of concomitant cardiovascular pathologies in patients who have undergone ASCA

Disease	Number of complications	Proportion of patients with concomitant diseases, %
Arterial hypertension	152	92.12%
Chronic heart failure	70, of them	42.42%, of them
Stage I	2	2.86%
Stage II	64	91.43%
stage III	4	5.71%
Diseases associated with irregular heart rhythm	36	21.82%
Diseases associated with impaired cerebral circulation	75	45.45%
Obliterating atherosclerosis of the lower extremities	10	6.06%
Pulmonary hypertension	13	7.88%
Diabetes mellitus	38, of them	23.03%, of them
Type 1	0	0.00%
Type 2 on oral hypoglycemic drugs	33	86.84%
Type 2 insulin-dependent	5	13.16%

Table 3 – Health status according to the patients' self-assessment

Indicators	Observation points			
	1 (n=165)	2(n=160)	3(n=148)	4(n=147)
How do you assess your current state of health in general?				
Excellent, %	–	3.6*	2.4*	2.4*
Very good, %	1.8	2.4*	3.0*	4.2*
Good, %	12.1	35.8*	38.2*	37.0*
Satisfactory, %	60.6	40.0*	33.9*	31.0*
Bad, %	25.5	15.2*	12.1*	14.5*
Missing values, %	0	3.0	10.4	10.9
How do you currently assess your health status in comparison with other people of your age?				
Better, %	15.2	14.5*	10.9*	10.9
The same, %	33.9	44.2*	44.8*	41.2
Worse, %	50.9	38.2*	33.9*	37.0
Missing values, %	0	3.0	10.4	10.9

Note: * p <0.05 in comparison with point 1; ** p <0.05 in comparison with point 2

Table 4 – Dynamics of the indicator of the life quality, determined by a visual analogue (100-point) scale

EQ VAS	Observation points			
	1 (n=165)	2 (n=160)	3 (n=148)	4 (n=147)
Mean value	52.52	64.94*	63.38*/**	62.82*/**
Standard deviation	19.72	19.61	19.31	20.95

Note: * p <0.05 in comparison with point 1; ** p <0.05 in comparison with point 2

Table 5 – Dynamics of indicators of the scales of the questionnaire EQ-5D-5L

EQ-5D dimension	The proportion of patients who have no problems on the scales of the questionnaire,%			
	1 (n=165)	2(n=160)	3(n=148)	4 (n=147)
Mobility	30.43	48.73*	50.68*	52.78*
Self-care	68.84	77.85*	80.82	81.25
Usual activities	35.51	67.09*	63.70*	64.58*
Pain / discomfort	42.03	72.78*	76.03*	79.86*
Anxiety / depression	49.28	70.25*	71.23*	71.53*

Note: * p <0.05 in comprison with point 1; ** p <0.05 in comparison with point 2

Table 6 – Dynamics of scales indicators of the SAQ questionnaire

Scales of the SAQ	Observation points			
	1 (n=165)	2(n=160)	3(n=148)	4 (n=147)
Physical loads limitation	63.38±20.20	75.71±15.12*	76.02±14.75*	76.07±14.45*
Stability of angina shoots	60.24±30.52	87.50±23.82*	86.76±27.36*	86.12±27.76*
Frequency of angina shoots	65.39±30.75	90.50±18.25*	90.54±21.18*	90.00±21.55*
Treatment satisfaction	67.14±21.03	72.00±19.08*	71.76±21.07*	71.58±21.11*
Disease perception	44.90±21.04	59.22±14.47*	59.35±15.30*	58.95±15.49*

Note: * p <0.05 in comparison with point 1; ** p <0.05 in comparison with point 2

Table 7 – Dynamics of dyspnea scoring (on the dyspnea scale)

