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PHARMACODYNAMICS OF ORAL ANTICOAGULANTS IN PATIENTS WITH ATRIAL FIBRILLATION IN THE ACUTE PERIOD OF ISCHEMIC STROKE

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Background. Every fifth ischemic stroke is caused by a patient's history of atrial fibrillation. Nowadays, direct and indirect oral anticoagulants are widely used to prevent thromboembolic complications in patients with atrial fibrillation. However, despite the prescription of this group of drugs, every year 1–2% of patients with atrial fibrillation have an ischemic stroke. In this situation, a number of questions take rise: if it is possible to carry out thrombolytic therapy in the patients who have been taking anticoagulants; if it is worth resuming anticoagulant therapy after a stroke; when exactly this should be done; and what drugs should be used to prevent another stroke.
The aim of this review was to summarize the available clinical guidelines and research results on the study of the anticoagulant therapy characteristics in patients with atrial fibrillation after an ischemic stroke.
Materials and methods. For this review, the information presented in the scientific literature from open and available sources, has been used. The information had been placed in the following electronic databases: PubMed, Scopus, Web of Science Core Collection, Cochrane Library, ClinicalTrials.gov; Elibrary, Cyberleninka, Google Academy. The covering period was 1997–2020.
The search queries were: "ischemic stroke + atrial fibrillation + anticoagulants"; "ischemic stroke + atrial fibrillation + direct oral coagulants" and "atrial fibrillation + ischemic stroke + warfarin" in both Russian and English equivalents.
Results and conclusion. Currently, the problem of the use of anticoagulants for the prevention of recurrent thromboembolic complications in patients with AF in the acute period of a stroke, is studied insufficiently. The difficulties are caused by the delivery of TLT in the patients who have been taking DOACs, first of all, due to the impossibility of an accurate assessment of the hemostasis state because of the unavailability of routine specific tests; and second, as a result of the lack of registered antidotes for most drugs, and their high costs. Besides, there are no RCTs dedicated to the study of the optimal time for the resumption or initiation of anticoagulant therapy in the acute period of an IS, and the optimal drugs for this group of patients. Most of the existing recommendations on these aspects, are based on the consensus of experts, and this fact indicates the need for further research in the area under review.
Keywords: atrial fibrillation, ischemic stroke, oral anticoagulants
Abbreviations: PTT – partial thromboplastin time; CI – confidence interval; IS – ischaemic stroke; INR – international normalized ratio; RR – risk ratio; DOAC – direct oral anticoagulant; RCT – randomized clinical trial; TT – thrombin time; TIA – transitory ischaemic attack; TLT – thrombolytic therapy; AF – atrial fibrillation; ECT – ecarin clotting time.

ФАРМАКОДИНАМИКА ОРАЛЬНЫХ АНТИКОАГУЛЯНТОВ У БОЛЬНЫХ С ФИБРИЛЛЯЦИЕЙ ПРЕДСЕРДИЙ В ОСТРОМ ПЕРИОДЕ ИШЕМИЧЕСКОГО ИНСУЛЬТА

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Введение. Каждый пятый ишемический инсульт обусловлен наличием у пациента в анамнезе фибрилляции предсердий. Для предотвращения тромбоэмболических осложнений у пациентов с фибрилляцией предсердий в настоящее время широко применяются прямые и непрямые пероральные антикоагулянты. Однако, несмотря на назначение данной группы препаратов, ежегодно у 1–2% пациентов с фибрилляцией предсердий возникает ишемический инсульт. В данной ситуации встает ряд вопросов: возможно ли проведение тромболитической терапии у больных, принимавших антикоагулянты, стоит ли возобновлять антикоагулянтную терапию после перенесенного инсульта, когда именно это нужно делать и какие препараты для этого использовать.

Цель. Целью написания данного обзора было резюмировать имеющиеся клинические рекомендации и результаты исследований, посвященные изучению особенностей антикоагулянтной терапии у пациентов с фибрилляцией предсердий, перенесших ишемический инсульт.

Материалы и методы. Для обзора использовали сведения научной литературы из открытых и доступных источников за период 1997–2020 гг., размещенных в электронных базах данных: PubMed, Scopus, Web of Science Core Collection, Cochrane Library, ClinicalTrials.gov; Elibrary, Киберленинка, Google-академия. Поисковые запросы – «ишемический инсульт+фибрилляция предсердий+антикоагулянты»; «ишемический инсульт+фибрилляция предсердий+прямые оральные коагулянты» и «фибрилляция предсердий+ишемический инсульт+варфарин» как в русском, так и английском эквиваленте.

Результаты и заключение. Проблема применения антикоагулянтов для профилактики повторных тромбоэмболических осложнений у пациентов с ФП в остром периоде инсульта в настоящее время изучена недостаточно. Сложности вызывает проведение ТЛТ у пациентов, принимавших ПОАК, в первую очередь, из-за невозможности точной оценки состояния гемостаза в виду недоступности рутинного проведения специфических тестов, во вторую очередь, отсутствие зарегистрированных антидотов для большинства препаратов и их высокая стоимость. Также отсутствуют РКИ, посвященные изучению оптимального времени для возобновления или инициации антикоагулянтной терапии в остром периоде ИИ и оптимальных препаратов для данной группы пациентов. Большинство существующих рекомендаций по этим аспектам основаны на согласованном мнении экспертов, что говорит о необходимости дальнейших исследований в данной области.

Ключевые слова: фибрилляция предсердий, ишемический инсульт, оральные антикоагулянты

Список сокращений: АЧТВ – активированное частичное тромбопластиновое время, ДИ – доверительный интервал, ИИ – ишемический инсульт, МНО – международное нормализованное отношение, ОР – относительный риск, ПОАК – прямые оральные антикогаулянты, РКИ – рандомизированное контролируемое исследование, ТВ – тромбинспецифическое время, ТИА – транзиторная ишемическая атака, ТЛТ – тромболитическая терапия, ФП – фибрилляция предсердий, ЭВС – экариновое время свертывания.

INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia with a cardioembolic stroke as the most severe complication. From 20 to 30% of ischemic strokes (ISs) are associated with AF. IS in patients with AF is characterized by a higher risk of death and more often leads to disability compared with strokes of another etiology [1].

As the asymptomatic nature of the course of arrhythmia is rather frequent, in some cases IS is the first symptom of AF. If AF is detected earlier, anticoagulant therapy is indicated for all patients with a high risk of thromboembolic complications.

Currently, doctors have a number of drugs with proven efficacy in the prevention of a stroke and other systemic embolisms; they can include direct (dabigatran, rivaroxaban, apixaban, edoxaban) and indirect (warfarin) oral anticoagulants.

Despite the success achieved in the prevention of thromboembolic complications of AF, the incidence of IS in the patients taking anticoagulants, is 1–2% per year [2]. In such patients, adherence to therapy as well as alternative causes of a stroke, should be assessed.

An effective method of treating an acute IS within 4.5 hours from the onset of symptoms is systemic thrombolysis with a recombinant tissue plasminogen activator [3]. However, the use of thrombolysis in patients receiving oral anticoagulant therapy, is difficult due to the high risk of bleeding. The question of the resumption timing of anticoagulant therapy after suffering an IS is also controversial.

THE AIM of this review was to summarize the available clinical guidelines and research results on the study of the anticoagulant therapy characteristics in the patients with atrial fibrillation after an ischemic stroke.

MATERIALS AND METHODS

For the review, the data from scientific literature from open and available sources over the period of 1997–2020 were used. The information was placed in the following electronic databases: PubMed, Scopus, Web of Science Core Collection, Cochrane Library, ClinicalTrials.gov; Elibrary, Cyberleninka, Google Academy. The search queries were: “ischemic stroke + atrial fibrillation + anticoagulants”; “ischemic stroke + atrial fibrillation + direct oral coagulants” and “atrial fibrillation + ischemic stroke + warfarin” in both Russian and English equivalents.

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RESULTS AND DISCUSSION

Systemic thrombolysis in patients taking anticoagulants

Systemic thrombolysis is contraindicated for the patients administrated with anticoagulants, on condition of effective hypocoagulation [4, 5].

Recombinant tissue plasminogen activator can be administered to the patients taking warfarin, if their international normalized ratio (INR) is lower than 1.7.

In this situation, thrombolytic therapy (TLT) did not lead to a significant increase in the risk of hemorrhagic complications [6, 7]. However, standard tests such as INR, activated partial thromboplastin time (APTT), prothrombin time are not suitable for assessing coagulation when using direct oral anticoagulants (DOCs) [8].

Plasma concentrations of dabigatran, causing a significant anticoagulant effect, did not lead to significant changes in prothrombin time and INR. The APTT index can be altered by the drug, but the relationship between the plasma concentration of dabigatran and the APTT is not linear [5].

To assess the effect of dabigatran, the best approach is to determine the thrombin clotting time (TT) and ecarin clotting time (ECT), but the dilute time of thrombin formation, which gives a quantitative assessment of the effect of dabigatran-Hemoclot (HyphenBioMed, "Neuville-sur-Oise", France), is especially highly sensitive to the action of the drug [9].

Although prothrombin time and APTT may be prolonged by direct Xa factor inhibitors (rivaroxaban, apixaban, edoxaban), these analyses do not quantify the anticoagulant effects of the drugs. Prothrombin time and APTT results can also depend on the reagent used, and must be calibrated in each laboratory to determine the effect of a particular drug [5]. The concentration of Xa factor inhibitors in plasma was most reliably reflected by the chromogenic anti-Xa assay [10, 11].

In real clinical practice, the above data are confirmed by the example of the German register RASUNOA (Registry of Acute Stroke Under New Oral Anticoagulants), which included 290 patients receiving DOACs therapy, who had been hospitalized with a diagnosis of an acute IS to neurological departments throughout Germany from 2012 to 2015 [2]. Coagulation indices were analyzed in patients by conducting nonspecific (INR, APTT, TT, ECT) and specific (analyses for anti-factor Xa or hemoclot) tests.

According to the data obtained, about half (56.2%) of patients receiving dabigatran had a slightly elevated INR level. In contrast, TT was above the upper limit in the majority (94%) of patients receiving dabigatran, and only in 14% of patients taking factor Xa inhibitors (rivaroxaban, apixaban). APTT was also more frequently prolonged with dabigatran (65%) than with rivaroxaban (32%) and apixaban (13%). A significant number of false negative results (11-44%) of INR and APTT, were detected even when the peak levels of DOACs concentration were exceeded.

Fewer than half of the patients receiving DOACs, were subjected to specific testing. The drug concentration levels varied greatly at admission even if similar intervals were observed, from the time of the last intake (according to patients). In 58% of patients, the concentration of drugs in the blood was within the expected range. In contrast, in 25% of the test persons, the concentration of the drug was below the minimum level. It is of interest that 17% of patients suffered a stroke even though their blood anticoagulant levels were above the peak range.

Based on the tests performed, only 9% of patients have gone systemic thrombolysis. The number of patients theoretically suitable for thrombolysis, based on the coagulation parameters. Hereby, the number of patients strongly depended on the parameters, the doctors would decide to use.

For example, if in the group of patients receiving rivaroxaban, the decision on TLT were made on the basis of normal anti-Xa values, then only 12% of the tested patients would be suitable candidates. Whereas, if doctors' decisions were based on normal APTT and TT values, the number of eligible patients would increase to 24%. This underlines the low sensitivity of nonspecific tests for determining the concentration of rivaroxaban.

It becomes apparent that standard coagulation tests are not reliable in predicting the actual level of DOACs in the blood. On the other hand, the possibility of conducting specific tests is not available in all medical institutions, even in large vascular centers. Thus, the use of DOACs for the prevention of thromboembolic complications in AF, is a barrier to TLT in the event of a stroke in patients.

According to the European Heart Rhythm Association's practical guidelines on the use of DOACs in AF, thrombolysis cannot be performed within 24 hours after the last dose of the drug due to its long half-life, which can also be prolonged in renal failure and in elderly patients [12]. The assessment of renal function is necessary for all patients receiving DOACs as antithrombotic therapy, in view of the presence of these drugs in varying degrees (27–80%) of the renal route excretion. Moreover, to calculate creatinine clearance, it is preferable to use the Cockcroft-Gault formula, since it was according

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to this formula that renal function was assessed in all phase III randomized controlled trials (RCTs) to study the efficacy and safety of DOACs [13].

In addition to assessing renal function, an assessment of the hemostasis system is required before TLT. In accordance with the Consensus Document of the Interdisciplinary Expert Group on Emergency and Urgent Care for Patients Receiving DOAC, the determination of APTT and TT is sufficient for a qualitative assessment of the residual anticoagulant effect of dabigatran, while for direct inhibitors of factor Xa, only the chromogenic method for determination of anti-Xa activity is recommended [14].

Based on the opinion of the experts from the European Heart Rhythm Association, the use of a recombinant tissue plasminogen activator is allowed in the patients treated with coagulation factor Xa inhibitors, if, according to the data of specific tests carried out without a significant delay, their concentration is less than 30 ng/ml (when measured later than in 4 hours after drug administration) [12].

According to the European and Russian clinical guidelines for the treatment of AF, systemic thrombolysis is allowed in patients with normal APTT treated with dabigatran if more than 48 hours have passed since the last intake of the drug (based on the experts' consensus) [1, 15].

TLT in the patients taking dabigatran, is also allowed in the presence of a specific inhibitor, idarucizumab [16]. Idarucizumab is a fragment of a human monoclonal antibody that binds to dabigatran with a high affinity that exceeds the binding capacity of dabigatran to thrombin by about 300 times [17]. Immediately after the administration of the drug, the concentration of unbound dabigatran in the plasma decreases by more than 99%, which is accompanied by a rapid normalization of indicators reflecting the anticoagulant activity of dabigatran (TB, APTT, ECT, diluted thrombin time). This effect persists for at least 24 hours. By itself, idarucizumab does not have any procoagulant effect.

The largest prospective study investigating the effectiveness of the drug to neutralize the action of dabigatran in patients with bleeding or before an urgent surgery, is the RE-VERSEAD study, but it did not include the patients who had been scheduled for thrombolysis [18]. But there are retrospective studies demonstrating the efficacy and safety of using idarucizumab in this category of patients [19, 20]. In 2018, a systematic review of a series of TLT cases following a reversal dabigatran action, was also published [21]. It presents 55 cases of IS in patients taking dabigatran; a prolongation of APTT and thrombin time were recorded at their admission. Thrombolysis after idarucizumab was successful in 81.9% of cases. Adverse outcomes (deaths/disabilities) were reported in 10.9% of patients. In Russia, a description of one successful case of using idarucizumab for reversing the action of dabigatran before thrombolysis, has been published [22].

Despite the fact that in the official instructions TLT is not an indication for the prescription of idarucizumab, the use of the drug in this clinical situation is regulated by the Protocol of Reperfusion Treatment of Acute Ischemic Stroke dated 2019 [16]. According to the instructions for medical use, the recommended dose of idarucizumab is 5 g (2 vials of 2.5 g). The drug is administered intravenously in the form of two successive infusions (2.5 g each) lasting no more than 5–10 minutes each, or as a bolus.

Since the specific antidote for Xa factor inhibitors, andexanet alfa, is not registered in the Russian Federation, thrombolysis is contraindicated in the absence of the possibility of determining anti-Xa activity.

The use of endovascular thrombectomy up to 7.3 hours after the onset of a stroke in patients with distal internal carotid artery occlusion or proximal middle cerebral artery occlusion who had not received anticoagulants, has been proven [12]. In the recommendations of the European Stroke Association, endovascular thrombectomy is mentioned as a first-line therapy in patients with contraindications to systemic thrombolysis [23]. Although the trials underlying these recommendations, included only a few patients on anticoagulants, a small amount of available data suggests that endovascular thrombectomy may be safe in these people as well [12].

The treatment tactics of a patient taking DOACs in the acute period of ischemic stroke, is shown in Fig. 1.

Resumption of anticoagulants after a transient ischemic attack or ischemic stroke

Patients with AF who have had a cardioembolic stroke, have a higher risk of a recurrent stroke within the first two weeks than the patients with other etiologies of ISs, and its frequency varies from 0.1 to 1.3% per day [24]. On the other hand, the presence of a large ischemic focus is a predisposing factor to the development of hemorrhagic transformation [25]. The CHA_2DS_2VASc and HAS-BLED scales, which are widely used to assess the risks of thromboembolic and hemorrhagic complications in AF for deciding on the prescription of anticoagulants, are not suitable for use in the acute period of a stroke [26]. Thus, the problem of prescribing anticoagulants after a previous AF is very hard.

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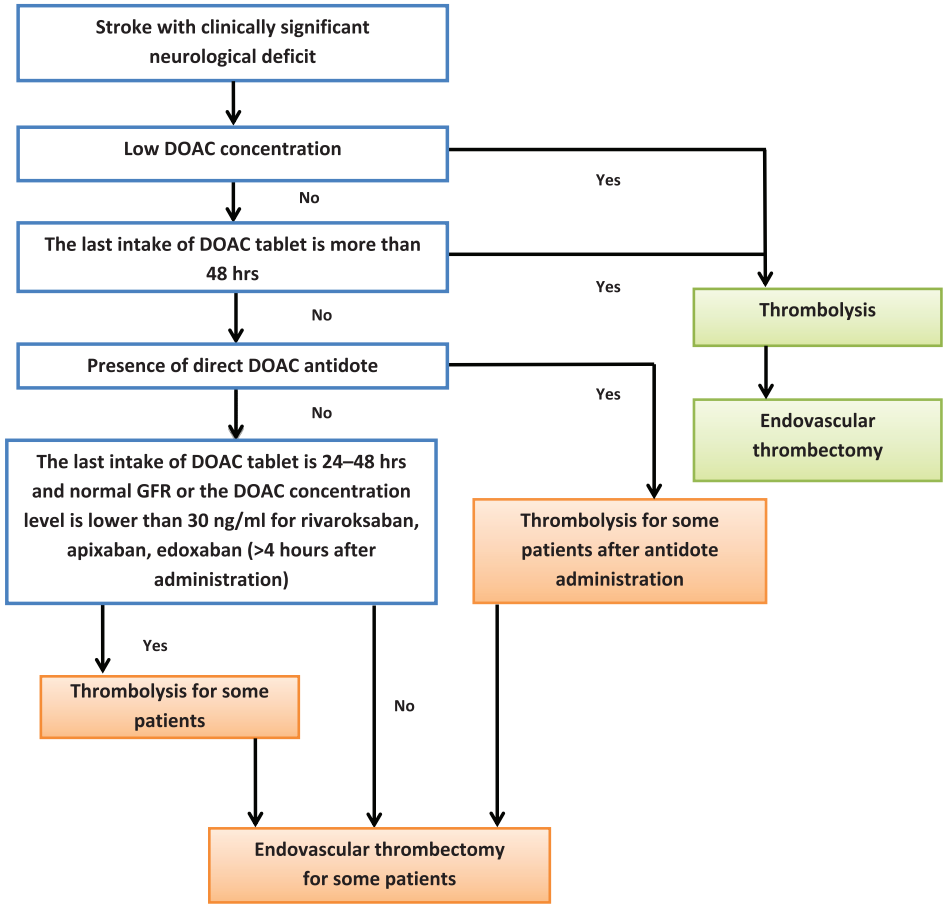

Figure 1 – Treatment tactics of a patient taking DOACs in the acute period of ischemic stroke (adapted by J. Steffeletal, 2018 [12])

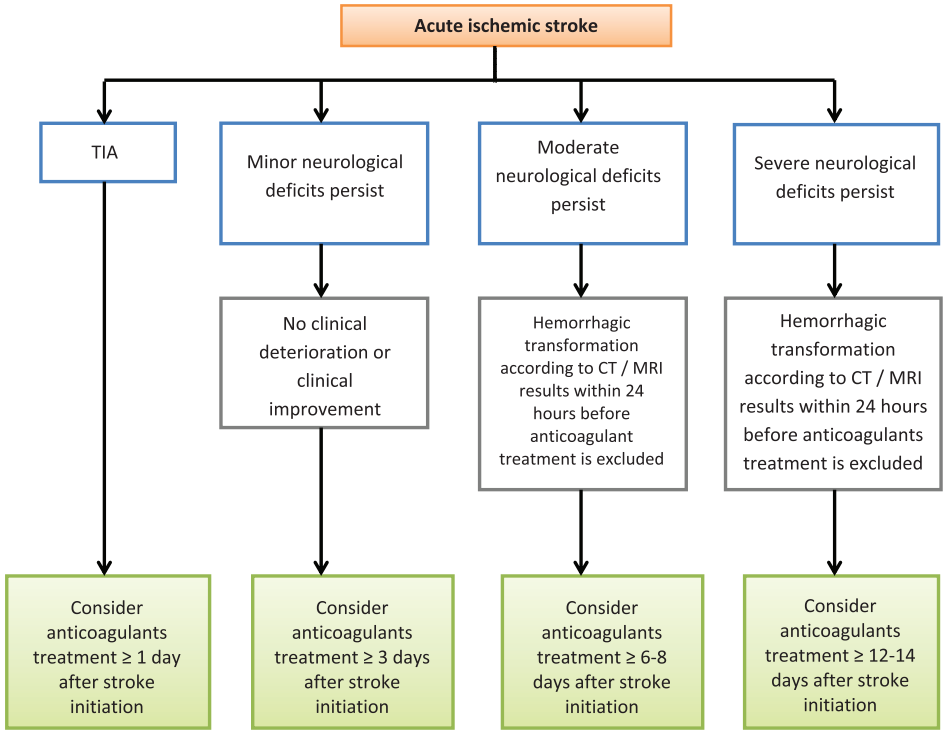

Figure 2 – Initiation/resumption of anticoagulant therapy after transient ischemic attack/ischemic stroke (adapted from J. Steffeletal, 2018 [12])

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In the meta-analysis by Paciaroni M. et al. [24], which included seven RCTs involving 4,624 patients with an acute cardioembolic stroke, the administration of unfractionated and low molecular weight heparin within 48 hours from the onset of stroke, was assessed.

It was shown that the prescription of parenteral anticoagulants within 7–14 days after a stroke contributes to a slight decrease in the risk of recurrent ISs (relative risk (RR) 0.68, 95% CI 0.44-1.06), while there is still a significant increase in the likelihood of intracranial hemorrhage (RR 2.89, 95% CI 1.19–7.01).

In 2015, the results of the international prospective study RAF were published [27]. They included 1037 patients with AF from 29 stroke departments across Europe and Asia. The patients who were participating in the study, were monitored for 90 days after the onset of ISs on the development of a recurrent stroke or a transient ischemic attack (TIA), as well as bleeding (both intracranial and extracranial).

All in all, 1029 people were included in the analysis (8 people were excluded due to the lack of data); 766 of them received anticoagulant therapy: 113 (14.7%) patients were prescribed low molecular weight heparin, 284 (37.1%) – vitamin K antagonists, 93 (12.1%) took DOAC, 276 people received low molecular weight heparin followed by switching on to vitamin K antagonists. Out of 263 patients who did not receive anticoagulants, 231 people took antiplatelet agents, 32 people did not receive any antithrombotic therapy.

It was established that the patients who had been receiving only oral anticoagulants, had a significantly lower risk of bleeding compared with the patients who had been receiving low molecular weight heparin followed by switching on to oral anticoagulants or only low molecular weight heparin (this regimen was associated with the highest risk of bleeding). The patients taking only DOACs, had a low risk of both intracranial bleeding (2.1%) and recurrent ischemic events (4.3%). In addition, the study determined the most optimal time for starting treatment with anticoagulants. It has been shown that the administration of anticoagulant therapy from the 4th to the 14th day of the IS development, is both safe and effective compared to the start of the treatment, before or after this period.

However, the RAF study had some limitations associated with the lack of randomization and, as a result, the influence on the selection: the patients with a smaller lesion in the brain and with a more favorable course of the disease, probably, began to receive anticoagulant therapy earlier than the patients with severe strokes. The administration of only low-molecular-weight heparin in the early post-stroke period, could be also associated with the development of dysphagia and, probably, a more severe course of the disease in such patients.

Several prospective observational studies have investigated the potential risks and benefits of the early DOACs prescription in patients with ISs associated with AF. Three studies included patients with a recent cardioembolic stroke who were followed up for at least 3 months before clinical outcomes (recurrent ISs and intracranial bleeding) [28–30]. In all three studies, a significant proportion of patients took DOACs: NOACISP-155 (75%), SAMRUAI-NVAF – 475 (41%), RAF-NOAC – 1127 (100%). Their average age was 76–79 yrs, the severity of a stroke was assessed using National Institutes of Health Stroke Scale – NIHSS – and ranged from 3 to 8 points. The start of taking anticoagulants was on average 5 days after the acute event. The annual risk of recurrent ISs was roughly equal in all the studies, and it was 7.7% in NOACISP, 8.5% in SAMRUAI-NVAF, and 7.8% in RAF-NOAC. The risk of intracranial bleeding was low in the NOACISP and SAMRUAI-NVAF studies (1.3% and 1.2% per year, respectively) while in the RAF-NOAC study it was 6.4% per year. Most of intracranial bleedings in RAF-NOAC were associated with a later initiation of anticoagulant therapy.

An early administration of DOACs after a mild stroke, was studied in two small RCTs. One of the studies conducted in 14 academic medical centers in South Korea, included 183 patients. They were prescribed anticoagulant therapy 5 days after suffering cardioembolic ISs (the severity averaged 2 points, according to the National Institutes of Health Stroke Scale (NIHSS) [31]. The participants were randomized into two groups: the first group took rivaroxaban 10 mg per day for 5 days, followed by 15 or 20 mg per day; the second – warfarin with a target INR of 2.0-3.0. The primary endpoint was the combination of a new ischemic lesion or a new intracranial hemorrhage as seen by magnetic resonance tomography in 4 weeks. The rivaroxaban group (n = 95) and the warfarin group (n = 88) showed no differences in the primary endpoint (47 [49.5%] vs 48 [54.5%]; RR 0.91; 95% CI 0.69–1.20) or in its individual components (a new ischemic lesion: 28 [29.5%] vs 31 of 87 [35.6%]; RR 0.83; 95% CI 0.54–1.26; new intracranial hemorrhage: 30 [31.6%] vs 25 of 87 [28.7%], RR 1.10; 95% CI 0.70–1.71).

In the DATAS II study, dabigatran was compared with aspirin in 301 patients with TIAs or minor strokes (up to 9 points on the NIHSS), but without confirmed AF [32]. The medication was started within 72 hours from the acute event and continued for 30 days. Magnetic resonance tomography was performed before randomiza-

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tion and repeated on day 30. Symptomatic hemorrhagic transformation was the primary endpoint. The symptoms of hemorrhagic transformation did not appear in any of the groups. Asymptomatic petechial hemorrhagic transformation developed in 11/142 (7.8%) patients administrated with dabigatran and in 5/142 (3.5%) patients administrated with aspirin (RR 2.301; 95% CI, 0.778–6.802). Thus, dabigatran has a risk of hemorrhagic transformation similar to aspirin in an acute non-severe noncardioembolic ISs or TIAs. Although these data cannot be extrapolated to patients with ISs associated with AF, the DATAS II study provides some confidence in the safety of an early initiation of anticoagulant therapy in these patients.

Currently, four RCTs are ongoing (ELAN, Switzerland; TIMING, Sweden; OPTIMAS, UK; START, USA). Up to 9000 people who had suffered ISs associated with AF, were involved into studies [26]. The study participants are prescribed DOACs in the acute period of a stroke. The endpoints in all RCTs are the onset of ischemic or hemorrhagic events, three of which also include cardiovascular or all-cause mortality. The research results are expected in 2021.

In 2013, the European Heart Rhythm Association from the European Society of Cardiology, proposed to use the rule of "1–3–6–12 days", according to which the start of taking an anticoagulant depends on the severity of the stroke [33]. The earliest initiation or resumption of anticoagulant therapy, is recommended for patients with TIAs – the day after the acute event, and a mild stroke (NIHSS<8) – 3 days after the acute event; in the patients with a moderate stroke (NIHSS 8-15) and a severe stroke (NIHSS≥16), it is recommended to refrain from prescribing anticoagulants after an acute event for 6 and 12 days, respectively.

This strategy for the secondary IS prevention is retained in the subsequent edition of the recommendations, dated 2016 [34]. A similar approach is reflected in the Russian clinical guidelines "Diagnosis and treatment of atrial fibrillation", dated 2017 [15]. However, both documents base their recommendations on the consensus opinion of experts, due to the lack of sufficient prospective studies.

In 2019, the recommendations of the European Stroke Organization for the secondary prevention of a stroke in patients with AF were published [35]. The authors of the document also point out the need for additional research to determine the optimal time to start anticoagulant therapy. In the absence of those, it was proposed to resume taking anticoagulants 3–4 days after a mild stroke, 7 days after a moderate stroke, and not earlier than 14 days after a severe one. In addition, within 48 hours after an IS before the start of anticoagulant therapy, it is recommended to prescribe aspirin at the dosage of 100–300 mg to prevent thromboembolic complications. These recommendations are based on two large non-blinding RCTs (IST and CAST), which demonstrated that when aspirin was given within 48 hours of an acute event, mortality and a stroke recurrence were minimal [36, 37].

The 2018 American Heart Association and American Stroke Association Guidelines for the Early Management of IS patients, recommend oral anticoagulants 4–14 days after the development of neurological symptoms, based on the results of the RAF study [38].

Thus, most guidelines for the treatment of patients with AF and ISs, recommend starting anticoagulant therapy in the first 14 days after the development of acute symptoms, focusing on the severity of the disease and the size of the lesion (the algorithm for prescribing anticoagulants after TIAs or ISs is shown in Fig. 2). These recommendations are based on the expert consensus and several prospective studies currently available. More data from large RCTs are necessary to determine a more accurate management of these patients.

Nowadays, we do not have comprehensive data as to whether drugs should be preferred in the acute period of ISs either. Acute stroke patients were not included in the RCTs of phase 3 that investigated the effectiveness of DOACs. However, in these studies, there was a subgroup of patients who had previously suffered ISs or TIAs. Thus, in the RE-LY study, which examined the effectiveness of two dosages of dabigatran (110 mg or 150 mg twice a day) in comparison with warfarin, the subgroup of stroke patients who had suffered ISs, consisted of 2,428 people with a median follow-up of 2 years [39]. In the ROCKET AF study with rivaroxaban at the doses of 20 mg or 15 mg (with creatinine clearance of 30–49 ml/min), 7,468 participants with previous ISs or TIAs were followed for 1.85 years [40]. In RCTs with apixaban (ARISTOTLE) at the doses of 5 mg or 2.5 mg twice a day (for the patients with two or more of the following data: age ≥80 years, body weight ≤60 kg, serum creatinine ≥133 μmol/L), the number of the patients with a previous stroke was 3,436; the average follow-up was 1.8 years. In the ENGAGE AF-TIMI 48 study, where the efficacy of edoxaban with a single dose of high (60/30 mg) or low (30/15 mg) doses with warfarin was compared, a subgroup of 5,973 people with previous ISs or TIAs was followed-up for 2.8 years [42]. A pooled analysis of the results of these studies showed that DOACs use was associated with a significant reduction in hemorrhagic strokes (RR 0.43; 95% CI 0.29-0.64) and death from any

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cause (RR 0.87; 95% CI 0.80–0.95) compared with warfarin. However, there was no significant difference in the risk of thromboembolic complications (RR 0.91; 95% CI 0.81–1.02) or recurrent ischemic stroke (RR 1.15; 95% CI 0.84–1.57) [35]. It should be notified that there was a significant reduction in thromboembolic complications and strokes in favor of DOACs when higher dose regimens of dabigatran and edoxaban were put into action, while the reduction in hemorrhagic strokes remained similar. Thus, based on the recommendations of the European Stroke Organization, DOACs should be given preference for the secondary prevention of ISs in comparison with varfarin because of their greater safety (a low risk of intracranial bleeding).

CONCLUSION

Summarizing all the above, it can be notified that the problem of the anticoagulants use for the prevention of recurrent thromboembolic complications in patients with AF in the acute period of the stroke, is currently insufficiently studied.

Difficulties are caused by TLT in the patients taking DOACs, first of all, due to the impossibility of the accurate assessment of the hemostasis state because of the inaccessibility of routine specific tests, and second, due to the lack of registered antidotes for most drugs, and their high costs.

There are no RCTs devoted to the study of the optimal time for resumption or initiation of anticoagulant therapy in the acute period of ISs and the optimal drugs for this group of patients, either. Most of the existing recommendations on these aspects are based on the consensus of experts. This factor indicates the need for further research in this area.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

V.I. Petrov – planning and editing the review; A.S. Gerasimenko – writing the review;
V.S. Gorbatenko – collection of materials for the review; O.V. Shatalova – collection of review materials.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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DEVELOPMENT OF A SOLID DOSAGE FORM WITH ADSORPTION ACTIVITY

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Enterosorbents are produced in various dosage forms – powders, tablets, pastes, etc., some of them are also manufactured in the form of capsules. A water-soluble polysaccharide complex (WSPC) manifesting a pronounced adsorption activity, which determines the prospects for the development of dosage forms of sorbents, was obtained from the cones of European Spruce *(Picea abies).*

The aim of the work is to develop a solid dosage form with an adsorption activity based on a water-soluble polysaccharide complex from the cones of European Spruce *(Picea abies).*

Materials and methods. The samples of European Spruce *(Picea abies)* cones were collected on the territory of Ilyinsky district of the Perm Krai and used as plant raw materials. A water-soluble polysaccharide complex was obtained from the raw materials. In order to improve the technological properties of the substance, (WSPC) granulates were obtained. The granulates were hand-made by wet granulation. The adsorption activity of the obtained granules was determined by the ability to bind methylene blue.

Results. As a result of the experiment it has been established, that the WSPC substance of European Spruce *(Picea abies)* cones needs to be improved in its technological properties. Granulation of the substance led to an improvement in technological properties and an increase in the adsorption activity in most of the selected compositions. It has also been shown that increased moisture content of granulate decreases its adsorption activity. A direct dependence of the adsorption activity on the concentration of the granulating liquid (with the exception of some granulates) has been revealed, but no significant effect of the size of the granulate particles on the manifestation of the adsorption effect has been reported. According to the results of the study, a dosage form “Capsules” has been proposed for the compositions that showed the best results of the adsorption activity, and their biopharmaceutical evaluation was carried out according to the disintegration test.

Conclusion. Thus, a solid dosage form with an adsorption activity has been obtained. The study shows the prospects for further research on the preparation of the drug with an adsorption activity based on the water-soluble polysaccharide complex of European Spruce *(Picea abies)* cones.

Keywords: sorbents, capsules, water-soluble polysaccharide complex, European Spruce *(Picea abies)* cones, adsorption activity, granulation

РАЗРАБОТКА ТВЕРДОЙ ЛЕКАРСТВЕННОЙ ФОРМЫ С АДСОРБЦИОННОЙ АКТИВНОСТЬЮ

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RESEARCH ARTICLE

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Энтеросорбенты выпускаются в различных лекарственных формах – порошки, таблетки, пасты и др., также некоторые наименования встречаются в виде капсул. Из шишек ели обыкновенной получен водорастворимый полисахаридный комплекс (ВРПК), проявляющий выраженную адсорбционную активность, что обуславливает перспективы разработки лекарственных форм сорбентов.

Цель работы – разработка твердой лекарственной формы с адсорбционной активностью на основе водорастворимого полисахаридного комплекса ели обыкновенной шишек.

Материалы и методы. В качестве растительного сырья использовали образцы ели обыкновенной шишек, собранные на территории Ильинского района Пермского края. Из сырья был получен водорастворимый полисахаридный комплекс. С целью улучшения технологических свойств субстанции ВРПК были получены грануляты ВРПК. Грануляты получали вручную, путем влажного гранулирования. Адсорбционную активность, полученных гранулятов, определяли по способности связывать метиленовый синий.

Результаты. В результате эксперимента установлено, что субстанция ВРПК шишек ели нуждается в улучшении технологических свойств. Гранулирование субстанции привело к улучшению технологических свойств и увеличению адсорбционной активности у большинства выбранных составов. Так же показано, что при повышенной влажности гранулята понижается его адсорбционная активность. Выявлена прямая зависимость адсорбционной активности от концентрации гранулирующей жидкости, за исключением некоторых гранулятов, но существенного влияния размера частиц гранулята на проявление адсорбционного действия, не было выявлено. По результатам исследования предложена лекарственная форма «Капсулы» для составов, показавших наилучшие результаты адсорбционной активности, и проведена их биофармацевтическая оценка по тесту «Распадаемость».

Заключение. Таким образом, была получена твердая лекарственная форма с адсорбционной активностью. Проведенное исследование показывает перспективность дальнейших исследований по получению лекарственного средства с адсорбционной активностью на основе водорастворимого полисахаридного комплекса шишек ели.

Ключевые слова: сорбенты, капсулы, водорастворимый полисахаридный комплекс, шишки ели обыкновенной, адсорбционная активность, грануляция

INTRODUCTION

Enterosorbents are group of drugs with various structures that bind and remove exo- and endogenous substances from the gastrointestinal tract (GIT) by adsorption, absorption, ion exchange, complexation [1].

Herbal polysaccharides have many useful properties, including adsorption [2–4]. They are able to “bind” heavy metals, some groups of secondary metabolites despite their solubility in water. These interactions are complex and described not only by adsorption, but also by intermolecular interactions or their ability to complexation. In addition, the use of the term “water-soluble polysaccharides – WSPS” is often criticized, since it is clear that water educes a complex of substances during the extraction of plant materials. Therefore, the uses of this term is a tribute to traditions than a description of specific physicochemical interactions [2–5]. The aqueous solutions of pectin polysaccharides from rowanberry fruits exhibit pronounced antioxidant and antiradical activity [6, 7]. Polysaccharides of the aquatic plant *Brasenia schreberi* J.F. Gmel. exhibit pronounced antiradical activity [8]. The sorption properties of herbal polysaccharides are being studied, such as: pectin substances of peach and peach gum [9–11], apples and sunflower baskets [9]; herbal polysaccharide complexes from milk thistle, fruits of sweet-brier, seeds of pumpkin, watermelon, grape, walnuts [13]. Polysaccharides of yellow melilot exhibit anti-inflammatory [13], immunocorrecting [14, 15], antianemic and adaptogenic effects [16]. *Stipa parviflora* polysaccharides exhibit hepatoprotective activity in experiments *in vivo* [17]. Polysaccharides of *Psidium guajava* leaves exhibit hypoglycemic activity in a model of diabetes induced by streptozotocin [18].

Plant-based sorbents (including polysaccharides), while entering the small bowel are full or partially fermented by the gut microbiome. It is known that some sources of prebiotics (inulin, resistant starches and some oligosaccharides) acting as a selective substrate for bacteria, which produce specific short-chain fatty acids and can lower intestinal pH. Short-chain fatty acids stimulate the proliferation of epithelial cells, which can healing the damaged part of the colonic wall [19]. Due to the formation of short-chain fatty acids, polysaccharides promote the growth of gut microbiome *Bifidobacteria and Lactobacilli*, and also inhibit the growth of pathogenic microorganisms [2, 20]. This is the advantage of sorbents based on polysaccharides in comparison with other sorbents obtained from non-plant raw materials.

At the Department of Pharmacognosy with a course of botany of the Perm State Pharmaceutical Academy, a water-soluble polysaccharide complex with adsorption activity was obtained from raw materials of spruce cones. In the experiment WSPC of spruce cones showed high adsorption activity for their ability to bind methylene blue, which known as a marker for many sorbents. The adsorption activity of spruce cones WSPC (232.88±4.17) turned out to be better than activity of comparator drug – activated carbon (230.9±1.12) and silicon diox-

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ide (211.5±1.42) [21]. The water-soluble polysaccharide complex of spruce cones exhibits high anti-inflammatory activity in the model of acute carrageenan edema caused by sub-plantar injection of 1% carrageenin solution into the rat's hind paw. The water-soluble polysaccharide complex of spruce cones reduced the increase of inflammatory edema by 65% compared to the control group, which is comparable to the activity of the comparator drug diclofenac sodium (68.3% inhibition of the inflammatory responses) [22].

The antioxidant activity has been established at the spruce cones' WSPC. The antioxidant activity was determined using a reaction with a stable radical – 2,2-diphenyl-1-picrylhydrazyl (DPPH). The IC50 value (the substance concentration which is bind a half of the concentration of the DPPH) for WSPC of spruce cones is 19.56 μg/ml and the same value for the comparator substance (ascorbic acid) is 9 μg/ml, which indicated the presence of antioxidant activity in the investigated substance [23].

Considering that fact, the development of a dosage form of WSPC is a relevant.

THE AIM of the work is to develop of solid dosage form in a capsule with adsorption activity based on a water-soluble polysaccharide complex from the cones of European Spruce.