Dyspnea / severity	Observation points			
	1 (n=165)	2 (n=160)	3 (n=148)	4 (n=147)
0/without, %	25.5	40.6*	33.3*/**	34.5*
1/light, %	23.6	28.5*	26.1*/**	24.8*
2/average, %	35.2	20.0*	22.4*/**	21.8*
3/heavy, %	14.5	6.7*	7.3*/**	6.7*
4/very heavy, %	1.2	1.2*	0.6*/**	1.2*

Note: * p <0.05 in comparison with point 1; ** p <0.05 in comparison with point 2

Table 8 – Patient satisfaction with the results of the operation

Satisfaction with the results of the operation	Observation points		
	2 (n=159)	3 (n=148)	4 (n=147)
Median	+8	+7*	+7*/**

Note: * p <0.05 in comparison with point 2; ** p <0.05 in comparison with point 3

Table 9 – Analysis of compliance of the operation results with the patients' expectations

Meeting expectations	Observation points		
	2 (n=159)	3 (n=148)	4 (n=147)
Satisfaction, %	71.5	58.2*	53.3*/**
No satisfaction, %-	24.8	31.5*	35.8*/**
Missing values, %	3.7	10.3*	10.9*/**

Note: * p <0.05 in comparison with point 2; ** p <0.05 in comparison with point 3

Table 10 – Structuring of generalized factors

Components		
1	2	3
Physiological status	Cardiological status	Rehabilitation status
Usual activity	Scale of angina stability	Health status on a five-point health scale
Mobility	Scale of angina frequency	Health status compared to peers
Self-care	Scale of disease perception	Visual analogue scale
Pain / discomfort		Dyspnea scale
Anxiety / depression		Treatment satisfaction
Physical loads limitation		Satisfaction with the results of the operation
		Meeting patients' expectations of the result of surgery

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Table 11 – Matrix of the factor coefficients component values

Factor	Indicators	Observation points			
		1	2	3	4
1	Usual activities	0.232	0.248	0.241	0.228
	Mobility	0.210	0.246	0.236	0.227
	Self-care	0.196	0.241	0.226	0.215
	Pain / discomfort	0.200	0.211	0.196	0.198
	Anxiety / depression	0.189	0.161	0.186	0.191
	Physical load limitation	–0.213	–0.173	–0.184	–0.186
2	Scale of angina stability	0.380	0.382	0.354	0.354
	Scale of angina frequency	0.386	0.376	0.353	0.354
	Disease perception	0.382	0.354	0.344	0.352
3	Health status on a five-point health scale	0.293	–0.199	–0.195	–0.190
	Health status compared to peers	0.258	–0.152	–0.156	–0.159
	Visual analogue scale	–0.302	0.218	0.206	0.203
	Dyspnea scale	0.240	–0.164	–0.155	–0.161
	Treatment satisfaction	–0.239	0.191	0.183	0.173
	Satisfaction with the results of the operation	–	0.208	0.194	0.188
	Meeting patients' expectations of the surgery result	–	0.194	0.179	0.180

Note: Factor extraction method: principal component analysis; Rotation method: Varimax with Kaiser normalization.

Table 12 – Results of life quality assessment using integrated index

Indicators	Observation points			
	1	2	3	4
Mean value	35.065	47.742*	44.511*/**	43.945*/**/***
Standard deviation	13.776	15.444*	15.566*/**	14.586*/**/***

Note: * p <0.05 in comparison with point 1; ** p <0.05 in comparison with point 2; *** p <0.05 in comparison with point 3

Table 13 – Variance analysis of qualitative factors influence on the integrated indicator of patients' with SBS after ASCA life quality