MATERIALS AND METHODS

The object of the research is a water-soluble polysaccharide complex, which is an amorphous light-brown powder, after micronization, with a characteristic odor. WSPC from spruce cones was obtained by the method described by N.K. Kochetkov [24]. A weighed sample of the air-dry raw material was reduced up to a particle size 2 mm in diameter. A water-soluble polysaccharide complex of spruce cones was obtained by purified water extraction in a ratio of 1:10 at a temperature of 80°C for 1.5 hours. The obtained extracts were evaporated under vacuum and the sedimented by the triple addition of 95 percent alcohol. The polysaccharides were precipitated with alcohol, then were removed by filtration and purified by multiple washing with 95 percent ethanol. The drying was carried out in a hot air at a temperature of 60°C and grinding.

The samples of spruce cones were collected before seed ripening in July 2017, on the territory of the Ilyinsky District of the Perm region for obtaining a polysaccharide complex. The drying of samples was carried out by a shade-drying method. The prepared and dried samples corresponded to the requirements of the State Pharmacopoeia of the XIV^{ed} on the next terms: essential oil, tannins and extractives (by water) [24].

In order to improve the technological properties of the WSPC, the granulate were obtained. The WSPC granulates of Spruce cone were obtained by wet granulation. Wet granulation was carried out by hand made on the next methodology: a weighed sample of the WSPC powder was weighed of an electronic analytic balance and placed in a mortar; the liquid for granules was weighed with cylinder. The powder was moistened by gradually adding the liquid for granulates and mixing. Then the mass of moistened granules was rubbed through a wire-mesh screen with the mesh size of 1 mm and 0.5 mm. The obtained granules were thinly spread over paper on a metal tray and dried in a drying at temperature of 60°C for 2 hours.

Determination of the technological properties of substance and the granulates was carried out according to the methods presented in the State Pharmacopoeia of the Russian Federation XIV^{ed} [25]: the flowability, the tapped density (weight), the powder compression, the moisture content.

The Latin square method to select the required rational composition, as a mathematical planning, was used [26]. The factors, that will affect the adsorption activity, were: the aqueous factor of granulates liquids (factor A): A1 – agar-agar; A2 – sodium alginate; A3 – methyl cellulose; A4 – pectin; concentration of granulates liquid (factor B): B1 – 1%; B2 – 5%; moisture content of the obtained granulate (factor C): C1 – from 6% to 8%; C2 – over 8%, granulate particle size (factor D): D1 – 1 mm; D2 – 0.5 mm, filler in the granutable mixture (factor E): E1 – without a filler; E2 – with the filler (lactose).

The sorption activity is determined by the ability to bind a substance and methylene blue by the method of V.I. Reshetnikov [27]. About 0.2 g of polysaccharides or granulates (accurately weighed) were placed in a 250 ml conical flask, 50 ml of 0.15% methylene blue solution was added, and mixed in a laboratory shaker with 140 vibrations per minute for 1 hour. The separation of the ratio solution after sorption was carried out by during centrifugation at 8000 rpm. One milliliter of the supernatant was placed into a 500 ml capacity measuring flask and made up to the mark by purified water. Next, the optical density on the SP 2000 spectrophotometer at 664 nm in a cuvette with a 10 mm layer thickness was measured. Purified water was used as a comparator solution. The index of sorption activity was calculated according to formula:

$$X = \frac{(A_0 - A) \times a \times 50}{A_0 \times b \times (1 - 0,01 \times W)},$$

where:

A_0 – optical density of reference standard methylene blue;

A – optical density of test solution;

a – the actual concentration of reference standard methylene blue, mg/ml;

b – the weighment of substance, g;

50 – the volume of reference standard methylene blue, ml;

W – the moisture content of substance, %.

The disintegration test was made on "Rotating Basket" ERWEKA ZT 223 apparatus by the basket method [24]. The eighteen samples of filled capsules were placed in each of the 6 tubes. Then the basket was put down into a vessel with water temperature of 36°C, then turned on the apparatus and noted the time. After 30 minutes, the basket was removed and the conditions of the capsules was examined. All samples must disintegrate. Not less than 16 out of 18 samples must disintegrate completely.

For part samples, the disc-based method was used – in these cases, a disc was placed on each of the six samples before determination was started. The disk method is identical the basket method.

The sample was considered completely disintegrated when there is no residue or the residue was a soft mass that collapsed with a light touch of a glass rod, apart from fragments of the insoluble capsule shell, which are on the reticle or adhered to the lower surface of the disk.

Seven replicate measurements were made. statistical manipulation was carried out according to GPM.1.1.0013.15 SP XIV[ed] "Statistics of the chemical experiment results. The *p*-value and Student *t*-test were calculated by the Microsoft Excel.

RESULTS AND DISCUSSION

The technological properties were researched and were obtained the following characteristics for assess the possibility of uses a solid dosage form (capsules), the result presented in Table 1.

The most important value of powder encapsulation process is flowability, because hard gelatin capsules filling and dosing by volumetric method. As shown in Table 1, the substance of WSPC doesn't have the capability for free poured out of the funnel under the gravity force (flowability). The vibration didn't have significance improvement of the score (Carr index = 22.78%, Hausner's ratio = 1.30). The value of the angle with natural repose of the substance also has turned out to be higher than recommended value (36–45°). It was found that the values of determined tapped density are lower than his specification limits. In this way, conducted researches has shown that the technological characteristics of substance WSPC are unsatisfactory: flowability (with and without vibration), angle of repose and tapped density. In this regard, in the future development of composition and technology an encapsulated dosage form with substance WSPC will be necessary to improve technological properties of this substance by adding excipients and using different technological methods (for example, wet granulation).

It is known that the transformation a powdery material into particles of a certain size (granulation) has a positive effect on the flowability. The classical method of granulation is compacting of powder particles by a binder (agar-agar, sodium alginate, methyl cellulose, pectin) and the next drying (wet granulation). In order to improve the flowability of the WSPC a row of granules in combinations with various excipients were obtained.

It is also known that the one of most important quality attribute of dry medicines from medicinal plant raw materials is moisture content. This quality attribute has a significant effect on different indicators: the stability of the drug, quantitatation, technological properties, such as flowability and tapped density, adsorption activity. A significant quantity of moisture can significantly reduce adsorption activity on surface and inside the raw material.

Therefore, for each granules, were researched technological properties, also the moisture content and adsorption activity were determined.

The results of research moisture content and adsorption activity of the granules are presents in Table 2.

As a result, it was found that the granulation by the wet granulation led to an increase of adsorption activity of most selected compositions, with the exception of compositions No.1 and No.3. The greatest adsorption activity from the granulates compositions No.1–8 was shown by the granulates No.8. The results of Table 2 shown that the granulates moisture content was confirmed on adsorption activity. The moisture content of the granulates increases that leads adsorption activity decreases, which can be explained by a decrease in the sorption capacity of granulates due to binding with water molecules. Considering this, the next granulates were obtained with a moisture content not more than 8% (factor C).

A direct dependence of the adsorption activity from the concentration of granulation liquid (factor B) was also revealed (the exception is granules No 4 and No 5). According to the results in Table 2, granulation liquids for next research were selected: sodium alginate, methyl cellulose and pectin at 5 percent concentration. Compositions granulated with agar-agar were excluded from next researches due to its low adsorption activity (1 percent solution) and a difficult granulation process (5 percent solution).

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In order for next research to granutable WSPC is added lactose, for optimally adjustment of technological properties. Lactose is a known such as excipient of the disaccharide group, widely used in solid dosage form technology. After preliminary studies of the technological properties of granutable WSPC and lactose in different correlations, an optimal ratio of 9:1 (WSPC/lactose) was proposed [27].

The technological properties of compositions and their adsorption activity were researched.

The influence of factors A (type of granulation liquid), B (concentration of granulation liquid), D (particle size of granules), E (presence or absence of lactose) on the adsorption activity of granulates was studied. The results of adsorption activity were rated with the initial WSPC substance and activated carbon powder. The activated carbon was used as a reference drug as a well-known drug with a broad-spectrum of action.

The results of the researches of granulation liquid, moisture content and particle size's influence on the technological properties of WSPC granulates and lactose are presented in Table 3.

According to the results of the researches, it was found that all granules (No.9–No.11) have next technological properties: satisfactory flowability with an acceptable angle of repose (Hausner's ratio 1.09–1.19; Carr index is 8.94–16.49 percent) and a fairly high tapped density.

After analyzing the results of Table 3, was noticed: a decrease of the particle size to 0.5 mm (No 10 and No 12) is led to insignificant changes in technological properties, in comparison with particle size granulates which has a of 1 mm (No.9, No.11, No.13) and had practically no effect in the initial technological properties of the WSPC substance. Thus, it was concluded that the particle size of granulates the in the range from 0.5 to 1.0 mm does not have significantly affect on technological properties of those granulates.

It was also found that the type of granulation liquid and the presence of lactose in granules doesn't change the technological properties of the test granulates (Table 4).

During the research of adsorption activity for test granulates (No.9 – No.11), some conclusions was established. Following from the results of Table 4, when lactose is added to the granutable mixture, the adsorption activity of the granulate increases, with the exception of granules of composition No.11, No.12. The change of the particle size didn't have any dependence reveal, since the adsorption activity of granulates No.13, No.14, No.11, No.12 decreases with decreasing particle size, and the adsorption activity of granulates No.9, No.10 increases with decreasing particles. The dependence an adsorption activity from particle size was established, for nextresearches.

For the development of a solid dosage form with adsorption activity, literature data was taken into account. It is known that there is a number of problems in the development of solid dosage forms with high adsorption activity. In case of using sorbents, there is no release and absorption into biological matrix of any active principle. Another singularity of sorbents is the possibility of partial or complete inactivation of pharmacologically active ingredient during the preparation of a dosage form, depending on a number of factors: heating or contact with excipients and solvents, physical impacts, dehydration, creation of protective coverings, etc. [28]. Considering the forgoing, hard gelatin capsules were chosen for the development of a solid dosage form. The advantage of capsules over other solid dosage forms (for example, tablets) is the reduction of technological stages, because there is no tabletizing (pressing) stage, where physical impacts are required, often leading to a decrease of the substance adsorption activity.

For filling, we chose STANDARD gelatin capsules of typical size 0 with a filling volume of 0.68 ml [29]. The large typical size of the capsules was chosen with the concideration of the expected dosage of the WSPC substance.

The capsules were filled with granulates No.9, No.10, No.11, No.12, No.13, No.14 by volumetric method. The disintegration of the obtained dosage form was determined according to the State Pharmacopoeia [25]. The test was carried out with the "Rotating Basket" apparatus with and without discs. The results are presented in Table 5.

In the course of research, it was found that factors A (granulation liquid) and D (granulate particle size) have a direct effect on the capsule's ability to disintegrate in a defined time (not more than 30 minutes). From the results presented in table 5, it can be seen that the capsules filled with granulate No.12 have disintegrated in 25 minutes according to the method without discs, capsules with granulates No.13, No.14, No.11, No.9, No.10 – in more than 30 minutes. To improve the indicators of the "Disintegration" test the method with discs was applied. According to the method with discs, the capsules with granulate № 12 have disintegrated in 18 minutes, with granulate № 10 – in 27 minutes, which corresponds to the defined time.

In further researches, it is possible to use the method with discs. Thus, the best results of the Disintegration test were obtained using a granulation liquid – methyl cellulose solution with a granulate particle size of 0.5 mm.

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Table 1 – The technological properties of the WSPC

Object	Moisture content, %	Flowability, g/s		Angle of repose, °	Tapped density, kg/m³		Hausner's ratio	Carr index
		Without vibration	With vibration		Bulk density (untapped)	Tapped density (tapped)		
WSPC	8.2	None	2.74±0.2	51	569.69±3.56	737.83±2.26*	1.30	22.78
Reference values	–	3.0–6.5		36–45	>600	> 600	1.19–1.25	16–20
Compliance with reference values	–	Not compliant	Not compliant	Not compliant	Not compliant	Compliant	Not compliant	Not compliant

Note: * p≥0.001 – compared to the sample before compaction

Table 2 – The influence of granulation liquid concentration and moisture content on the adsorption activity of granulates

No.	Object	Moisture content, %	Adsorption activity, mg/g $\bar{x}\pm\bar{\Delta x}$
1	WSPC	8.2	180.03 ± 0.88
2	Activated carbon powder	7.8	232.64±0.51
3	No.1 – $A_1B_1C_1D_1E_1$	8.5	112.28±0.54
4	No.2 – $A_1B_2C_1D_1E_1$	9.7	195.91±0.85**
5	No.3 – $A_1B_2C_2D_1E_1$	18.3	94.13±0.52
6	No.4 – $A_2B_1C_1D_1E_1$	7.3	221.91±0.51**
7	No.5 – $A_2B_2C_1D_1E_1$	7.5	192,25±0.91**
8	No.6 – $A_3B_1C_1D_1E_1$	6.6	191.41±0.41**
9	No.7 – $A_3B_2C_1D_1E_1$	7.2	215.44±0.76**
10	No.8 – $A_4B_2C_1D_1E_1$	7.7	266.95±0.83*

Note: * – accurate with a confidence range p≥0.001 compared with activated carbon powder; ** – p≥0.001 compared with WSPC

Table 3 – The influence of granulation liquid, moisture content and particle size on the technological properties of WSPC granulates with lactose (9:1)

No.	Object	Particle size, mm	Moisture content, %	Flowability with vibration, g/s	Angle of repose, °	Tapped density, kg/m³		Hausner's ratio	Carr index, %
						Bulk density (untapped)	Tapped density		
1	WSPC	1.0	8.2	2.74±0.2	51	569.69±3,56	737.83±2.26	1.30	22.78
2	No.9 – $A_4B_2C_1D_1E_2$	1.0	7.3	8.47±0.82*	35	537.72±2,89	590.54±2.34	1.09	8.94
3	No.10 – $A_4B_2C_1D_2E_2$	0.5	7.3	7.44±0.39*	40	575.16±3.04***	662.30±2.58***	1.15	13.16
4	No.11 – $A_3B_2C_1D_1E_2$	1.0	6.8	6.02±0.30*	60	458.45±3.25	529.80±2.19	1.16	13.47
5	No.12 – $A_3B_2C_1D_2E_2$	0.5	7.4	5.03±0.79**	40	488.24±2.97***	578.14±2.47***	1.18	15.50
6	No.13 – $A_2B_2C_1D_1E_2$	1.0	6	7.16±0.54*	38	481.60±3,16	542.38±2.32	1.12	11.20
7	No.14 – $A_2B_2C_1D_2E_2$	0.5	6	6.10±0.42*	40	511.50±3.05***	612.54±2,28***	1.19	16.49
8	Reference values	–	–	3.0–6.5	36–45	> 600	> 600	1.19–1.25	16–20

Note:* – accurate with a confidence range p≥0.001 compared with WSPC; ** – p≥0.05 compared with WSPC; *** – p≥0.001 compared with the particle size of 1 mm

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Table 4 – The influence of granulation liquid, moisture content and particle size on the adsorption activity of WSPC granulates with lactose (9:1)

No.	Object	Partical size, mm	Moisture content, %	Adsorption activity, mg/g $(\bar{x}\pm\bar{\Delta x})$
1	WSPC	1	8.2	180.03±0.88
2	Activated carbon powder	1	7.8	232.64±3.12
3	No.9 – $A_4B_2C_1D_1E_2$	1	7.3	215.73±0.43*
4	No.10 – $A_4B_2C_1D_2E_2$	05	7.3	271.54±0.10**
5	No.11 – $A_3B_2C_1D_1E_2$	1	6.8	150.80±0.84
6	No.12 – $A_3B_2C_1D_2E_2$	05	7.4	115.66±0.74**
7	No.13 – $A_2B_2C_1D_1E_2$	1	6.0	250.58±0.95*/***
8	No.14 – $A_2B_2C_1D_2E_2$	05	6.0	232.70±0.94*

Note: * – accurate with a confidence interval p≥0.001 compared with WSPC; ** – p≥0.001 compared with the particle size of 1 mm; *** – p≥0.001 compared with the particle size of 0.5 mm

Table 5 – The results of the "Disintegration" test of capsules filled with WSPC granulates with lactose (9:1)

No.	Object	Particle size, mm	Disintegration, min	
			Without disks	With disks
1	No.13 – $A_2B_2C_1D_1E_2$	1	> 30	–
2	No.14 – $A_2B_2C_1D_2E_2$	0.5	> 30	–
3	No.11 – $A_3B_2C_1D_1E_2$	1	> 30	–
4	No.12 – $A_3B_2C_1D_2E_2$	0.5	25	18
5	No.9 – $A_4B_2C_1D_1E_2$	1	> 30	–
6	No.10 – $A_4B_2C_1D_2E_2$	0.5	> 30	27

CONCLUSION

The WSPC substance of spruce cones has been obtained and technological properties (flowability with and without vibration, angle of repose, bulk and tapped density, Carr index and Hausner's ratio) have been determined in laboratory conditions.

The granules of spruce cones WSPC and compositions with lactose in ratio (9:1) have been obtained under laboratory conditions with granulation liquids: agar-agar, sodium alginate, methyl cellulose, pectin in various concentrations with particle sizes of 1 mm and 0.5 mm and moisture content from 6 percent and higher. A total amount of 14 granules have been obtained. The technological properties (flowability, angle of repose, bulk and tapped density, Carr index and Hausner's ratio), adsorption activity of the granules and the dependence of these indicators from the factors of the granulation process have been determined.

The capsules with granules of a composition WSPC spruce cones with lactose (9:1) have been obtained and the «Disintegration test» of dosage form was carried out in laboratory conditions.

According to the disc – method, capsules with granules of two compositions have been shown a satisfactory result.

The studies of factor A have shown that pectin has become a promising granulating liquid

Thus, after researches and validation of results, the optimal composition for encapsulation to hard gelatin capsules STANDARD of typical size 0 has been chosen: the spruce cones WSPC substance and lactose in a ratio at 9:1; as a binding liquid was used 5% pectin with moisture content of no higher than 8% and a particle size of 0.5 mm, which can be used for next researches in the development of a new herbal medicine with high adsorption activity.

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CONFLICT OF INTERESTS

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AUTHOR'S CONTRIBUTIONS

All authors have contributed to the research equally.

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RESEARCH ARTICLE

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STUDY OF THE STABILITY OF THE SUBSTANCE 3-[2-(4-PHENYL-1-PIPERAZINO)-2-OXOETHYL]QUINAZOLINE-4(3*H*)-ONE UNDER STRESSFUL CONDITIONS

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The aim of the research was to study the stability of a new pharmaceutical substance 3-[2-(4-phenyl-1-piperazino)-2-oxoethyl]quinazoline-4(3*H*)-one under stress conditions.

Materials and methods. The study was conducted in accordance with the recommendations of the ICH guidelines. The object of the study was a previously unknown derivative of quinazoline-4(3*H*)-one: 3-[2-(4-phenyl-1-piperazino)-2-oxoethyl] quinazoline-4(3*H*)-one synthesized in Volgograd state medical university. The following laboratory equipment was used: HPLC chromatograph, HPLC-MS, centrifuge, electronic scales, pH meter, thermostat, laboratory filters.

The computational experiment was conducted on a computer with an Intel Xeon E3-1230 processor using the programs ORCA 4.1. and GROMACS 2019.

Results. The influence of the most unfavorable environmental factors, such as high temperature, light, oxidants, hydrolysis in acidic and alkaline environments, affect the stability of the test substance. The results of the computer-based stability prediction were confirmed by HPLC and HPLC-MS, and the degradation products of the substance under stressful conditions were determined. The conducted studies showed that the test substance is stable to UV radiation at the wavelength of 365 nm, at the elevated temperature (80°C), to the action of oxidants. But it is unstable to hydrolysis: in an alkaline medium of sodium hydroxide 1M, a break in the amide group occurs with the formation of 2-(4-oxoquinazoline-3-yl)acetic acid and 1-phenylpiperazine. And in an acidic environment, hydrochloric acid 1M is also destroyed, but it is significantly reduced, presumably due to the protonation and stabilization of tertiary nitrogen atoms in the molecule.

Conclusion. The conducted research makes it possible to conclude that the test substance 3-[2-(4-phenyl-1-of piperazino)-2-oxoethyl]quinazoline-4(3*H*)-one is stable to aggressive environmental factors, with the exception of hydrolysis in an alkaline environment that will be further considered in the preparation of regulatory documents for this pharmaceutical substance.

Keywords: quinazoline-4(3*H*)-one derivative, pharmaceutical substance, stress testing, stress test modeling, thermolysis, photolysis, hydrolysis, high-performance liquid chromatography, photostability, thermalstability, GROMACS, ORCA

Abbreviations: State Pharmacopoeia (SF), high performance liquid chromatography (HPLC), high performance liquid chromatography with mass spectrometry (HPLC-MS), ultraviolet radiation (UV radiation).

ИЗУЧЕНИЕ СТАБИЛЬНОСТИ СУБСТАНЦИИ 3-[2-(4-ФЕНИЛ-1-ПИПЕРАЗИНО)-2-ОКСОЭТИЛ]ХИНАЗОЛИН-4(3*H*)-ОНА В СТРЕССОВЫХ УСЛОВИЯХ

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Цель работы – изучение стабильности новой фармацевтической субстанции 3-[2-(4-фенил-1-пиперазино)-2-оксоэтил]хиназолин-4(3*H*)-она под воздействием стресс-условий.
Материалы и методы. Исследование выполнено в соответствии с рекомендациями руководства ICH. Объектом исследования было ранее не изученное производное хиназолин-4(3*H*)-она: 3-[2-(4-фенил-1-пиперазино)-2-оксоэтил] хиназолин-4(3*H*)-он, синтезированное в Волгоградском государственном медицинском университете. Было использовано лабораторное оборудование: ВЭЖХ-хроматограф, ВЭЖХ-МС, центрифуга, электронные весы, pH-метр, термостат, лабораторные фильтры. Вычислительный эксперимент проводился на компьютере с процессором Intel Xeon E3-1230 с использованием программ ORCA 4.1. и GROMACS 2019.
Результаты. Изучено и определено влияние неблагоприятных факторов внешней среды, таких как: высокая температура, свет, действие окислителей, гидролиза в кислой и щелочной среде на стабильность исследуемого вещества. Результаты компьютерного прогнозирования стабильности были подтверждены с помощью ВЭЖХ и ВЭЖХ-МС, а также определены продукты деструкции субстанции в стрессовых условиях. Проведенные исследования показали, что исследуемое вещество стабильно к воздействию УФ-облучения при длине волны 365 нм, повышенной температуры (80°С), действию окислителей и нестабильно к гидролизу: в щелочной среде натрия гидроксида 1М происходит разрыв по амидной группе с образованием 2-(4-оксохиназолин-3-ил)уксусной кислоты и 1-фенилпиперазина; а в кислой среде кислоты хлористоводородной 1М также происходит деструкция, но она значительно снижается, предположительно, за счет протонирования и стабилизации третичных атомов азота в молекуле.
Заключение. Проведенные исследования позволяют сделать вывод о том, что исследуемая субстанция 3-[2-(4-фенил-1-пиперазино)-2-оксоэтил]хиназолин-4(3*H*)-он стабильна к агрессивным факторам внешней среды, за исключением гидролиза в щелочной среде, что в дальнейшем будет учитываться при составлении нормативной документации этой фармацевтической субстанции.
Ключевые слова: производное хиназолин-4(3*H*)-она, фармацевтическая субстанция, стресс-тестирование, моделирование стресс-тестов, термолиз, фотолиз, гидролиз, высокоэффективная жидкостная хроматография, фотостабильность, термостабильность, GROMACS, ORCA
Список сокращений: Государственная фармакопея (ГФ), высокоэффективная жидкостная хроматография (ВЭЖХ), высокоэффективная жидкостная хроматография с масс-спектрометрией (ВЭЖХ-МС), ультрафиолетовое излучение (УФ-излучение).

INTRODUCTION

Stress tests of drugs are artificially recreated unfavorable environmental conditions in order to establish degradation products of medicinal substances.

Various factors that accelerate the rate of chemical reactions are used: high temperatures, light (in the ultraviolet and visible regions of the spectrum), high humidity, changes in the acidity/alkalinity of the medium, exposure to various oxidizing agents and other air components. The study and analysis of the degradation products and half-life of drugs during stress testing should be taken into account when developing methods for determining foreign impurities, quantitative determination, production, storage, transportation, and other regulatory documents being developed for a particular drug [1].

The results of stress tests also allow us to assess the impact of short-term deviations from the declared storage conditions and various ways of destruction of the studied substances, to determine the most unfavorable environmental factor, to which the test substance is more sensitive. Stability data for the resistance of drug substances to air components, also allow a more rational approach to the choice of its primary packaging [2–4].

In particular, the GPM.1.1.0009.18 "Stability and shelf lives of medicinal products" of the State Pharmacopoeia of the Russian Federation (XIVth edition) indicates that the study of drug stability should include stress, accelerated and long-term trials; special attention should be also paid to the development of a program for studying the stability of new drugs. For new pharmaceutical substances, the SP of the XIVth edition recommends conducting stress tests on a compulsory basis [1].

THE AIM of the research was to study the stability of a new pharmaceutical substance 3-[2-(4-phenyl-1-piperazino)-2-oxoethyl]quinazoline-4(3H)-one under stress conditions.

MATERIALS AND METHODS

The work was performed in accordance with the recommendations of the ICH manual and the SP (XIVth edition) [1, 5].

The object of the study was one series of the substance 3-[2-(4-phenyl-1-piperazino)-2-oxoethyl]quinazoline-4(3*H*)-one (laboratory code: VA-10-21) synthesized in Volgograd State Medical University. The structural formula is shown in Fig. 1.

HPLC studies were performed using the UltiMate 3000 system ("Dionex", CCA) with a special spectrophotometric detector with an operating wavelength range from 190 to 900 nm. The data collection and processing were performed using a chromatographic data collection and processing system Chromeleon, version 7 ("Dionex", USA).

It is shown that when using acetonitrile and 0.05 M phosphoric acid as the mobile phase, pH=3.5 in the ratio of 25:750, it is possible to provide optimal chromatography conditions. The experimental parameters were as follows: a stainless steel chromatographic column 150×4.6 mm size LunaC 18 with a particle size of 5 microns, a flow rate of 1 ml/min, a column temperature of 25°C, detection at 226 nm, a sample volume of 20 µl, and an analysis time of 40 minutes. HPLC-MS analysis was performed on a Bruker mass spectrometer (Germany) by electrospray ionization in the "Positive" mode. The temperature control of the samples was carried out in the TS-1/20 SPU thermostat (Russia).

As a mobile phase, acetonitrile and a 0.05 M phosphoric acid solution in the ratio of 25:75, with a pH value of 3.5±0.05, were selected. An aqueous solution of phosphoric acid was adjusted to the specified pH value by adding a triethylamine solution. The pH was controlled potentiometrically. The samples were centrifuged before the HPLC analysis using a laboratory centrifuge with Sigma 2–16P accessories (Germany). All the sample solutions had been centrifuged at 8000 min^{-1} for 3 minutes before being placed in the device. The tested solutions had been pre-filtered through Nylon Membrane, 0.2 µm 25 mm Syringe Filters (USA). The samples were weighed on laboratory electronic scales LV 210-A (Russia). The pH value of the solutions was measured on the C axis and using the pH meter pH-150MI (Russia). All the solvents and reagents used in the study, met the requirements of the GPM.1.3.0001.15 GP (XIVth ed.)

Computer modeling of stress tests, was performed on a workstation with an Intel Xeon E3-1230 processor and 16 GB of RAM. The optimization of the VMA-10-21 substance geometry was performed in the ORCA 4.1 program by the density functional theory (UB3LYP) method using the 3–21G* basis. To study the structure of the solid aggregate state of the substance under study, molecular dynamics was modeled by the method of molecular mechanics in the CHARMM36 force field using the GROMACS 2019 program [6, 7]. The Internet service SwissParam was used for parameterization of the molecule and the studied substance [8]. 10 randomized molecules of the test substance were included in the simulated system. Next, the geometry was optimized using the gradient method.

To study the effect of temperature on the stability of the VMA-10-21 substance, a precisely weighed quantity (about 50 mg) was placed in a conical flask with a capacity of 100 ml, 50 ml of 95% ethyl alcohol was added, and placed on an ultrasonic bath for 15 minutes until the substance was completely dissolved. The resulting solution was boiled in the flask with a return refrigerator in a thermostatically controlled water bath (the temperature of 80^0C) for 3 hours, taking the samples every 45 minutes. In order to search for the solid state structure, molecular dynamics was simulated using simulated annealing with a temperature decrease from 1000 K to 273 K for 200 ns with the use of a thermostat at Nose-Hoover [9]. Further on, in order to study the stability of the studied substance to thermolysis, the molecular dynamics of a system of four molecules was modeled using the unlimited Hartree-Fock method with a base set of 3–21G* for 5000 fs with 1 fs step in the ORCA 4.1 program [10]. The temperature control was performed by scaling velocities with a temperature of 400 K and spherical boundary conditions of constant volume.

The effect of UV light on the stability of the VMA-10-21 substance was studied by the following method: a precisely weighed quantity (about 50 mg) was placed in a conical quartz glass flask with a capacity of 100 ml, 50 ml of 95% ethyl alcohol was added, and placed on the ultrasound bath for 15 minutes until the substance was completely dissolved. The resulting solution was exposed to UV light at a wavelength of 365 nm, and the radiation source fully met the ICH requirements. The sampling was performed every 3 hours. Computer modeling of the effect of light was performed using the unlimited Hartree-Fock method, a set of basic functions 3–21G*, a temperature of 400 K, a simulation step of 1 fs, a simulation duration of 5 ps, and a molecule multiplicity of 3.

To study the effect of acids on the stability of the substance, a precisely weighed quantity (about 50 mg) was placed in a conical flask with a capacity of 100 ml, 40 ml of 95% ethyl alcohol was added, and placed on an ultrasonic bath for 15 minutes until the substance was completely dissolved. After that, 5 ml of a 1 M hydrochloric acid solution was added. The resulting solution was boiled in the flask with a return refrigerator in a thermostatically controlled water bath (the temperature of 80°C) within 45 minutes. On completion the time, the solution was cooled down and 5 ml of a 1 M sodium hydroxide solution was added.

The influence of alkalies on the stability of the substance was studied by the following method: a precisely weighed quantity (about 50 mg) was placed in a conical flask with a capacity of 100 ml, 40 ml of 95% ethyl alcohol was added, and placed on an ultrasonic bath for 15 minutes until the substance was completely dissolved. After that, 5 ml of a 1 M sodium hydroxide solution was added. The resulting solution was boiled in the flask with a return refrigerator in a thermostatically controlled water bath (the temperature of 80°C) within 45 minutes. On completion the time, the solution was cooled down and 5 ml of a 1 M hydrochloric acid solution was added.

In order to calculate the most probable hydrolysis products in acid and alkaline media, vibration analysis was performed, and thermodynamic characteristics using the density functional theory (UB3LYP) method and the 6-311G** basis set in the ORCA 4.1 program, were calculated. During acid hydrolysis, the reaction products calculated with protonated tertiary nitrogens. Since the equivalence factor of 0.5 by titrimetric methods had been determined before, for the molecule VMA-10-21, the Gibbs energies of two simultaneously protonated nitrogen atoms of the VMA-10-21 molecule were calculated for the VMA-10-21 molecule in all possible combinations. During alkaline hydrolysis, the reaction products were presented in the form of carboxylic acid salts – COONa. The Gibbs energy of hydrolysis reactions (ΔG_r) was calculated from the difference between the sum of the Gibbs energies of the reaction products (ΔG_{prod}) and the initial compounds (ΔG_{reac}) in accordance with the Hess' law: $\Delta G_r = \sum \Delta G_{prod} - \sum \Delta G_{reac.}$

The thermodynamic characteristics of water, OH^- ions and the analyzed substance VMA-10-21 without protonation, were also determined. The calculation was performed at a temperature of 310K.

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To study the effect of the oxidation process on the stability of the substance, a precisely weighed quantity (about 50 mg) was placed in a conical flask with a capacity of 100 ml, 45 ml of 95% ethyl alcohol was added, and placed on an ultrasonic bath for 15 minutes until the substance was completely dissolved. Then 5 ml of 3% hydrogen peroxide solution was added. The resulting solution was boiled in the flask with a return refrigerator in a thermostatically controlled water bath (the temperature of 80°C) within 45 minutes. On completion the time, the solution was cooled down and analyzed.

RESULTS AND DISCUSSION

The chemical stability of pharmaceutical molecules is of a serious concern, as it affects the safety and effectiveness of the drug. The FDA and ICH guidelines establish requirements to these tests to determine the effect of various environmental factors on the quality of a pharmaceutical substance due to the passage of time. Knowing the stability of the molecule, helps in choosing the right composition and packaging, as well as ensuring proper storage conditions and shelf life, which is important for regulatory documentation. Artificial degradation involves the destruction of drugs and their semi products under more stringent conditions than accelerated tests, which makes it possible to study the stability of the molecule more fully, and determine the most probable degradation ways [11].

Information on the stability of molecules, helps in the manufacture of dosage forms and determination of storage conditions, so it is rational to start degradation studies at an early stage of drug development [12].

The question of whether the degree of degradation of a substance is sufficient, has been a topic of a lot of discussions among pharmaceutical scientists. Decomposition of a drug substance between 5% and 20%, was considered reasonable for chromatographic analyses [13, 14]. Some pharmaceutical scientists believe that 10% decomposition is optimal for the substances with a low molecular weight [15]. It is not necessary that a forced decomposition should lead to a complete decomposition of a substance. The study can be discontinued if a pharmaceutical substance or a drug form is not decomposed after the exposure to stressful conditions [16]. This indicates the stability of the tested molecule. An excessive exposure to stress tests on the sample, may lead to the formation of secondary decomposition products that will not be identified in storage stability studies, and insufficient exposure may not produce decomposition products [17].

Stability study of VMA-10-21 when exposed to high temperatures (thermolysis)

When modeling molecular dynamics in order to search for the structure of the solid state of the substance under study, the VMA-10-21 substance molecules were arranged systematically, forming elements of the crystal lattice (Table 1).

These data were used as the initial location of the molecules for further calculations by the molecular dynamics method for a system of 4 molecules of the substance under study.

Table 2 shows the state of the system in the process of molecular dynamics simulation by the Hartree-Fock method with a basis set of 3-21G**.

As Table 2 shows, according to the results of computer modeling, the chemical structure of the VMA-10-21 molecule did not change, which makes it possible to assume the stability of the molecule when exposed to elevated temperatures.

ICH recommends performing thermal treatment at temperatures of 60–80°C, i.e. at higher temperatures than in the accelerated tests [18]. The maximum recommended temperature was selected.

Fig. 2 shows a chromatogram of the VMA-10-21 alcohol solution before stress testing.

Fig. 3 shows a chromatogram of the VMA-10-21 alcohol solution after stress testing.

The data on the stability of VMA-10-21 for a time of 0–180 minutes, are presented in Table 3.

As follows from the presented data, the substance VMA-10-21 is almost completely resistant to high temperatures. The results of computer modeling coincided with the practical data.

Study of the VMA-10-21 stability under UV light (photolysis)

Photolytic degradation is believed to be caused by wavelengths in the range of 300–800 nm [19]. Photostability studies are an important aspect of drug studies on substance stability, since photolysis can cause photooxidation by a free-radical mechanism, so we chose the most “hard” UV at a wavelength of 365 nm. Fig. 4 shows the obtained chromatogram after 24 hours of UV exposure.

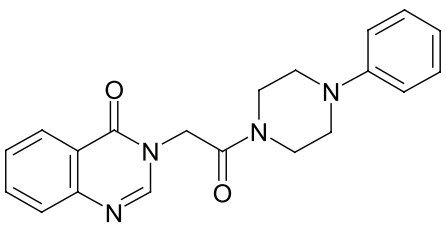

Figure 1 – Structural formula of substance VMA-10-21

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Table 1 – State of the system in the process of molecular dynamics simulation of 10 molecules by the method of molecular mechanics

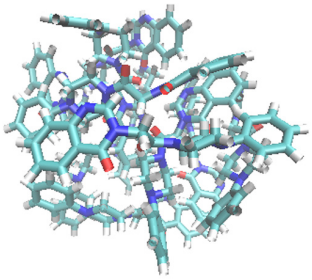
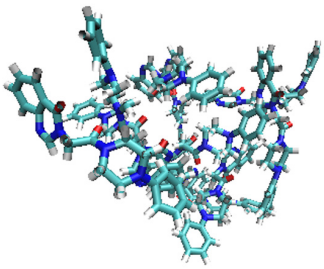
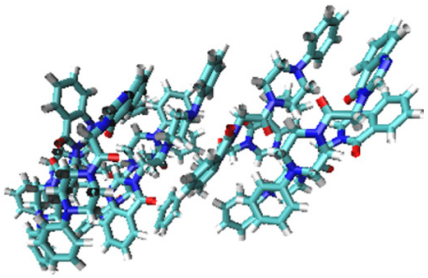
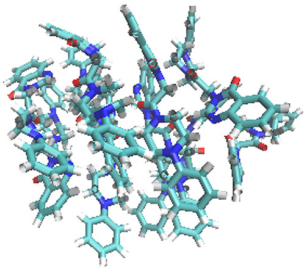
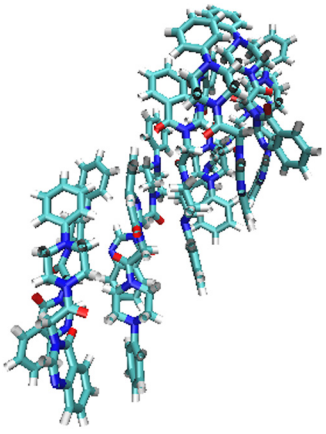
Time, ns	System image
0	
50	
100	
150	
200	

Table 2 – State of the system in the process of molecular dynamics simulation 0–5000 fs

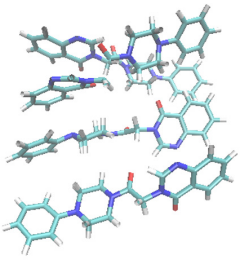
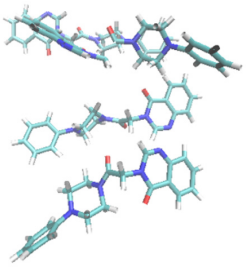
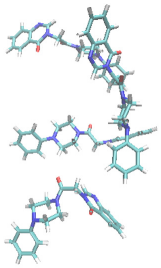
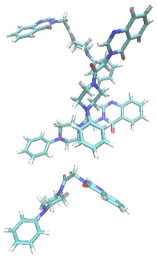
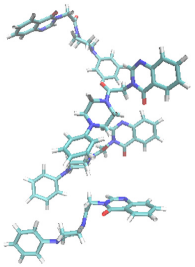
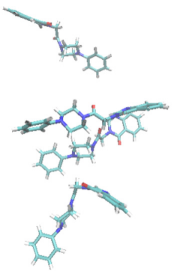
Time, ns	System image
0	
1000	
2000	
3000	
4000	
5000	

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Table 3 – Results of the influence of the stress test (elevated temperature) on the VMA-10-21 stability

Stress test (heating at 80°C)	Contents of VMA-10-21	Degradation percentage
VMA-10-21 without heating	99.68%	–
VMA-10-21 after 45 minutes	99.60%	0.08%
VMA-10-21 after 90 minutes	99.58%	0.1%
VMA-10-21 after 135 minutes	99.54%	0.14%
VMA-10-21 after 180 minutes	99.50%	0.18%

Table 4 – Results of the impact of the stress test (UV light) on the VMA-10-21 stability

Stress test (UV light 365 nm)	Contents of VMA-10-21	Percentage of degradation
VMA-10-21 without UV irradiation	99.68%	–
VMA-10-21 after 3 hours	99.66%	–
VMA-10-21 after 6 hours	99.65%	–
VMA-10-21 after 9 hours	99.61%	–
VMA-10-21 after 12 hours	99.60%	–
VMA-10-21 after 15 hours	99.60%	–
VMA-10-21 after 18 hours	99.59%	–
VMA-10-21 after 21 hours	99.58%	–
VMA-10-21 after 24 hours	99.56%	–

Table 5 – Results of vibrational analysis of all possible combinations for the doubly protonated VMA-10-21 molecule

Molecule	Enthalpy, a.u.	Entropy, cal/mol*K	Enthalpy, kJ/mol	Entropy, kJ/mol*K	Gibbs energy, kJ/mol
VMA-10-21	–1143.540907	35.914	–3002366.237	0.150364735	–3002366.237
I + III	–1144.153274	35.731	–3003974.007	0.149598551	–3003974.007
I + IV	–1144.189237	35.721	–3004068.428	0.149556683	–3004068.428
II+ III	–1144.061293	35.952	–3003300.432	0.150272626	–3003300.432
II + IV	–1144.12641	35.742	–3003732.511	0.150523834	–3003732.511
III + IV	–1144.119529	35.663	–3003903.475	0.149644606	–3003903.475

Table 6 – Results of the conducted vibrational analysis and calculated thermodynamic characteristics and hydrolysis products

The molecule	Enthalpy, a.u.	Entropy, cal/mol*K	Enthalpy, kJ/mol	Entropy, kJ/mol*K	Gibbs energy, kJ/mol
Product 1	–687.469271	106.648	–1805019.32	0.44651	–1805157.74
Product 1 (– COONa)	–686.943269	114.886	–1803638.25	0.48100	–1803787.36
Product 2	–532.492603	93.828	–1398112.58	0.39284	–1398234.36
Product 3	–498.863275	92.872	–1309815.41	0.38884	–1309935.95
Product 4	–721.096936	99.055	–1893312.12	0.41472	–1893440.68
Product 4 (– COONa)	–720.566026	96.379	–1891918.16	0.40352	–1892043.25
OH^-	–75.751778	41.417	–198893.87	0.1734	–198947.62
Water	–76.422293	46.469	–200654.37	0.19456	–200714.68

The data on the stability of the VMA-10-21 when exposed to UV for 0–24 hours, are presented in Table 4.