Factors	Number of degrees of freedom	Observation points					
		2		3		4	
		F	P	F	P	F	P
Sex	1	2.096	0.150	0.268	0.606	0.004	0.952
Age	5	0.558	0.732	0.156	0.978	0.317	0.902
Body mass index	4	0.728	0.574	0.034	0.998	0.504	0.733
Stent type *	1	0.461	0.498	0.463	0.498	0.198	0.657
History of myocardial infarction	1	0.288	0.592	0.002	0.966	0.037	0.849
Arterial hypertension	1	1.232	0.269	0.195	0.660	0.134	0.715
Chronic heart failure	1	0.945	0.333	0.250	0.618	0.890	0.347
Diseases associated with irregular heart rhythm	1	0.454	0.502	0.031	0.861	0.289	0.592
Diseases associated with impaired cerebral blood formation	1	0.343	0.559	1.982	0.162	0.076	0.783
Pulmonary hypertension	1	0.932	0.336	2.895	0.091	1.945	0.166
Diabetes mellitus	1	0.002	0.967	0.044	0.834	0.177	0.674
Obliterating atherosclerosis of the lower extremities	1	0.050	0.823	0.229	0.633	0.002	0.964
R-squared value	–	0.092		0.068		0.063	

Note: * – accepted division into uncoated stent / stents and stent / stents with drug-induced antiproliferative coating; F – Fisher's criterion, p – significance level

As a result of transformation, with the results of the factor analysis taken into account, formula (1) took the following form (Table 12):

$$I = k_{UA} * w_{UA} * x_{UA} + k_{AS} * w_{AS} * x_{AS} + k_{VAS} * w_{VAS} * x_{VAS} \quad (2),$$

where: I – an integrated index of treatment efficiency of patients with SCHD after ASCA; k – a weight coefficient of the indicator "Usual activities"; w_{UA} – a coefficient of importance / significance of the indicator "Usual activities"; x_{UA} – a value of the patient's indicator "Usual activities"; k_{AS} – a weight coefficient of the indicator "Angina stability"; w_{AS} – a coefficient of importance / significance of the indicator "Angina stability"; x_{AS} – a value of the patient's indicator "Angina stability"; k_{VAS} – a weight coefficient of the indicator "Visual analogue scale"; w_{VAS} – a coefficient of importance / significance of the indicator "Visual analogue scale"; x_{VAS} – a value of the indicator "Visual analogue scale".

Thus, patients' quality of life assessment after ASCA, based on the dynamics of indicators of the complex questionnaire, includes the data of the non-specific questionnaire EQ-5D-5L, the scales of the specific SAQ questionnaire, the scale of dyspnea, the general self-assessment of the patients' health state and the analysis of the integrated index at every point. These factors make it possible to assert the certainty improvement of health before and after the surgery at all observation points, i. e.: 1, 6, 12 months after the hospital discharge. Attention is drawn to the fact that in 53% of patients, the results of treatment meet their expectations.

Taking into account the clinical pathology features and the approaches to the pharmacotherapy of patients, it seemed expedient to assess the influence of the qualitative factors on the value of the integrated indicator of the patients' life quality ~~of~~ with SCHD after ASCA at the outpatient stage. Sex, age, type of stent, and the presence of concomitant cardiovascular pathologies were analyzed as independent variables. The results of a multifactorial univariate analysis of the qualitative factors variance in the studied sample, make it possible to assert that there is no statistically significant influence of the qualitative factors on the integrated indicator (Table 13).

It has been established that the value of the Livigne criterion (the criterion for the homogeneity of samples) at the points under study, shows the equality of variances, i.e. the combination of factors does not affect the dependent variable. At the same time, the value of the R-squared is low at all points, i.e., it confirms the statistical insignificance of the influence of independent variables. Therefore, for subsequent studies, the homogeneity of the study group was justified, which, in turn, mediated its assessment without dividing patients into groups.

DISCUSSION

All patients with stable coronary artery disease admitted for planned percutaneous coronary intervention, i.e. for angioplasty and stenting of coronary arteries, had standard indications (a burdened anamnesis, a functional class of the underlying disease) for performing a surgery for myocardial revascularization. The incidence of cardiovascular and major cerebrovascular pathologies was typical for the patients with stable angina pectoris [14–17].

It is worth emphasizing a high importance of the rehabilitation stage after a surgical intervention on the coronary arteries, which is indicated in the works by national and foreign scientists [18–25]. The results of the study indicate an improvement in the patients' health status after ASCA by the annual follow-up point compared to the baseline, including an increase in mobility, no problems associated with personal care, habitual daily activities, a decrease in pain and discomfort, a decrease in anxiety and depression. A decrease in frequency and stability of angina attacks. Such conclusions about the improvement in the patients' life quality after myocardial revascularization, are consistent with the literature data [26–28].

In the light of these considerations, one cannot ignore the allocation of state quotas for performing surgical interventions on the vessels that nurse the heart. Thus, according to the data of the Federal Mandatory Medical Insurance Fund in 2019, the average standard of financial costs per unit of medical care in the territory of the Russian Federation within the framework of the program "Coronary myocardial revascularization with the use of angioplasty in combination with stenting for coronary heart disease" was 221,645 rubles. Depending on the stage of the disease, the pathophysiological characteristics of the organism, the patient's previous anamnesis, modern technologies for myocardial revascularization make it possible to choose the most effective and safe method for the patient, taking into account his individual characteristics. An assessment of the spectrum of state quotas with a high prevalence of surgical interventions on the vessels supplying the heart, indicates a significant burden on the federal budget. In its turn, it justifies the feasibility of conducting rational rehabilitation measures aimed at improving the prognosis of patients, improving their life quality, as well as increasing life expectancy of the patients with stable angina pectoris in the context of limited funding for the health care system.

In a lot of countries, there is an improvement in the system of providing medical care to the population towards an emphasis on involving patients in making decisions about treatment, as well as evaluating the methods of prevention, diagnosis and therapy used. According to the creators of the value-based medicine system, the calculation should be based on the results of achieving a positive outcome of the disease from the patient's point of view [29, 30]. Changes in the terms of contracts for payment of medical services in terms of expanding the choice of medical organizations will slightly reduce the

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annually progressing budgetary funds for health care. These are due to the loss of benefits to organizations. They are caused by an increase in the number of services provided and their rise in costs. The achievement of a favorable outcome of the disease with the lowest costs of treatment will be commercially viable. According to experts, such an approach will reduce healthcare costs by 10% or more due to-limiting / eliminating the use of unnecessary medical and diagnostic procedures, increasing the effectiveness of treatment, replacing ineffective and expensive medical technologies, as well as the widespread use of primary and secondary kinds of prevention [31]. For the first time in 2006, the FDA published Guideline for assessing patient reported outcomes (PRO). It indicates the value of assessing patient outcomes as any assessment of a patient's health status given by the patient himself without any interpretation by a doctor or third parties. At the same time, it is important to notify that the subjective opinion expressed by the patient is not questioned and is taken into account as it is. The use of PRO helps to assess all patient's symptoms; the general state of his health; his usual activity; psychological condition; the general life quality and the life quality associated with health. The study of health state indicators in dynamics, the satisfaction with the treatment process and its result are of no small importance. PRO is a tool that makes it possible to measure the effect of a medical intervention from a patient's point of view. The study of the evaluation of the patient's treatment results contributes to the achievement of three main goals of the patient's treatment: a patient satisfaction with the treatment, improving his health status and reducing the costs of treatment, which improves the patient's life quality through the use of effective and affordable methods of diagnosis and treatment [32-34].

Currently, coronary heart disease still occupies a leading place in the structure of mortality in the population worldwide, which underlines the need to improve the provision of medical care to patients at every stage of treatment. Thus, summarizing all of the above, due to the wide variability of the disease and the significant influence of comorbidities, it is appropriate to apply a personalized approach in each clinical situation for the treatment of patients in conditions of rational use of budgetary funds [35]. The following studies will be devoted to the use of tools for optimizing health care resources in the treatment of coronary artery disease.

CONCLUSION

Evaluation of patient treatment outcomes after undergoing planned percutaneous coronary intervention demonstrates an improvement in health status according to the analyzed parameters of the complex questionnaire, which is confirmed by statistical analysis tools.