As follows from the presented data, the substance VMA-10-21 is resistant to UV light, which corresponds to the results of modeling.

Study of VMA-10-21 stability under the influence of acids and bases (hydrolysis)

Hydrolysis is one of the most common chemical decomposition reactions over a wide pH range. It is assumed that high humidity, as one of the parameters of the water content in the ambient air, is a potential threat to the implementation of hydrolytic cleavage reactions. In the study of hydrolysis reactions, the influence of acidic and basic conditions on it is considered as a stress factor for the primary destruction of drugs.

For acid hydrolysis, hydrochloric or sulfuric acids with concentrations of 0.1–1M are used, and for alkaline hydrolysis – sodium or potassium hydroxides with concentrations of 0.1–1M are used [17, 20]. In our study, we selected hydrochloric acid and sodium hydroxide in the maximum allowable concentrations of 1M.

Theoretically, several hydrolysis ways are possible for the VMA-10-21 substance under study. They are shown in Fig. 5.

By the first hydrolysis way, 2-(4-oxoquinazoline-3-yl) acetic acid (product 1) and 1-phenylpiperazine (product 2) are produced, and by the second way, these are 3-methylquinazoline-4-one (product 3) and 4-phenylpiperazine-1-carboxylic acid (product 4).

The results of the vibrational analysis on the search for the most possible tertiary nitrogen atoms involved in protonation, are presented in Table 5.

The protonated nitrogen atom in position 1 of the quinazoline nucleus was denoted as I, in position 3 of the quinazoline nucleus – as II, 1-piperazino – as III, 4-phenyl – as IV. As the results of Table 5 show, protonation of nitrogen atoms in the VMA-10-21 molecule is most probable in positions I and IV. This Gibbs energy was used by the authors in the vibrational analysis for VMA-10-21 in acid hydrolysis reactions.

Table 6 shows the obtained vibrational analysis data for hydrolysis products.

The calculated Gibbs energies of the reactions are presented in Table 7.

As the results obtained show, hydrolysis in an alkaline environment is the most probable for the first hydrolysis way, since the Gibbs energy value is lower for this reaction. It can also be seen that in the acidic environment, due to the higher Gibbs energy of the protonated I + II molecule, the Gibbs energy of hydrolysis in the acidic reaction has increased, so hydrolysis in the acidic environment can be assumed to be less pronounced.

The chromatogram obtained as a result of the interaction of VMA-10-21 solution with hydrochloric acid, is shown in Fig. 6.

The data on the VMA-10-21 stability under the influence of acids are presented in Table 8.

As the presented data show, under the action of a 1 M solution of hydrochloric acid, a partial decomposition of the VMA-10-21 molecule occurs.

The data obtained are shown in Fig. 7.

VMA-10-21 stability data are presented in Table 9.

As the presented data show, under the action of 1 M sodium hydroxide solution, the VMA-10-21 molecule decomposes with a degradation percentage of 92.61%.

Under the action of an alkali solution, the VMA-10-21 molecule decomposes to form two predominant products with retention times of 2.12 min. (about 18%) and 2.98 min. (about 67%). HPLC-MS was used to determine the structural fragments formed as a result of hydrolysis of the products. Fig. 8 and 9 show the obtained mass spectra.

The peak, with a retention time of 2.12 min., corresponds to the molecular ion with a molar mass of 162 g/mol, and the peak with a retention time of 2.98 min., corresponds to the molecular ion with a mass of 186.9 g/mol. The most probable hydrolysis ways calculated as a result of a computational experiment, confirmed the mass detector data.

Based on the obtained data, it can be concluded that the decomposition of the VMA-10-21 molecule occurs at the amide group (way 1 in Fig. 5) with the formation of two main degradation products, which are separated from each other under the selected chromatographic conditions.

Hydrolysis of the VMA-10-21 molecule in the presence of 1M hydrochloric acid occurs with a significantly less decomposition of the molecule. This is probably due to the stabilization of the VMA-10-21 molecule in an acidic environment due to the formation of salts with hydrochloric acid. The main decomposition product in an acidic medium is a molecular ion with a mass of 186.9 g/mol, which also coincides in the retention time (2.98 min.)

Study of VMA-10-21 stability under the influence of oxidizing agents

Hydrogen peroxide, metal ions, oxygen, initiators of radical reactions (azocompounds, N-nitrozoanilides, triazenes, dibenzyls, etc.) are widely used for the forced oxidation of drug substances. It was established that exposure to solutions of 0.1–3% hydrogen peroxide at pH=7 and a temperature of 20°C for seven days, can potentially lead to the appearance of corresponding degradation products [21]. In this study, the maximum allowable hydrogen peroxide solution of 3% was used, but with a shorter time interval.

First, a 3% hydrogen peroxide solution was analyzed without adding the analyzed substance. The resulting chromatogram is shown in Fig. 10.

The data on the stability of VMA-10-21 to oxidizing agents are presented in Table 10.

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Table 7 – Calculated Gibbs energies of hydrolysis reactions.

Way of reaction	Hydrolysis product	Calculated Gibbs energies of reactions, kJ/mol
First	No.1 + No.2 (acidic environment)	1391.01
	No.1 + No.2 (alkaline environment)	–707,86
Second	No.3 + No.4 (acidic environment)	1406.48
	No.3 + No.4 (alkaline environment)	–665.343

Table 8 – Results of the stress test influence (hydrolysis, acid) on the VMA-10-21 stability

Stress test (1M hydrochloric acid)	The contents of the VMA-10-21	Percentage of degradation
VMA-10-21 without hydrolysis	99.68%	–
VMA-10-21 after 45 minutes	98.14%	1.54

Table 9 – Results of the influence of stress test (hydrolysis, alkaline) on the VMA-10-21stability

Stress test (1M sodium hydroxide)	Contents of the VMA-10-21	Percentage of degradation
VMA-10-21 without hydrolysis	99.68%	–
VMA-10-21 after 45 minutes	7.07%	92.61%

Table 10 – Results of the stress test (oxidation) effect on the VMA-10-21stability

Stress test (3% hydrogen peroxide solution)	**Contents of VMA-10-21**	**Percentage of degradation**
VMA-10-21 without addition of hydrogen peroxide	99.68%	–
VMA-10-21 after 45 minutes	98.56%	1.12%

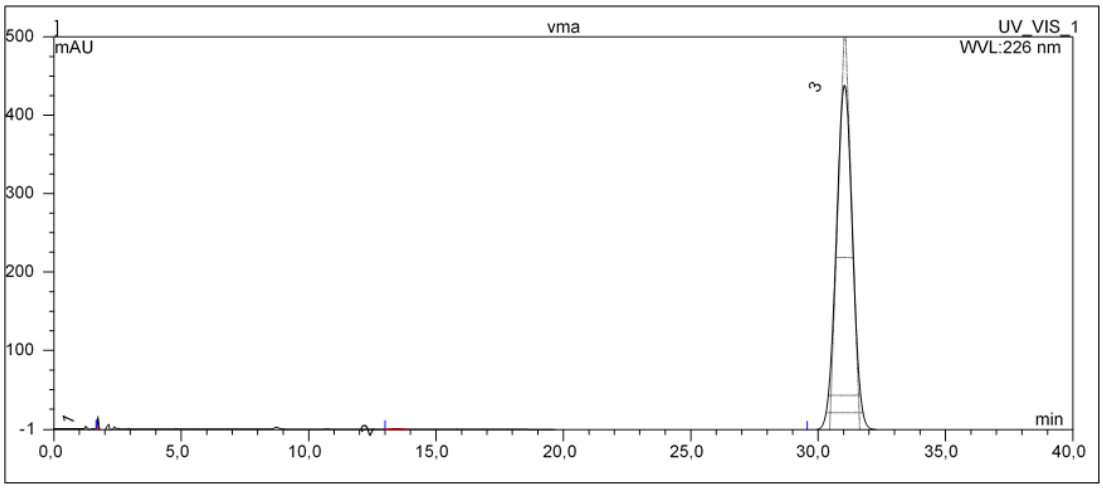

Figure 2 – Chromatogram of VMA-10-21 alcohol solution before stress testing

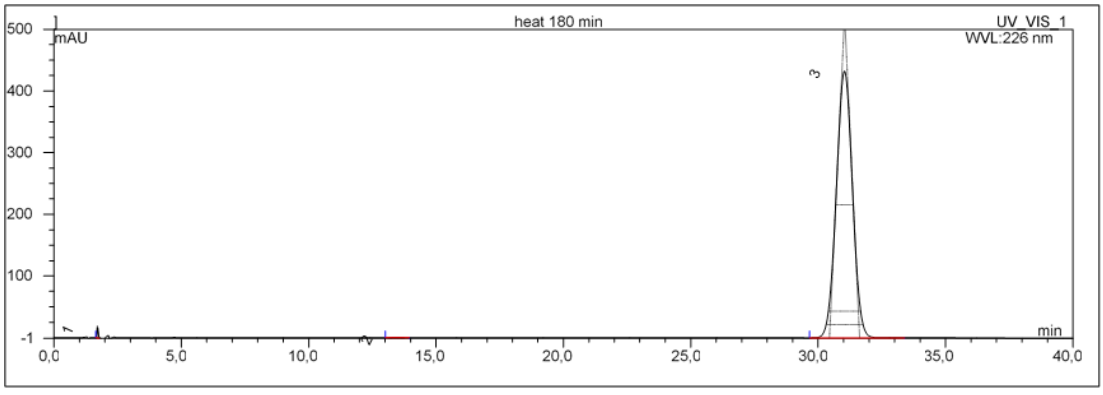

Figure 3 – Chromatogram of VMA-10-21 alcohol solution after heating at 80°C for 180 minutes

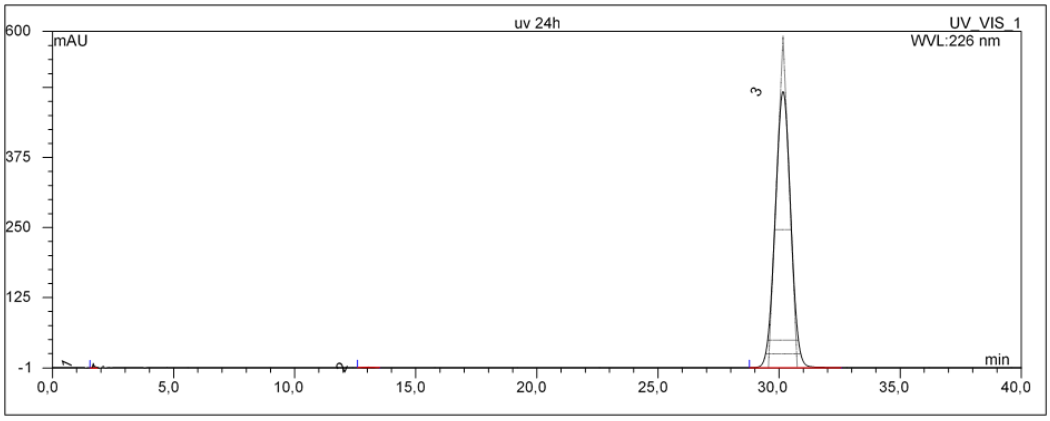

Figure 4 – Alcohol solution VMA-10-21 exposed to UV light after 24 hours

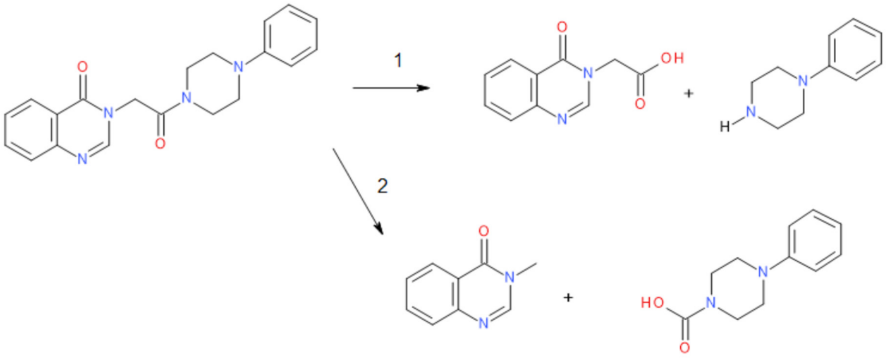

Figure 5 – Suggested ways of hydrolysis of VMA-10-21 substance

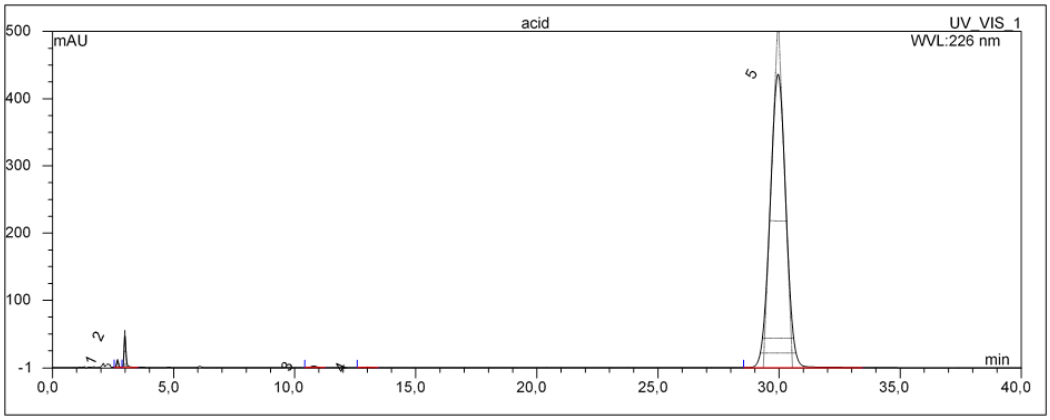

Figure 6 – Chromatogram of VMA-10-21 alcohol solution during hydrolysis with 1M solution of hydrochloric acid after 45 minutes

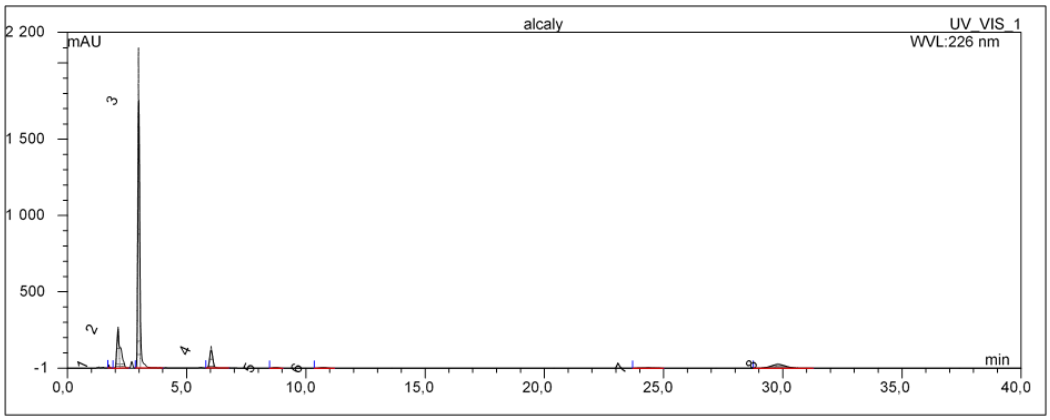

Figure 7 – Alcohol solution of VMA-10-21 during hydrolysis with 1M sodium hydroxide solution after 45 minutes

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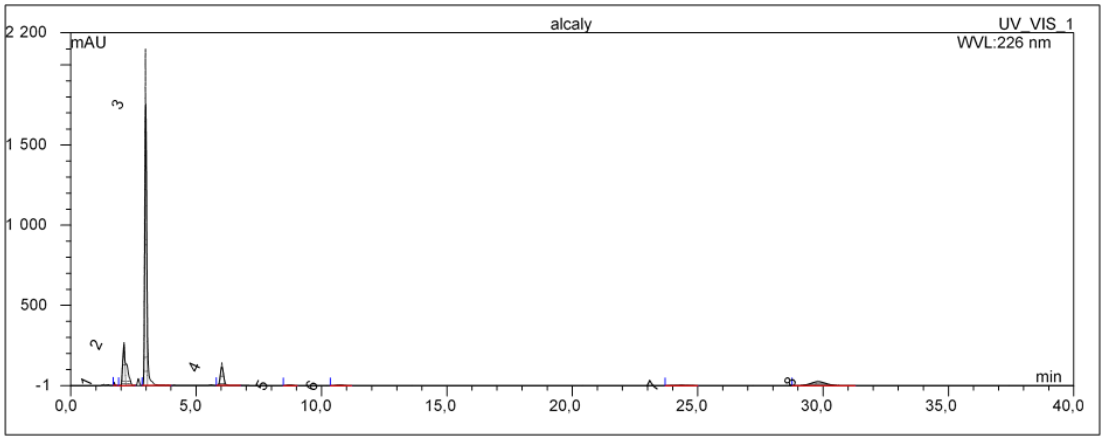

Figure 8 – Mass spectra of a structural fragment with a molecular weight of 162 g/mol

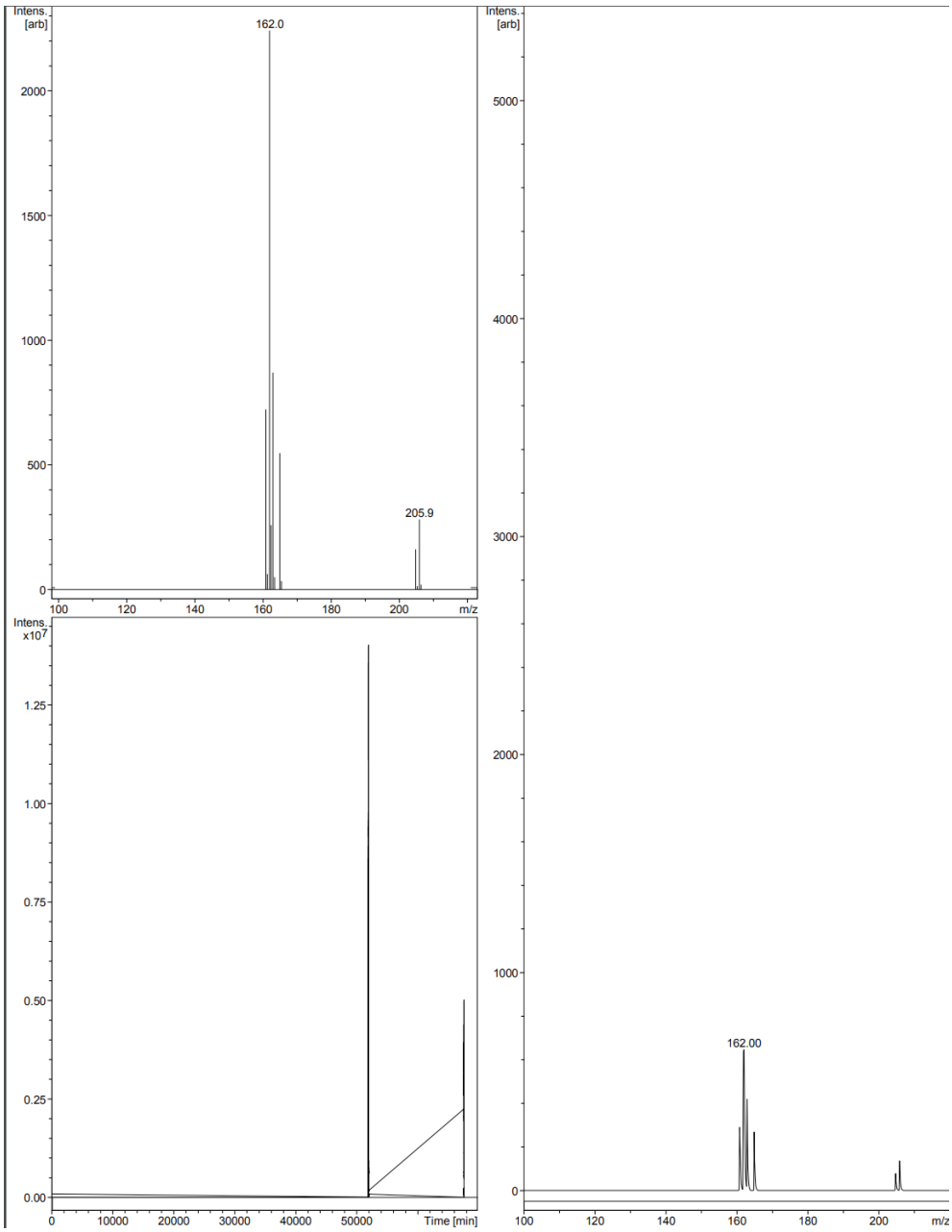

Figure 9 – Mass spectra of a structural fragment with a molecular weight of 186.9 g/mol