In the context of the value-oriented paradigm of health care development, the calculation of an integrated indicator of the treatment effectiveness of patients with stable coronary artery disease after angioplasty with stenting of coronary arteries has been proposed and scientifically substantiated. It is based on the results of a two-stage factor analysis, which will be further used in the framework of the pharmacoeconomic «cost-benefit» analysis of patients' therapy in the study group. In addition, the proposed calculation of the quality of life indicator can be used to assess the effectiveness of medical technologies in the treatment of a number of other pathologies.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

I.A. Narkevich – choice of the research direction, idea; O.D. Nemyatykh – formulation of research aims and objectives, development of the research algorithm, consultations at all research stages, article writing; K.A. Kovaleva– literature analysis, article writing, conducting all research stages, processing the results; L.G. Ratova – collection of primary data, consultations at separate research stages, article writing; I.O. Trushnikova – consultations on mathematical and statistical data processing; E.N. Parizskaya – collection of the primary data; A.O. Konradi – consultations on carrying out separate stages of the study.

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CASE REPORT

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NUMBER OF RUNS VARIATIONS ON AUTODOCK 4 DO NOT HAVE A SIGNIFICANT EFFECT ON RMSD FROM DOCKING RESULTS

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The aim. The number of runs in the docking process with AutoDock 4 is known to play an important role in the validity of the results obtained. The greater the number of runs it is often associated with the more valid docking results. However, it is not known exactly how the most ideal runs in the docking process with AutoDock 4. This study aims to determine the effect of the number of runs docking processes with AutoDock 4 on the validity of the docking results.
Materials and methods. The method used is the redocking process with AutoDock 4.2.6. The receptor used is an estrogen receptor with ligand reference estradiol (PDB ID 1GWR). Variations were made on the number of runs from 10 to 100 in multiples of 10. The parameters observed were RMSD, free energy of binding, inhibition constants, amino acid residues, and the number of hydrogen bonds.
Results. All experiments produce identical bond free energy, where the maximum difference in inhibition constant is only 0.06 nM. The lowest RMSD is indicated by the number of runs of 60, with a RMSD value of 0.942. There is no linear relationship between the number of runs and RMSD, with R in the linear equation of 0.4607.
Conclusion. Overall, the number of runs does not show a significant contribution to the validity of the results of docking with AutoDock 4. However, these results have only been proven with the receptors used.
Keywords: AutoDock 4; docking; number of runs; RMSD, variation

ИЗУЧЕНИЕ ВЛИЯНИЯ КОЛИЧЕСТВА ЗАПУСКОВ AUTODOCK 4 НА СРЕДНЕКВАДРАТИЧЕСКОЕ ОТКЛОНЕНИЕ РЕЗУЛЬТАТОВ ДОКИНГА

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Цель. Известно, что количество запусков докинга с AutoDock 4 играет важную роль в достоверности получаемых результатов. Чем больше количество запусков, тем больше достоверных результатов докинга. Однако точно не известно, как наиболее оптимально работает докинг с AutoDock 4. Это исследование направлено на определение влияния количества запусков процесса докинга в AutoDock 4 на достоверность результатов.
Материалы и методы. В качестве метода исследования использовали процесс редокинга с AutoDock 4.2.6. Исполь-

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КРАТКОЕ СООБЩЕНИЕ
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зуемый рецептор представляет собой рецептор эстрогена с эталонным лигандом эстрадиола (PDB ID 1GWR). Варьировали количество прогонов от 10 до 100, кратные 10. Наблюдаемыми параметрами были RMSD, свободная энергия связывания, константы ингибирования, аминокислотные остатки и количество водородных связей.

Результаты. Все эксперименты вырабатывают идентичную свободную энергию связи, где максимальная разница в константе ингибирования составляет всего 0,06 нМ. Наименьшее среднеквадратичное отклонение (RMSD) определяется количеством прогонов, равным 60, при значении среднеквадратичного отклонения, равного 0,942. Установлено, что между количеством прогонов и пространственным выравниванием (RMSD) нет линейной зависимости, так как коэффициент корреляции (R) равен 0,4607.