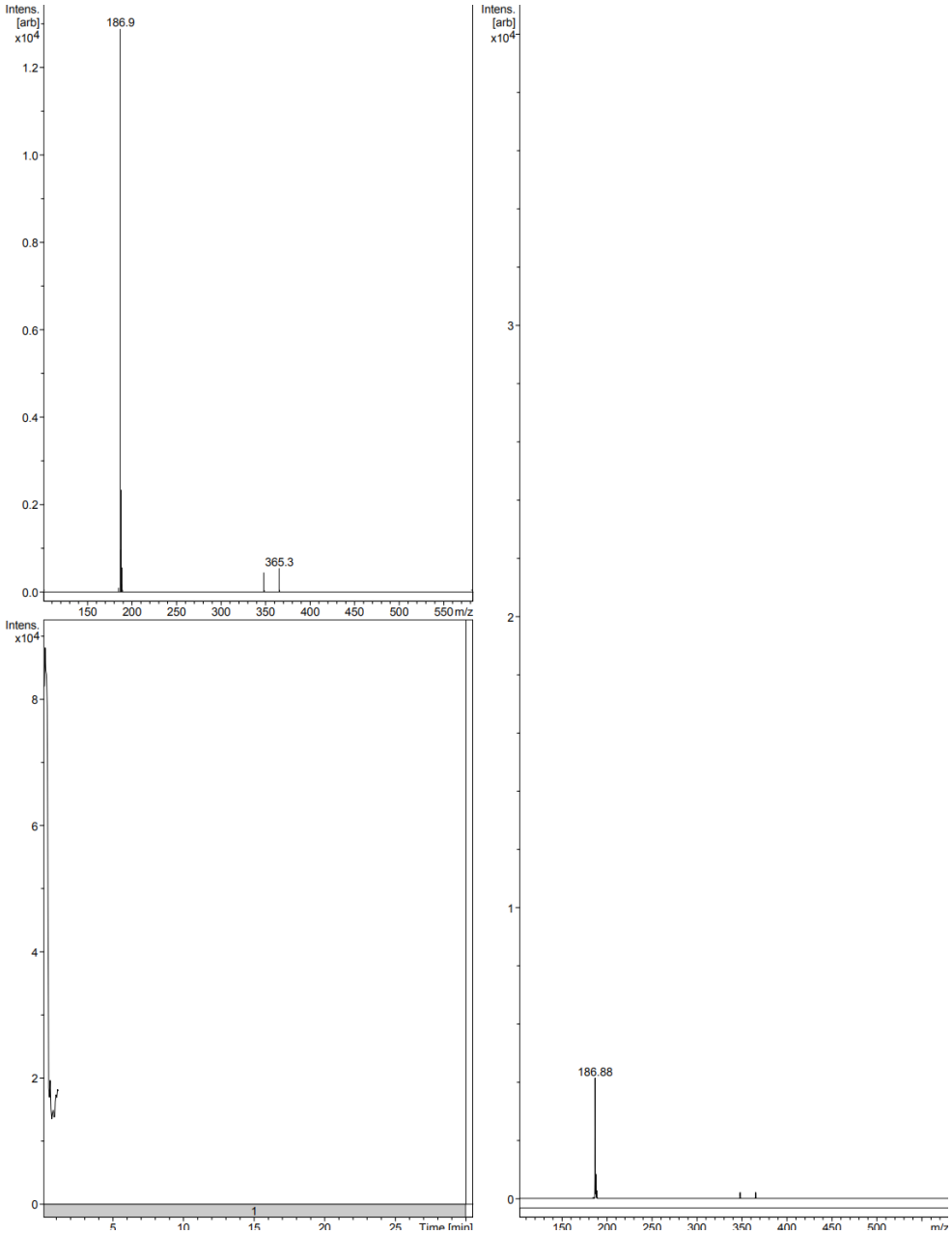

Figure 10 – 3% hydrogen peroxide solution without VMA-10-21

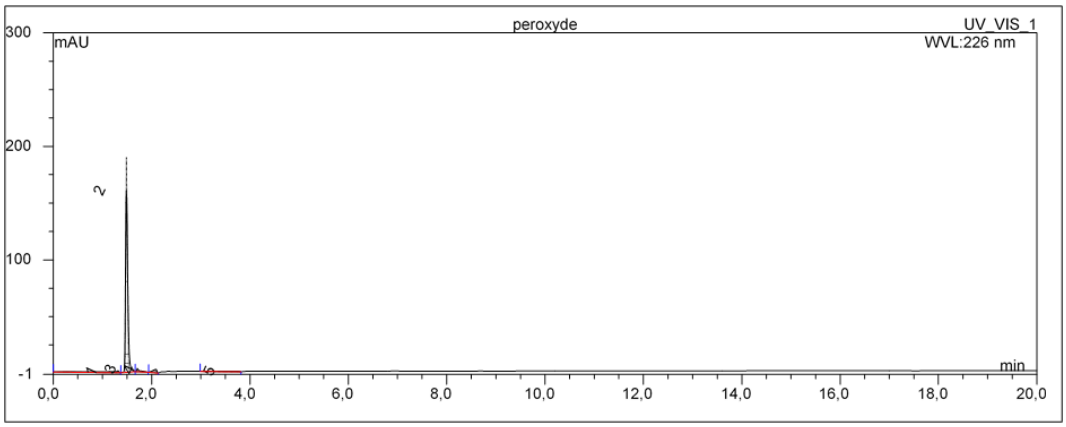

Figure 11 – Alcohol solution of VMA-10-21 with the addition of 3% hydrogen peroxide solution after 45 minutes

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As follows from the presented data, under the action of a 3% hydrogen peroxide solution, the VMA-10-21 molecule is partially decomposed with a degradation percentage of 1.12%.

CONCLUSION

The stability of the new pharmaceutical substance VMA-10-21 was studied in the course of stress tests. As a result of the experiment it was established, that the substance is stable under the action of high temperatures and UV radiation. When conducting the hydrolysis, the investigated substance hydrolyses in alkaline environment at the amide group with the formation of 2 main products, the structural fragments of which were established using the mass detector. In the acid medium, decomposition of the product is greatly reduced, which is likely associated with the increased stability of the molecule due to the formation of salts with hydrochloric acid and the protonation of the two tertiary nitrogen atoms. When the substance is exposed to oxidizing agents (a 3% hydrogen peroxide solution), there is a slight destruction of the molecule (about 1%), which shows the relative stability of the molecule under the action of oxidizing agents. The presented computer calculations have also made it possible to predict the stability and most likely hydrolysis ways of the studied substance, which correspond to practical results. These results will be taken into account in the future when developing regulatory documentation for the substance 3-[2-(4-phenyl-1-piperazino)-2-oxoethyl]quinazoline-4(3*H*)-one.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTION OF AUTHORS

T.A. Gendugov – computer analysis and processing of the results obtained, carrying out the practical part of the work; A.A. Glushko – computer analysis and processing of the results; A.A. Ozerov – research conception and strategy, text editing, synthesis and purification of the VMA-10-21substance; L.I. Shcherbakova – research conception and strategy, text editing.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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ESTIMATION OF THE EFFICIENCY OF HORMONE-REGULATING SYNCHRONIZATION OF OVULATION IN FEMALE MICE

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The aim of the wok is to assess the efficiency of hormone-regulating synchronization of ovulation in female mice, to increase the number of simultaneously fertilized individuals and obtain their offspring in the planned time frame.
Materials and methods. The study was carried out on 180 female mice of three lines – CBA/lac, C57BL/6, BALB/c (n = 60), divided into three subgroups: intact (mating without confirmation of the estrous phase) (n = 20), cytological examination of vaginal secretions before mating with the determination of the estrous phase (n = 20), hormone-regulating synchronization of the estrous cycle with the introduction of progesterone (4.5 mg/100 g) on the 1st and prostaglandin F2α (0.083 mg/100 g) on the 7th day, once from the beginning of the experiment followed by immediate mating (n = 20). The planned date of delivery was considered the 22nd day from the moment of mating. The ovulation synchronization index (OSI) was assessed on the 14th day after mating.
Results. On the 14th day from the beginning of the experiment, the ovulation synchronization index in the intact groups of the CBA/lac, C57BL/6, BALB / c lines, was 25%, 25%, 40%, respectively. On the 14th day, the number of pregnant individuals admitted to mating after the established estrus by the method of cytological assessment of vaginal secretions according to OSI, was 65%, 60%, 75%, respectively. In the experimental groups, OSI was 80%, 75%, 100%, respectively. On the 22nd day, the number of delivered females of CBA/lac, C57BL/6, BALB/c lines in the intact group, was 3, 1, 3 individuals; in the control group – 10, 6, 9, and in the experimental group – 16, 15, 17, which is significantly higher than in the control and intact groups ($p<0.05$).
Conclusion. Hormone-regulating synchronization of ovulation in female mice significantly increases the number of delivered individuals on the 22nd day, relative to those synchronized by estrus by 53%, and to intact groups by 85.5%. It has been revealed that an additional effect of hormonal synchronization of ovulation is an increase in the number of offspring by 120% in comparison with the control groups and by 390% in comparison with the intact groups. This method of timing planning of the offspring birth of the experimental animals reduces the time spent on preclinical studies of drugs for the following types of assessment of toxic effects: reproductive toxicity, embryotoxicity, teratogenicity, effects on fertility.
Keywords: estrous cycle, estrus, progesterone, vaginal cytology, ovulation synchronization, prostaglandin F2α

ОЦЕНКА ЭФФЕКТИВНОСТИ ГРУППОВОЙ ГОРМОН-РЕГУЛИРУЮЩЕЙ СИНХРОНИЗАЦИИ ОВУЛЯЦИИ У САМОК МЫШЕЙ

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RESEARCH ARTICLE

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Цель. Оценить эффективность гормон-регулирующей синхронизации овуляции у самок мышей, для увеличения количества одновременно оплодотворенных особей и получения потомства в запланированные сроки.
Материалы и методы. Исследование было проведено на 180 самках мышей трех линий – CBA/lac, C57BL/6, BALB/c (n=60), разделенные на три подгруппы: интактные (спаривание без подтверждения фазы эструса) (n=20),цитологическое исследование вагинального секрета перед спариванием с определением фазы эструса(n=20), гормон-регулирующей синхронизации эстрального цикла с введением прогестерона (4,5 мг/100 г) на 1-е и простагландина F2α (0,083 мг/100 г) на 7-е сутки однократно от начала эксперимента с последующим немедленным спариванием(n=20). Запланированной датой родов считались 22 сутки с момента спаривания. Индекс синхронизации овуляции (ИСО) оценивался на 14 сутки с момента спаривания.
Результаты. На 14-й день с начала эксперимента индекс синхронизации овуляции в интактных группах линий CBA/lac, C57BL/6, BALB/c составил 25%, 25%, 40% соответственно. Количество беременных особей на 14 сутки, допущенных к спариванию после установленного эструса методом цитологической оценки вагинального секрета согласно ИСО, составило 65%, 60%, 75% соответственно. В экспериментальных группах ИСО составил 80%, 75%, 100% соответственно. На 22 сутки количество родивших самок линий CBA/lac, C57BL/6, BALB/c в интактных группе составило 3, 1, 3 особи, в контрольных 10, 6, 9, а в экспериментальной группе 16, 15, 17 что достоверно выше чем в контрольных и интактных группах ($p<0,05$).
Заключение. Гормон-регулирующая синхронизация овуляции у самок мышей достоверно увеличивает количество разродившихся особей на 22 сутки относительно синхронизированных по эструсу животных на 53% и интакта на 85,5%. Выявлено, что дополнительным эффектом гормональной синхронизации овуляции является увеличение количества приплода в 2,2 раза в сравнении с контрольными группами и в 3,9 раз в сравнении с интактными группами. Данный способ планирования сроков рождения потомства экспериментальных животных сокращает временные затраты проведения доклинических исследований лекарственных препаратов по следующим видам оценки токсических эффектов: репродуктивная токсичность, эмбриотоксичность, тератогенность, влияние на фертильность. Кроме того, данный способ расширяет возможности экспериментального моделирования патологий беременности и плода с последующей оценкой их фармакологической коррекции.
Ключевые слова. эстральный цикл, эструс, прогестерон, вагинальная цитология, синхронизация овуляции, простагландин F2α

INTRODUCTION

Currently, the productivity of laboratories with mice of various lines as their main model organisms, is increasing, This species has gained its great popularity relatively recently, and, as a result of the efforts of many scientists over the past decades, a large number of inbred lines of mice, have been created and maintained. These events influenced a further research, thereby, making a huge contribution to modern ideas about immunology, oncology, embryology and neurobiology [1].

To prepare pregnant female mice for the experiment, the group of authors initially tested the method of vaginal cytology. This technique is based on the identification of the phases of the estrous cycle, followed by sampling the individuals in the estrous phase, and their subsequent placing to males to copulate [2].

The estrus determination in the group of individuals is important in the sampling of the animals; the aim is their subsequent mating and obtaining the offspring for experimental purposes [3]. However, this requires a long-term screening of the entire animal population, the experimenter has special skills and knowledge, which implies making a mistake. Vaginal cytology is a non-invasive and inexpensive way to determine the phase of the cycle, requiring certain skills in interpreting the morphological picture of vaginal secretion cells. This method is tedious and time-consuming [4].

A group hormone-regulating synchronization of ovulation is very popular in livestock farming. The basis of this method is a pharmacological correction of the hormonal cycle in order to induce ovulation within necessary time limits.

The progesterone used in the proposed scheme, has a strong antigonadotropic effect. The increased progesterone levels alter the characteristics of the two outer layers of the endometrium.

There is thickening of the cervical mucus, which leads to desynchronization of the endometrial changes necessary for an egg cell implantation, and significantly suppresses the penetration of spermatozoa [5]. The progesterone levels peak in the middle of the secretory phase, reducing the level of the luteinizing hormone (LH) and the follicle stimulating hormone (FSH), so that the secondary oocyte does not leave the dominant follicle and does not pass into the lumen of the fallopian tube. These changes make it impossible to fertilize an egg cell with sperm.

Prostaglandin F2α (PGF) is a biologically superpotent substance that plays an important role in the control of reproduction. The use of the drug in cattle is based on its luteolytic properties [6].

In addition, the experimental data on cattle indicate that in the peri-ovulatory period, intrafollicular prostaglandin is necessary for the ovulation process [7].

THE AIM of the study was to estimate the number of pregnancies in the female mice subjected to the hormone-regulating synchronization of ovulation, compared with planning the timing of pregnancy and the birth dates in the animals using the determination of the estrus phase.

MATERIALS AND METHODS

The research protocol was reviewed and approved of, at the meeting of the commission for work with labo-

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ratory animals of the Research Institute of Pharmacology of Living Systems, Belgorod State University. The carried out work, met the requirements of the Law of the Russian Federation "On the Protection of Animals from Cruelty" dated 24 June, 1998, the rules of laboratory practice when conducting preclinical studies in the Russian Federation (GOST 3 51000.3-96 and GOST R 53434-2009), European Community directives (86/609 EU), and the Rules of Laboratory Practice adopted in the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 708 dated 29.08.2010).

Animals

The females, regardless of the group, were placed to the males in ratio of 2:1 to copulate. The planned birth date was considered 22 days later from the beginning of mating. Excluding the tribal selection, the following female mice were selected for the experiment: they were the same age and weight of CBA/lac, C57BL/6, BALB/ lines, each line was represented by 60 individuals, and males of the corresponding lines in the amount of 30 individuals from the laboratory mouse bank of "Stolbovaya" (Moscow region).

The choice of individuals of these lines was justified by their most frequent use in biomedical research [8].

The animals were kept in individual ventilated cages. Non-coniferous sawdust was used as a bedding material. The animals were given standard granulated complete feed for laboratory animals – extruded LBK-120 GOST R 50208-92. (ZAO "Tosno animal formula-feed plant"). Feeding of the animals was carried out according to the standards in accordance with the species of the animals. Purified tap water was given *adlibitum* in standard drinkers.

Study design

The animals of three lines were divided into three groups: intact (natural mating) (n=20), estrous synchronization (cytological examination of vaginal secretions before mating with the determination of the estrous phase) (n=20), hormonal synchronization of the estrous cycle (n=20). The individuals belonging to the control group, were selected if estrus had been established in them. The females of the intact and experimental groups had not been preselected. The females, regardless of the group, were placed to the males in ratio of 2:1 to copulate. The planned birth date was considered 22 days later from the beginning of mating.

In the control groups, the animals were admitted to mating after confirming their estrous phase by assessing the vaginal secretions.

To carry out the first stage of the hormonal synchronization of ovulation in the mice, progesterone (suspension for the injection, ZAO “Mosagrogen”, RF) was injected intramuscularly at the dose of 4.5 mg/100 g, regardless of the phase of the estrous cycle of the females. 7 days after the administration of progesterone, an intramuscular injection of prostaglandin F2α (ZAO “Mosagrogen”, RF) was carried out at the dose of 0.083 mg/100 g. The estimated time of the onset of ovulation was 34–72 hours after the administration of the second drug.

The pharmacological correction of the estrous cycle of the female mice was evaluated by examining the cytological picture of vaginal secretions.

The ovulation synchronization index (OSI) was calculated after the established fact of pregnancy on the 14th day from the beginning of mating. The birth rate index (BRI) was analyzed by the fact of birth.

$$OSI = \frac{\text{The number offertilized females}}{\text{The number of females placed to malestocopulate}} \times 100\%;$$

$$BRI = \frac{\text{The number of offspring}}{\text{The number offertilized females}} \times 100\%$$

Vaginal smear/cytology

The manipulation was carried out the next day after the progesterone injection, 3 days later, immediately before the injection of prostaglandin F2α and the next day after it.

Vaginal secretions were collected from a fixed female, for the purpose of cytological assessment of the estrous cycle phase. A small amount (20 µl) of distilled water was gently injected into the vagina using a pipette, followed by drawing the previously injected liquid into the pipette. This procedure was repeated 4–5 times. It is important to make sure that the pipette is placed at the entrance of the vaginal canal and does not penetrate the vaginal opening. The liquid containing a few drops of the cell suspension, is placed then on a slide, air-dried, and stained according to the Romanovsky-Giemse method [9, 10]. After that, the slide was covered with a cover glass, and the quantitative and qualitative composition of the secretion cells was immediately examined under a light microscope (Biomed 5) at 40× magnification (Fig. 1).

The vaginal secretions consist of three types of cells. These include leucocytes, keratinized epithelial cells, and nucleated epithelial cells. The estimation of the estrous cycle phase, is based on the proportion of these cells in the vaginal secretions [11].

Numerous rounded nucleated cells that are uniform in size and appearance, are the hallmark of the proestrus phase (A). The estrus phase shows abundant non-nuclear keratinized epithelial cells (B). Nucleated epithelial cells are present in the late metestrus (C). Diestrus is characterized by the presence of polymorphonuclear leukocytes and several epithelial and keratinized cells within the field of view [12].