Заключение. В целом, количество запусков не оказывает значительного вклада в достоверность результатов процесса докинга с AutoDock 4. Однако эти результаты справедливы только с рецепторами, использованными в данном исследовании.

Ключевые слова: AutoDock 4; докинг; количество запусков; RMSD; вариация

INTRODUCTION

Molecular docking (or simply called docking) is one of the most common *in silico* experimental methods. The ease in the process of preparation, implementation, and analysis of results is one of the attractions of the method [1]. However, docking is one simple, yet complex method. As one of the most popular *in silico* methods, docking has several advantages and disadvantages. In addition to the speed and practicality, docking also offers a fairly complete observation to analyze complexes between drug-receptors, which are even very difficult to do in vitro or in vivo. However, the results obtained generally have a large degree of variation, where reproducibility is one of the important issues in the level of confidence in the results of docking [2]. For this reason, the validation of docking results is absolutely necessary.

The results of the validation docking are influenced by various factors, both related to the conditions and settings of the software-hardware and protocol used. The different settings and variations of various factors can cause differences in the value of Root-mean-square deviation (RMSD), which shows the degree of difference in the position of each atom before and after the docking process is carried out [3]. The RMSD value itself is the main indicator of the docking validation process, where the smaller the RMSD shows the position of the atom which is getting closer to the original position before the docking process is carried out. Ideally, to be declared valid, the RMSD value docking from the crystallographic ligand should not be greater than 2 Å [4]. This provision also applies to AutoDock 4, one of the most popular docking software.

In AutoDock 4, there are several parameters that can affect the RMSD value of docking results, such as position and size of grid box, regulation of rigid/flexible amino acid residues, presence of water molecules, and number of runs of docking processes [5]. Of these factors, the number of runs is interesting for further investigation because different from other factors, the number of runs does not depend on the type of ligand or the receptor used [6]. In general, number of runs shows the number of repetitions of the Lamarckian Genetic Algorithm performed to obtain variations in the ligand pose on the grid box that has been determined [7]. Logically, the larger number of runs will provide an opportunity for the ligand to reach the most ideal pose and approach the crystallographic pose, although this will certainly increase the time needed [8]. However, there is currently no clear limit on how many number of runs are ideal for obtaining the smallest RMSD with the shortest docking time.

This study aims to determine the effect of the number of runs docking processes with AutoDock 4 on the validity of the docking results as indicated by the RMSD value. We will try to prove whether the larger number of runs will also give smaller RMSD or vice versa. In addition, we also determined the linearity of the relationship between number of runs to the RMSD value seen from the correlation coefficient (R).

MATERIALS AND METHODS

The hardware used is the ASUS A46CB series Ultrabook with an Intel™ Core i5-3337U@1.8 GHz and Windows 7 Ultimate 64-bit SP-1 operating system. The software used is AutoDockTools 1.5.6 and Autodock 4.2.6 software from The Scripps Research Institute, Inc (USA). Information on 3D dimensional structures of receptor proteins obtained from the website of Protein Data Banks (http://www.rcsb.org).

The receptor used is an estrogen receptor with ligand reference estradiol (PDB ID 1GWR). Receptors are downloaded in the format. pdb then the unused part including water molecules is removed, added non-polar hydrogen, given charged, and arranged size and coordinate grid box using AutoDockTools 1.5.6 [9]. The reference ligand, estradiol, is extracted and then reused for the redocking process after added non-polar hydrogen, given charged, and set torque. The size and coordinates of the grid box are adjusted automatically with the ligand co-crystal position of each receptor by making the ligand position the center of the grid box [10]. The grid

coordinate used for x, y, and z dimensions is –7.351, –4.233 and 12.804, respectively. While the grid box size is 40 x 40 x 40 Å.

Docking search parameter used are Lamarckian Genetic Algorithm with the number of genetic algorithm varies from 10 to 100 runs with multiples of 10, population size 150, the maximum number of energy evaluation is medium with 2500000, the maximum number of generations 27000, with the default docking parameter used for run options.

The main parameter observed is the RMSD value to determine the validity of the docking results, where the linearity of the relationship between number of runs as a control variable and RMSD as the dependent variable is determined in the form of a correlation coefficient (R). In addition, observations of other parameters such as the free energy of binding (ΔG), the inhibition constant (K_i), amino acid residues, and the number of hydrogen bonds [9]. The selection of poses for each run is done by looking at the re-rank score of the ΔG and K_i value, where the conformation with the most negative ΔG and the smallest K_i value is representative for the conformation of the number of runs. Observation of these parameters is done to observe differences in the results of docking that occur due to differences in number of runs.

RESULTS AND DISCUSSION

The docking results show that the difference in number of runs does not give a significant difference to all observed parameters, even parameters such as ΔG and the number of hydrogen bonds show identical results for all number of runs. The RMSD value of each number of runs is in the range 0.942 to 0.97, with the smallest RMSD produced at number of runs 60. This result is interesting, because it turns out that the smallest value of RMSD is not indicated by the largest number of runs. These results become information updates, where researchers generally use a standard number of runs of at least 100 in the docking process which requires a longer processing time [9, 11], where the number of runs that are lower can actually provide a better RMSD value. This result provides a new dimension for the docking process with AutoDock 4, where in addition to conducting orientation to determine the ideal coordinates and grid box size [12], the number of runs that provide the smallest RMSD must also be determined in advance.

On the other hand, other parameters such as K_i and amino acid residues also did not show a significant difference. The K_i value shown varies in the range 15.57 to 15.63 nM with a difference of only 0.06 nM and the lowest value produced on number of runs 90, indicating there is no difference in the K_i value of the difference in number of runs used. Amino acid residues show almost the same results for each number of runs with interactions shown in the amino acids 353-Glu, 384-Leu, 388-Met, 391-Leu, 404-Phe, 424-Ile, 521-Gly, 524-His, and 525-Leu. The difference in amino acid residues is shown on number of runs 20, 30, 40, and 90, with variations in interactions occurring in amino acids 384-Leu and 391-Leu. These results indicate that variations in the number of runs can lead to small variations in the K_i value and the residual amino acids obtained. Again, these results emphasize the importance of carrying out an orientation to determine the most ideal number of runs before carrying out the docking process [11]. The complete results of the docking process include the values of RMSD, ΔG, K_i, amino acid residues, and the number of hydrogen bonds from each number of runs can be seen in Table 1.

Table 1 – The results of redocking 1GWR receptors with variations in number of runs

Parameter	Number of runs									
	100	90	80	70	60	50	40	30	20	10
RMSD (Å)	0.950	0.948	0.951	0.953	0.942	0.948	0.956	0.970	0.958	0.951
ΔG (kcal/mol)	–10.65	–10.65	–10.65	–10.65	–10.65	–10.65	–10.65	–10.65	–10.65	–10.65
Amino acid residues	353-Glu	353-Glu	353-Glu	353-Glu	353-Glu	353-Glu	353-Glu	353-Glu	353-Glu	353-Glu
	384-Leu	-	384-Leu	384-Leu	384-Leu	384-Leu	–	–	384-Leu	384-Leu
	388-Met	388-Met	388-Met	388-Met	388-Met	388-Met	388-Met	388-Met	388-Met	388-Met
	391-Leu	391-Leu	391-Leu	391-Leu	391-Leu	391-Leu	391-Leu	391-Leu	–	391-Leu
	404-Phe	404-Phe	404-Phe	404-Phe	404-Phe	404-Phe	404-Phe	404-Phe	404-Phe	404-Phe
	424-Ile	424-Ile	424-Ile	424-Ile	424-Ile	424-Ile	424-Ile	424-Ile	424-Ile	424-Ile
	521-Gly	521-Gly	521-Gly	521-Gly	521-Gly	521-Gly	521-Gly	521-Gly	521-Gly	521-Gly
	524-His	524-His	524-His	524-His	524-His	524-His	524-His	524-His	524-His	524-His
	525-Leu	525-Leu	525-Leu	525-Leu	525-Leu	525-Leu	525-Leu	525-Leu	525-Leu	525-Leu
Number of hydrogen bonds	3	3	3	3	3	3	3	3	3	3