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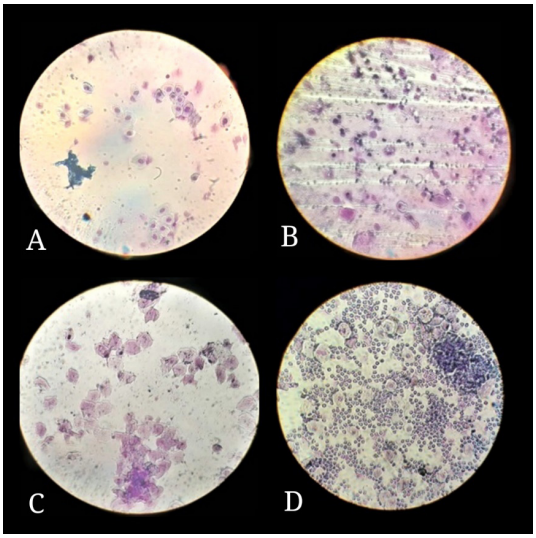

Figure 1 – Microscopic assessment of cell suspension drops of vaginal secretions in animals without hormone-regulating ovulation synchronization

Note: A – proestrus, B – estrus, C – metestrus, D – diestrus

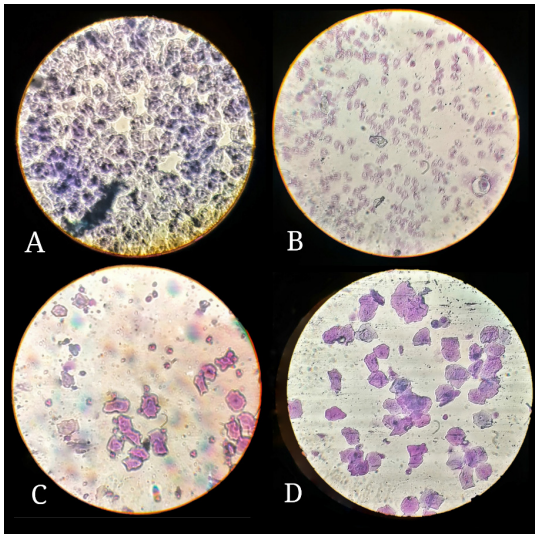

Figure 2 – Microscopic assessment of cell suspension drops of vaginal secretions in females after hormone-regulating ovulation synchronization

Note: A – cytological picture of the contents of the vaginal secretion on the next day after the administration of progesterone; B – on the 3rd day; C – on the 7th day before the administration of prostaglandin F2α; D – on the next day after the administration of prostaglandin F2α

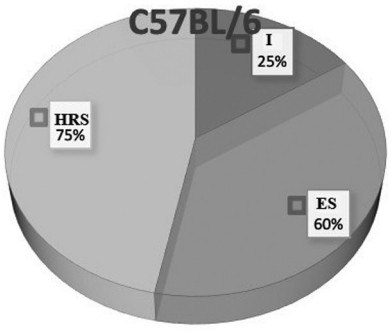

Figure 3 – The ratio of the ovulation synchronization index of individuals of the C57BL/6 line experimental groups

Note: I – intact group; ES – estrous synchronization; HRS – hormone-regulating synchronization

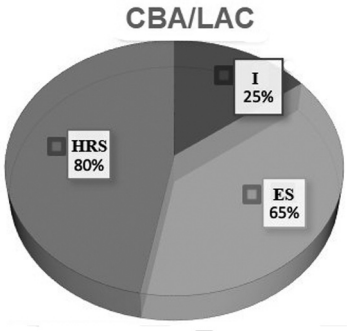

Figure 4 – The ratio of the ovulation synchronization index in individuals of the CBA/lac line experimental groups

Note: I – intact group; ES – estrous synchronization; HRS – hormone-regulating synchronization

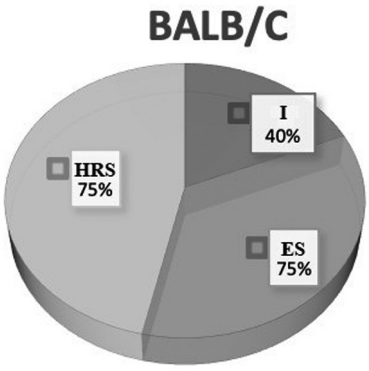

Figure 5 – The ratio of the ovulation synchronization index of individuals of the line BALB/C experimental groups

Note: I – intact group; ES – estrous synchronization; HRS – hormone-regulating synchronization

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Table 1 – Ovulation synchronization index

Line name / Groups	CBA/lac			C57BL/6			BALB/c		
	(I)	(ES)	(HRS)	(I)	(ES)	(HRS)	(I)	(ES)	(HRS)
Number of pregnant females on the 14th day	5	13	16	5	12	15	8	15	20
Number of females placed to males to copulate	20	20	20	20	20	20	20	20	20
OSI, %	25%	65%	80%	25%	60%	75%	40%	75%	100%

Note: I – intact group; ES – estrous synchronization; HRS – hormone-regulating synchronization.

Table 2 – The number of females which gave birth on the 22nd day

Line name / Intact group	CBA/lac (n=20)	C57BL/6 (n=20)	BALB/c (n=20)
	3	1	3
Estrous synchronization	10*	6*	9*
Hormone-regulating synchronization	16*	15*	17*

Note: * – p<0.05 compared to control and experimental groups

Table 3 – Birth rate index

Line name / Groups	CBA/lac			C57BL/6			BALB/c		
	(I)	(ES)	(HRS)	(I)	(ES)	(HRS)	(I)	(ES)	(HRS)
Number of pregnant females on the 14th day	5	13	16	5	12	15	8	15	20
Number of offspring	34	77	145	28	67	126	74	123	193
BRI, %	6,8	5,9	9,0	5,6	5,5	8,4	9,25	8,2	9,65

Hormonal regulation of the estrous cycle

To implement the first stage of the hormonal synchronization of ovulation in mice, progesterone was intramuscularly administered (the suspension for injection, ZAO “Mosagrogen”, RF “Progestomag”, Reg. 32-3-4.15-2649 No. PVR-3-4.15/03139 dated 27.06.2018) at the dose of 4.5 mg/100 g, regardless of the estrous cycle phase of females. 7 days after the administration of progesterone, the intramuscular injection of prostaglandin F2α (ZAO "Mosagrogen", RF "Magestrofan", Reg. 32-3-4.15-2649 No. PVR-3-4.15/03139 dated 06/11/15) was carried out at the dose of 0.083 mg/100 g. The probable time of the ovulation onset is 34–72 hours after the administration of the second drug. The regimen for the use of drugs is presented in the instructions for the veterinary use of drugs.

Statistical analysis

A statistical analysis comparing the number of births on day 22 in the groups, was carried out using *Pearson's Chi-square test.* Differences were identified at a significance level of 0.05. Statistical analysis was performed using Statistica 10.0 software. The differences were determined at the significance level of 0.05. The statistical analysis was performed using the Statistica 10.0 software.

RESULTS

Vaginal smear/Cytology

The next day after the progesterone injection to the female mouse (Fig. 2), a mixed cytological picture takes place; it does not make it possible to attribute the visible result to a certain cycle phase (A). Polymorphonuclear leukocytes and a small number of keratinized cells are observed 3 days after the administration of progesterone, which corresponds to the diestrus phase (B). On the seventh day before the injection of prostaglandin F2α, there is a predominance of rounded nucleated cells with a small impregnation of keratinized epithelial cells and polymorphonuclear leukocytes between them (C). In a vaginal smear taken the next day after the injection of prostaglandin F2α, there is a predominance of abundant non-nuclear keratinized epithelial cells with cells of irregular shape and granular cytoplasm (D).

Hormone-regulating estrous cycle synchronization

During the study it was found out that the hormonal synchronization of a group of females, increases the number of fertilized individuals by 55% relative to the intact groups, and by 18.3% relative to the control groups. The selection of individuals based on the cytological examination of vaginal secretions, increases the number of pregnant females by 36% (p <0.05) (Table 1). On day 22, the hormone-regulating synchronization increases the probability of giving birth by 53% (p<0.05) in comparison with the control group, and by 85.5% (p <0.05) in comparison with the intact group (Table 2). The ratio of the ovulation synchronization index is shown in Fig. 3, 4, 5.

On the 14th day after mating, the pregnancy was confirmed in 25% of females of the C57BL/6 line in the intact group. The estrous synchronization of the cycle increased the number of pregnant individuals relative to the control ones, by 35%. The hormone-regulating synchronization of the ovulatory cycle increased the number of fertilized individuals by 50% relative to the intact

ones, and by 10% relative to the estrous synchronization ($p<0.05$).

On the 14th day after mating, the pregnancy was confirmed in 25% of females of the CBA/lac line in the intact group. The estrous synchronization of the cycle increased the number of pregnant individuals relative to the control ones, by 40%. The hormone-regulating synchronization of the ovulatory cycle increased the number of fertilized individuals by 55% relative to the intact ones, and by 15% relative to the estrous synchronization ($p<0.05$).

On the 14th day after mating, the pregnancy was confirmed in 25% of females in the CBA/lac line intact group. The estrous synchronization of the cycle increased the number of pregnant individuals relative to control ones by 35%. The hormone-regulating synchronization of the ovulatory cycle increased the number of fertilized individuals by 35% relative to intact ones and did not change in the estrous synchronization ($p<0.05$).

Assessment of the birth rate index

The number of offspring in the experimental animals of different groups is not the same. In the groups of the hormone-regulating stimulation, the average number of the offspring is higher in comparison with intact and estrous synchronization groups (Table 3).

DISCUSSION

The results of the study confirmed the hypothesis that the hormone-regulating correction of the ovulatory cycle in female mice, makes it possible for the experimenter to obtain a larger number of fertilized individuals within necessary time limits, with a minimum error in the date of birth.

According to the instructions for the veterinary use of the drugs "Progestomag" and "Magestrofan", progesterone inhibits the hypothalamic-pituitary system. As a result, there is no release of gonadotropic hormones – follicle-stimulating (FSH) and luteinizing (LH), hence, follicle maturation and ovulation do not occur.

This leads to the induction of the synthesis of cervical mucus by the epithelial cells of the cervix, its edema as a result of an increase in its blood supply. It also leads to the proliferation of the endometrium and an increase in the extensibility of the myometrium, reduces the release of gonadoliberin, thereby inhibiting new ovulations, preventing the maturation of follicles in the ovaries, and makes the ovulation impossible. From the sixth to seventh day, there is a decrease in the concentration of progesterone and estrogen, causing a natural increase in LH and FSH, as well as an increase in the content of estrogen in the blood plasma.

All the follicles growing in a cohort, have specific receptors for FSH and require gonadotropin, which is necessary for their growth. At this stage, the growing follicles do not have enough LH receptors to respond to stimulation, that is why this growth stage is often referred to as FSH-dependent.

The second stage of the hormone-regulating synchronization of ovulation, begins with the administration of prostaglandin F2α on the seventh day. The main effect of this biologically active substance is to stimulate the transition of the estrous cycle from the diestrus phase to estrus by overcoming the progesterone blockade of the cycle. In addition, prostaglandin F2α promotes the development of folliculogenesis, estrogen synthesis and, as a consequence, the onset of estrus. Prostaglandin F2α supports luteinolysis caused by the upsurge in LH, which leads to the ovulation and the release of egg cells from the dominant follicle within 16–32 hours.

During the ovulation, which occurs approximately 34–36 hours after the LH upsurge, the secondary oocyte in metaphase II leaves the dominant follicle and enters the lumen of the fallopian tube, where it can be fertilized [12].

The upsurge in luteinizing hormone (LH) stimulates the preovulatory follicles to form local autocrine and paracrine mediators, which coordinate complex intra- and extracellular molecular mechanisms, subsequently causing ovulation and luteinization. The key local mediators include progesterone and its nuclear receptor (PGR), prostaglandins (PTG) (PGE2 and PGF2α).

An increase in LH levels, increases progesterone production and PGR expression in periovulatory follicles, which is necessary for successful ovulation in various animal models [13]. For example, blocking progesterone biosynthesis [14], the inhibition of PGR activity by chemical inhibitors [15, 16] or knockout of genes encoding PGR synthesis [17, 18], led to anovulation in various experimental animal models.

All the above listed changes, lead to the onset of ovulation on the 8th–9th days after the first stage of the hormone-regulating ovulation synchronization.

It was confirmed that the selection of animals for mating, based on the cytological picture of the estrous cycle phase, increases the number of fertilized individuals. However, the range in the birth dates was 2–4 days, which complicates planning of the experimental protocol for studying the pharmacological correction of pregnancy pathology. There are known models in which the drug is administered at different periods of pregnancy, studying its effect on different periods of developing the offspring: from the 1st to the 6th days (the pre-implantation period), from the 6th to the 16th days (organogenesis), and from the 16th to the 19th days of pregnancy (fetogenesis) [19]. In this case, the range of the due birth dates of 2–4 days is a problem that should be minimized. According to the authors' observations, the date of placing females to males to copulate immediately after the injection of prostaglandin F2α, can be considered the first day of pregnancy, since 21–22 days after the two-stage hormone-regulating estrus synchronization, 80% of females gave birth, whereas in the estrus synchronization group, only 42% females gave birth.

In addition, the number of fetuses born to females

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whose ovulation induction was artificial, is 30% higher in comparison with natural. The authors hypothesize that this is due to an increased release of hormones from the hypothalamic-pituitary system into the bloodstream, which stimulates the release of more egg cells from the ovaries into ovulation. [20].

According to the degree of impact on the body, the drugs used in this scheme, are classified as low-hazard substances (hazard class 4 according to GOST 12.1.007).

In the subcutaneous or intramuscular administration of the drug Progestamag, the required exogenous level of progesterone in the blood for the manifestation of the therapeutic effect, is maintained for 6–7 days, but it does not exceed the physiological content in the body of the animals. In accordance with the instructions, there are no side effects and complications in farm animals, caused by the drug Magestrofan; as a rule, they are not observed.

Thus, the administration of progesterone to a female mouse at the dose of 4.5 mg/100 g, stimulates the transition of the ovulatory cycle phase to the secretory phase of the female's ovulatory cycle. The subsequent administration of prostaglandin F2α at the dose of 0.083 mg/100 g after 34–36 hours, provides the release of LH, which stimulates the release of the secondary oocyte into the lumen of the fallopian tube, where it can be fertilized.

In the authors'opinion, the proposed scheme has the following advantages:

The use of a combined hormone-regulating synchronization of ovulation is justified when conducting preclinical studies of embryotoxicity of drugs due to the absence of toxic effects of the components used on the fetus.

An accurate prediction of the birth date minimizes the risks of delayed research, makes it possiblet to plan the experiment rationally.

A sequential administration of two drugs with a fairly long interval, minimizes labor costs and greatly facilitates planning of further experiments.

Taking into account the fact of adopting the first day of pregnancy of an individual since the moment of mating, prospects for the study of pathologies of the pre-implantation period, organogenesis, fetogenesis, and intrauterine pathologies open up.

CONCLUSION

Hormonal ovulation stimulation in female mice, significantly increases a number of births on the 22nd day relative to the control group with a cytological confirmation of the estrous phase and the intact group. This method of planning the birth timing of the offspring of the experimental animals, reduces the time spent on preclinical studies of drugs for the following types of assessment of toxic effects: reproductive toxicity, embryotoxicity, teratogenicity, effects on fertility. In addition, this method expands the possibilities of experimental modeling of pathologies of pregnancy and fetus, with the subsequent assessment of their pharmacological correction.

Thus, the administration of progesterone to a female mouse at the dose of 4.5 mg/100 g, stimulates the transition of the ovulatory cycle phase to the secretory phase of the female's ovulatory cycle. The subsequent administration of Prostaglandin F2α at the dose of 0.083 mg/100 g after 34–36 hours, provides the release of LH, which stimulates the release of the secondary oocyte into the lumen of the fallopian tube, where it can be fertilized.

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AUTHORS' CONTRIBUTION

All authors have contributed equally to the research work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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THE SEARCH FOR NEUROPROTECTIVE COMPOUNDS AMONG NEW ETHYLTHIADIAZOLE DERIVATIVES

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The aim of the study is to search compounds with neuroprotective properties among new ethylthiadiazole derivatives in simulated traumatic brain injury.
Materials and methods. The experiment was carried out on 78 white male rats 270±20 g line "Wistar" 5–6 months of age and 120 outbred sexually mature mice weighing 20±2 grams. The article describes the search for compounds with neuroprotective properties among new ethylthiadiazole derivatives under the codes LKHT 4–15, LKHT 10–18, LKHT 11–18, and LKHT 12–18 in experimental traumatic brain injury in rats. Acute toxicity of the compounds was studied. Pharmacological screening was performed using behavioral and neurological research methods. The McGraw stroke score scale modified by I.V. Gannushkina and the mNSS psychometric scale were used in the study. The open field and Rota-rod tests were used to assess the behavioral status of the animals.
Results. The compound-LKHT 12–18 at a dose of 50 mg/kg was detected as a leader. In pharmacological correction of pathology, this compound had the lowest percentage of fatality among the studied compounds (8%), the severity of neurological deficit was significantly reduced, the lowest scores and a higher level of motor activity of the limbs were registered. The number of rearing in the group of animals receiving the compound LKHT 12–18 at the dose of 50 mg/kg increased by 1.5 times, statistically significant ($p<0.05$) in comparison with the control group. Based on the results of the "Rota-rod" test, the total time of holding animals on the rod for 3 attempts was statistically significantly different in the groups administered with LKHT 12–18 derivatives (1.5 times longer) at the dose of 50 mg/kg compared with the control ($p<0.05$).
Conclusion. Based on the results obtained in this study, it is planned to study in more detail the compound LKHT 12–18 at the dose of 50 mg/kg
Keywords: traumatic brain injury, ethylthiadiazole derivatives, neuroprotection
Abbreviations: TBI – traumatic brain injury; ATP – adenosine triphosphate; DAI – diffuse axonal injury; tSAH – traumatic subarachnoid hemorrhage; BBB – blood-brain barrier; LP – latency period

ИССЛЕДОВАНИЕ НЕЙРОПРОТЕКТИВНЫХ СОЕДИНЕНИЙ В РЯДУ НОВЫХ ПРОИЗВОДНЫХ ЭТИЛТИАДИАЗОЛА

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RESEARCH ARTICLE

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Цель: Поиск нейропротекторов в ряду новых производных этилтиадиазола в условиях экспериментальной черепно-мозговой травмы

Материалы и методы. Эксперимент проведен на 78 белых крысах-самцах 270±20 г линии «Wistar» 5–6-месячного возраста и 120 аутбредных половозрелых мышах массой 20±2 грамма. Исследована острая токсичность соединений. Произведен фармакологический скрининг производных этилтиадиазола с изучением поведенческого статуса и неврологических реакций. Тяжесть черепно-мозговой травмы оценивалась по шкале неврологического дефицита McGraw в модификации И.В. Ганнушкиной и шкале mNSS. Для оценки поведенческого статуса животных использовали тесты «Открытое поле» и «Rota-rod».

Результаты. Выявлено соединение-лидер – ЛХТ 12–18 в дозе 50 мг/кг. При фармакологической коррекции черепно-мозговой, данное соединение имело самый низкий процент летальности среди исследуемых соединений (8%), статистически значимо снижалась тяжесть неврологического дефицита, регистрировали самые низкие баллы и более высокий уровень моторной деятельности конечностей. Количество стоек в группе животных, получавших соединение ЛХТ 12–18, увеличивалось в 1,5 раза статистически значимо ($p<0,05$), относительно группы контроля. Исходя из результатов теста «Rota-rod», суммарное время удержания животных на стержне за 3 попытки статистически значимо отличалось в группах с применением производных ЛХТ 12–18 (в 1,5 раза дольше) в сравнение с контролем ($p<0,05$).

Заключение. Экспериментальным путем было доказано наличие нейропротективных свойств у производного этилтиадиазола ЛХТ 12–18 в дозе 50 мг/кг у крыс.

Ключевые слова: черепно-мозговая травма, производные этилтиадиазола, нейропротекция

Список сокращений: ЧМТ – черепно-мозговая травма; ЛХТ – шифр производного этилтиадиазола (4–15, 10–18, 11–18, 12–18); АТФ – аденозинтрифосфат; ДАТ – диффузная аксональная травма; тСАК – травматическое субарахноидальное кровоизлияние; ГЭБ – гематоэнцефалический барьер; ЛП – латентный период

INTRODUCTION

Home and industrial traumatism, including traumatic brain injury (TBI) is which are the main public health problem diseases in all industrialized countries and lead to persistent disability, as well as high mortality, disability and lead to high treatment costs [1].

The development of effective pharmacological correction ways of traumatic brain injury consequences is one of the main tasks of experimental research, which for highly developed countries spend billions dollars [2]. Unfortunately, many substances give excellent results only at the stage of research in the laboratory. The reason for this is the complex pathogenesis of TBI, which includes a complex of interrelated factors that affect the primary and secondary “wave” of damaging brain processes [3]. Therefore, the searching for innovative neuroprotectors is relevant in the modern science.

Traumatic brain injury can be classified into severity: mild, moderate, and severe categories. Also the special forms of contusion are distinguished: diffuse axonal injury (DAI) and traumatic subarachnoid hemorrhage. The most common is moderate brain contusion.