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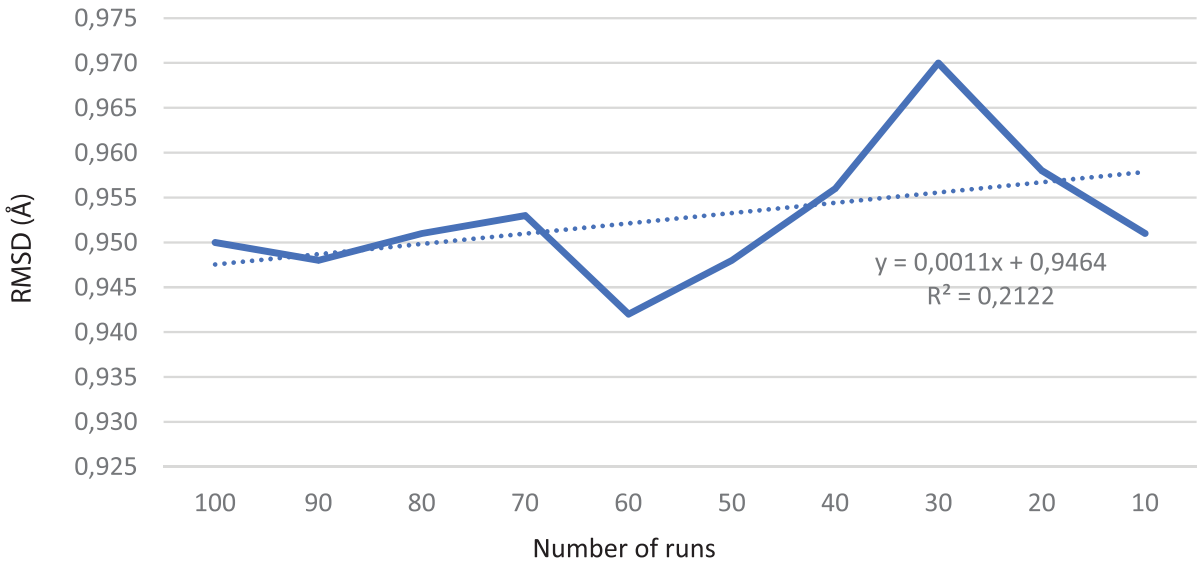

Figure 1 – The relationship between number of runs to the RMSD value at the 1GWR receptor

To observe the linearity of the relationship between the number of runs and the RMSD obtained, a comparison was made with the chart to obtain the R. The results of the observation show that the equation of linearity between the number of runs and RMSD is y = 0.0011x + 0.9464 with the value R = 0.4607, as shown in Figure 1. These results confirm that the relationship between the number of runs and RMSD is indeed not linear, where increasing the number of runs does not necessarily increase the validity of the docking results. In other words, an increase in number of runs does not guarantee the better validity of docking results. This result also supports opinions which state that the most ideal number if runs are in the range of around 50 to 60, where RMSD in that range is at the smallest value [9].

CONCLUSION

This simple study successfully proved that even though number of runs has an influence on the RMSD value of the docking results, the influence shown does not have a linear relationship. The most ideal number of runs in the range 50 to 60, with larger numbers of runs not showing a smaller RMSD value. However, this study has limitations, including the type of receptor and the docking software used. Further research using other types of receptors in large quantities and other docking software such as AutoDock Vina can be done to increase confidence in these findings. Still, this discovery can be a basic guideline for research using the docking method with AutoDock 4.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Pratama M.R.F. – conceptualization, data curation, formal analysis, investigation, validation, visualization, writing original draft; Siswandono – conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, writing review & editing

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