The overwhelming majority of patients with TBI are diagnosed with deviations of varying severity – from minor disorders to pronounced neurological symptoms. TBI in the long-term period can be a trigger mechanism for the development of diseases such as Parkinson’s and Alzheimer’s. In the first 10–12 months after an injury, the risk of an epileptic seizure is very high (12 times). Post-traumatic epilepsy is detected in more than 10% of patients with moderate pathology [5].

TBI are classified into primary and secondary. Primary brain injury is a result of a traumatic factor on the bones of the skull including structures and vessels of brain [6]. These lesions occur due to various kinds of impact, which characterizes a wide range of damage reactions.

TBI causes damage to the cells of the nervous system, the constituent structures of the vessels, the structures of white matter. This entails the onset of the second wave of damage – stress for metabolic processes, as well as disturbances in ion exchange, biochemical and molecular levels of neuronal regulation [7–9].

The metabolism in nerve cells after TBI is increased: the reserves of adenosine triphosphate (ATP) are depleted, the Ca^{2+} pump are disrupted. The increased permeability of cell membranes for Ca^{2+} leads to the release of calcium from the intracellular calcium depot. Cell depolarization and glutamate release of nerve endings leads to a violation of the membranes integrity of nerve cells and vascular endothelium [6, 10, 11]. The neurotransmitter (glutamate) provokes the activated postsynaptic glutamate receptors. The increase in the Na^{+} influx leads to a further depolarization. More Ca^{2+} start entering the cell through ion channels. The consequence of calcium overloading cells damaged by the activation of phospholipases, proteases and nucleases are leads to the loss of membrane integrity, genome expression, and destruction of the structural components in cell [12, 13].

TBI primary injuries includes local brain contusion, cerebral trunk contusion, axonal and vessel cerebral injuries. Primary injury affects of the neurons body and astrocytes, synaptic breaks, in the vessels formed blood clots and the walls of the vessels is disrupted. At the end of the pathological process of primary trauma, a decrease in the supply of adenosine triphosphate (ATP) occurs due to a violation of the permeability of cell membranes, which at the next stage leads to cytotoxic edema and cell death. [14, 15].

A penumbra zone forms along the periphery of the primary injury. All cells remain viable. Only their sensitiv-

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ity to even minor deviations in the normal operation of oxygen and nutrient delivery increases [16, 17].

Mechanical neuronal membranes destruction is a triggering factor leading to the depletion of ionic reserves of cell, free radicals and lipid peroxidation are formed. The next pathological stage is an increase Ca^{2+} content in cell, the triggering of phospholipases and calpain. All these pathological factors activate secondary injury to the membrane and cytoskeleton of neurons. Plasma movement of the axon is slowed down and leads to delayed cell death [18].

Due to TBI apoptosis of nerve cells is triggered. This process begins with an action of damaging agent on the cell genome or with the destructive action of inflammatory mediators. The influence of secondary brain injury factors, the transport of O_2 and nutrients to neurons is disrupted, as well as their destruction begins in an unnecessary volume [19, 20]. The prenumbra area is more susceptible to pathological changes due to TBI [21].

The inflammatory response is triggered out as a result of TBI primary injury. These reactions are damaging and neuroprotective. The primary pathological processes in TBI are triggered due to any mechanical damage of the cerebral tissue. Scientists attach great importance to secondary trauma in animal experiments. And that's why, pharmacological approaches to the treatment of the consequences of TBI, affecting the secondary pathology mechanisms with further apoptosis of brain cells, require in-depth study [9].

The development of secondary disorders after TBI are cause why inhibition of cerebral microcirculation, violations of oxygenation and metabolic processes in nerve cells are observed, and also occurs edema and cerebral ischemia (CI). These damages occurs on a 40% of people who had sufferred moderate TBI and 85% of severe TBI [22].

People who had suffered from an TBI, the risk of an unfavorable outcome in the event of secondary brain injury increases, since that worsens the severity of the patient's condition and restoration of cognitive functions. Therefore, timely prophylaxis and correctly chosen treatment of secondary brain injury is the main task of therapy for victims of TBI severe [9].

The inflammatory response occupies one of the most important parts, an evolutionarily process of tissue reactions. A membrane-destroying process at the cels, due both mechanical damage and autolytic processes. The end of such pathological processes can be necrosis and apoptosis or regeneration and repair [23]. The reconstructions of cells involves all factors of inflammatory response. These factors including: edema, inhibition of blood circulation and protein, carbohydrate, fat metabolism. The fact that sanogenic pathological reactions such as edema and hyperemia, in the case of generalization, can have a pathogenic or even thanatogenic character.

In case of primary brain injury, activation and release of large volumes of cytokines begins throughout the human body. These cytokines can be inflammatory and anti-inflammatory. Also, an activation of resident macrophages from astrocytes with microglia in brain, the movement of neutrophils to the injury and to the violations of BBB permeability. The cytokines consists of: growth factors, interleukins, neuropoietins, chemokines, interferons, tumor necrosis factors, neurotropins. All these components are involved in the inflammatory response [24].

Despite the rapid development of experimental pharmacology [4, 25–28], the improvement of methods of directed synthesis, allowing the creation of highly selective drugs, remains highly relevant for new compounds. Ethylthiadiazole derivatives may be potential candidates with neuroprotective properties. Among them, a large number of compounds with anti-inflammatory, antimicrobial, anticonvulsant, hypotensive, antioxidant, and antitumor effects have been identified [29–34].

THE AIM of the study: to study the neuroprotective properties of new ethylthiadiazole derivatives under the conditions of experimental traumatic brain injury (TBI).

MATERIALS AND METHODS

Test samples

LKHT 4–15, LKHT 10–18, LKHT 11–18, and LKHT 12–18 were synthesized at the Russian Research Center for Safety of Bioactive Substances (Staraya Kupavna, Russia).

Animals

The experiment was conducted on 78 white male Wistar rats aged 5–6 months, body weight 270±20 gramms and in 120 outbred white mice with body weight 20±2 gramms. The animals were buyed at the Federal State Institution of Science Scientific Center of Biomedical Technology of the Federal Medical-Biological Agency of Russia. Conditions of detention: under standard conditions in accordance with Sanitary and epidemiological requirements for the device, equipment and maintenance of experimental biological clinics (vivariums) No2.2.1.3218-14 and Federal Standard of Russia No.33044-2014.

Study design

The acute toxicity of LKHT 4–15, LKHT 10–18, LKHT 11–18 and LKHT 12–18 was studied in male mice. The next dose range was studied: 500 mg/kg, 1000 mg/kg, 2500 mg/kg, 5000 mg/kg. The choose of doses was carried out by the experiment. The injection of the samples was made fractionally. Each mouse were observed keep watching for the first 60 minutes after injection of the test samples. Then the observation was performed once per day.

The manipulations on rats were carried out under general anesthesia by intraperitoneal injection of chloral hydrate (300 mg/kg). In this work, the technology of modeling TBI on rats was reproduced by the method of free-fall of block with weight 155 grams from height at 0.6 meters [33]. The setup consist of a stand with a 1.1 meters long hollow tube clamped in a stand in a vertical position. A firing pin with stopper is locate at lower edge of the pipe. The striking surface area of firing pin was 0.5 cm^2. The movement of the firing pin was limited in any conditions to no more than 5 mm. With the help of a return spring, upon impact, the firing pin returns to starting point. Restriction in the movement of the firing pin allows to escape depressed fractures of the cranial vault. The block was placed in the pipe cavity at a prearranged height. The displacement of the firing pin at the point of impact was excluded if using this function in experiment. Before modeling the pathology, it was checked the position of the pipe (installed vertically) and the table (horizontally). The localization of the impact was carried out according to the anatomy of the rat brain. The impact was simulated in the field zone Fr1, Fr3, FL, HL, Par1, Pag2. The impact site was located in the fronto-parietal-temporal region of the left hemisphere of the brain. The rat's head was not rigidly fixed. This model made it possible to reproduce the TBI model as close as possible to that in humans [33].

The pharmacological screening was carried out using the severity of neurological deficits by the McGraw point scale modified by I.V. Gannushkina [35] and the mNSS scale [36].

This McGraw scale as modified by I.V. Gannushkina (Table 1) is presented with a list of neurological disorders. The analyzed indicators were summarized. Depending on the total amount, the neurological deficit can be designated in different ways: the amount of points 0.5-2.0 corresponds to a mild degree of deficiency; 2.5–5.0 – moderate severity; 5.5-10 severe neurological deficits. The evaluation is carried out on the 1st, 3rd and 7th days of the experiment.

To assessment the neurological deficit, a modified test for neurological deficit was carried out 48 h after modeling the TBI. The mNSS scale (modified neurological severity score) is a special system for interpreting neurological deficits in the presence of a brain injury. It is used to assess motor, sensory, balance, and reflex behavior of animals [36].

According to this neurological scale, rodents were suspended by the tail (to determine the presence of paresis and paralysis), motor activity in the home cage (to register gait disturbances and stereotypical movements) and the peculiarities of movement on a horizontal bar (to assess the coordination of movements) were assessed, the safety of the main reflexes (startle reflex, reflex of the external auditory canal, corneal reflex). The results of the study to determine the neurological deficit were formulated based on the sum of points scored in each test. A higher score indicates a more severe injury. The total number of points in the range from 1–6 indicates the presence of a mild TBI, from 7 to 12 – moderate, and a total of 13–18 indicates the presence of severe TBI.

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To the behavioral assessment of animals, the Open Field [37] and Rota-rod [38] tests were used.

Open Field Test

This behavioral test is used to register the characteristics of behavioral reactions in pharmacology and psychogenetics. The main task of this testing is to study the motor component of the orienting reaction and emotional reactivity of rodents. The setup for the Open Field Test has a large number of modifications, and the parameters of determination in this test depend from objectives of study. In rodents without pathologies, exploratory behavior prevails over fear, therefore, if the rodent is placed in the arena, with normal levels of horizontal and vertical activity, exploratory behavior dominates over fear (grooming and bowel movements). The analysis of the behavioral status with the exploratory activity assessment of the rats was investigated in an "open field" installation (Open Field LE800S, PanLab Harvard Apparatus, Spain) for 10 minutes. The room was illuminated with a 100W lamp, which hung at a height of 1.5 m from the bottom of the arena. The rodents were placed an "open field" around the periphery of the arena. The arena was wiped with a damp cloth after each animal. For analyzing the data obtained, the Smart v.3.0.03 program was used. (Panlab Harvard Apparatus, Spain). The results of the data obtained were summarized.

For statistical calculations of the "Open Field" test, the following indicators were taken: horizontal activity in the center, horizontal activity on the periphery, stances, mink reflex, number of defecation acts, number of urination acts, grooming.

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Rota-rod Test

The Rota-rod test is used characterizing motor coordination of movements [13]. In this experiment, a constant rod rotation speed of 20 rpm was used. The latency period (LP) of first fall (the time of first fall of rat from the rotating rod) and the total holding time on the rotating rod for 3 attempts were recorded [33, 39–42].

Based on the objective, all animals were divided into the next groups (n=13):

1. Intact group (animals with oral administration of NaCl in equivalent doses)
2. Simulation of experimental TBI (control)
3. Simulation of experimental TBI + LHT 4–15
4. Simulation of experimental TBI + LHT 10–18
5. Simulation of experimental TBI + LHT 11–18
6. Simulation of experimental TBI + LHT 12–18.

All substances were injected at dose of 50 mg/kg (dose selection was carried out by the experiment), 30 minutes before the modeling of the pathology. Samples were dissolved with sodium chloride and injected intragastrically.

A day later, the indicator assessments of neurological deficit and behavioral status was researched [15].

Statistical processing of results

Descriptive statistics were used for all data. The data obtained were checked for normality of distribution. The type ща distribution was determined by the Shapiro-Wilk test. In the case of a normal distribution, the mean value (M) and the standard error of the mean (m) were calculated. Inter-group differences were analyzed using parametric (Student's *t*-test) or non-parametric (Mann-Whitney test) methods, depending on the type of distribution. Statistical analysis was performed using IBM SPSS Statistics 23 (USA) and Microsoft Office Excel 2010.

RESULTS AND DISCUSSION

During the experiment, it was found that injection of LKHT 4–15, LKHT 10–18, LKHT 11–18, LKHT 12–18 to mice at the dose range of 500-5000 mg/kg did not lead to changes compared with the usual behavior of rodents. When LKHT 4–15, LKHT 10–18, LKHT 11–18, and LKHT 12–18 were injected at dose of 10000 mg/kg, a decrease in behavioral activity was observed and, visually, a slight increase of respiratory rate in mice was recorded. Animals were localized on the periphery of the cage. There was no change in the skin and hair, mucous membranes of mice. The amount and quality of urinations and defecations were unchanged.

Based on the data obtained during the study of acute toxicity of LKHT 4–15, LKHT 10–18, LKHT 11–18, LKHT 12–18, it was not possible to determine LD_{50}, since no deaths of mice were recorded. Maximum injected dose 10000 mg/kg was selected as LD_{50} for further experiment, according to the protocol and design of the study.

The investigated derivatives of ethylthiadiazole were injected to rats at a dose of 50 mg/kg (1/200 of maximum injected dose).

The effect of ethylthiadiazole derivatives on indicators of neurological deficit in experimental animals with simulated TBI

For all animals of the experimental groups, the characteristic symptoms were: lethargy, tremor, weakness of the limbs, paresis. The cognitive dysfunction rats was recorded, pathological work of the forelimbs was observed: the animal pulled the forelimbs along with it, the fingers were clenched to the palm.

At the first days of experiment, the mortality rate was equal to 0% in all groups. On the 3rd day, the highest percentage of mortality – 23%, was in the groups with TBI without correction, with correction of LKHT 10-18 and LKHT 11–18 at the dose of 50 mg/kg. The lowest percentage (8%) of fatality on the 3rd day was registered in the group of animals injected by LKHT 12–18.

The highest mortality rate of animals in experimental groups was observed for 3 to 7 days. TBI caused the death of a high percentage of control group animals (46%). Injection of LKHT 11–18 at the dose of 50 mg/kg did not lead to a significant reduction in the number of deaths (38%). Average mortality rates were recorded in groups with LKHT 10–18 (31%) and LKHT 4–15 (23%). The lowest mortality rate was in group injected by the LKHT 12–18 (8%).

The effectiveness of LKHT 4–15, LKHT 10–18, LKHT 11–18, and LKHT 12–18 at the dose of 50 mg/kg on the neurological deficit of animals after TBI simulation was evaluated using the McGraw stroke score scale modified by I.V. Gannushkina and the psychometric neurological deficit score scale mNSS. The intact group didn't have neurological deficit.

The group without pharmacological treatment was taken as a control.

In this group, a neurological deficit of moderate severity was observed on 1st day – 4.04 points, with a tendency to worsen the severity by day 7th to 6.08 points.

One day after modeling the pathology, a severe degree of neurological deficit was recorded in the control group (4.04 points). In the group LKHT 12–18, the neurological deficit was mild (2.96 points). In groups with correction, LKHT 4–15, LKHT 10–18 and LKHT 11–18 occupied intermediate values (3.19, 3.65 and 3.88 points).

On the 3rd day, a neurological status decrease was observed in all groups, with the exception of the group with correction LKHT 12–18 (2.73 points).

On the 7th day, a neurological deficit decrease was observed in the groups with LKHT correction 12–18 (2.46 points) and using of Citicoline at a dose of 500 mg/kg (2.97 points). In the group LHT 4–15, the severity did not change (4.27 points). In the control groups LHT 10–18 and LHT 11–18, the severity changed to severe (6.08, 5.54 and 5.73 points).

Table 1 – Scale for neurological deficit according to McGraw modified by I.V. Gannushkina (1996)

Symptoms	Point
Lethargy, slowness of movement	0.5
Tremor	1.0
Unilateral half-tosis	1.0
Bilateral half-tosis	1.5
Unilateral ptosis	1.5
Bilateral ptosis	1.5
Manege movements	2.0
Paresis of 1–4 limbs	2.0–5.0
Paralysis of 1–4 limbs	3.0–6.0
Coma	7.0
Fatal outcome	10.0

Table 2 – Modified mNSS neurological symptom severity scale

Test	Point	Manifestations	Point
Hanging by the tail	0–3	Flexion of the forelimb	1
		Flexion of the forelimb	1
		Head displacement >10° from vertical axis for 30 sec	1
Physical activity	0–3	Without features	0
		Impossibility to move in a straight line	1
		Arena movements	2
		Falling to one side	3
Sensory tests	0–2	Front limb placement test	1
		Resistance to passive flexion of the limb in the ankle joint	1
Walking the crossbar	0–6	Steady posture	0
		Pinching one side of the bar	1
		Grasping the bar with sliding one of the limbs	2
		Grasping the bar with slipping of two limbs or rotating on the bar (> 60 sec)	3
		Unsuccessful attempt to stay on the bar, fall (>40 sec)	4
		Unsuccessful attempt to hold onto the bar, fall (> 20 sec)	5
		Falling without trying to hang or hold onto a beam (<10 sec)	6
Loss of reflexes, specific movements	0–4	Reflex of the external auditory canal	1
		Corneal reflex	1
		Startle reflex	1
		Convulsions, myoclonus, muscular dystonia	1

Table 3 – The effect of ethylthiadiazole derivatives on the dynamics of neurological disorders severity in accordance with McGraw score scale modified by I.V. Gannushkina (1996) (by the average score) (n=13)

Groups	Days		
	1	3	7
Intact animals	0	0	0
TBI	4.04	5.35	6.08
LKHT 4–15 (50 mg/kg)	3.19	4.00	4.27
LKHT 10–18 (50 mg/kg)	3.65	4.69	5.54
LKHT 11–18 (50 mg/kg)	3.88	4.85	5.73
LKHT 12–18 (50 mg/kg)	2.96	2.73*	2.46*
Citicoline(500 mg/kg)	3.97	4.33	2.97*

Note: * – $p<0.05$ in comparison with the control group of rats

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Table 4 – The effect of ethylthiadiazole derivatives on the dynamics of neurological disorders severity in accordance to the mNSS neurological assessment deficit scale (based on the average score) (n=13)

Groups	Period of time
	2nd day
Intact animals	0
TBI	10.69
LKHT 4–15 (50 mg/kg)	8.38*
LKHT 10–18 (50 mg/kg)	8.92
LKHT 11–18 (50 mg/kg)	9.83
LKHT 12–18 (50 mg/kg)	7.85*
Citicoline(500mg/kg)	8.00

Note: * – p<0.05 in comparison with the control group of rats

Table 5 – Results of the Open Field Test with intragastric injecttion of ethylthiadiazole derivatives (M±m; n=13)

Test indicatirs / Groups	Horizontal activity in the center, (m)	Horizontal activity in the outer zone, (m)	Rearing behavior	Hole Exploratory behavior	Grooming	Defecation	Urination
Intact animals	0.66±0.23	71.72±3.30	10.77±0.60	4.69±0.36	14.15±0.96	2.00±0.30	0,92±0,21
TBI	0.58±0.13	38.34±7.98	3.55±0.53	2.90±0.31	15.72±0.93	0.73±0.24	0.55±0.20
LKHT 4–15 (50 mg/kg)	1.25±0.20*	53.57±5.90	4.42±0.81	2.83±0.47	13.67±0.85	1,08±0,26	0.42±0.20
LKHT 10–18 (50 mg/kg)	0.77±0.09	55.92±6.29	3.90±0.45	3.09±0.65	14.18±0.86	0,91±0,25	0.55±0.21
LKHT 11–18 (50 mg/kg)	0.69±0.07	42.09±2.44	3.82±0.54	2.63±0.53	15.09±0.62	0,82±0,26	0.72±0.25
LKHT 12–18 (50 mg/kg)	1.5±0.11*	59.47±3.41*	5.92±0.38*	3.41±0.37	14.50±0.72	1,00±0,25	0.83±0.21
Citicoline (500 mg/kg)	1.44±0.22*	49.8±3.00*	4.60±0.53	3.40±0.35	12.78±0.60*	1,00±0,28	0.74±0.21

Note: * – p<0.05 in comparison with the control group of rats

Table 6 – Results of the Rota-rod Test with intragastric administration of the ethylthiadiazole derivatives (M±m; n=13)

Group	The latent period of the first fall	The total retention time of animals for 3 attempts
	72 hours	
Intact group	83.31±2.86	158.69±2.13
TBI	7.64±0.61	68.90±4.54
LKHT4–15	41.25±3.20*	95.58±1.09*
LKHT10–18	30.67±2.46*	81.18±1.33
LKHT11–18	24.18±1.98*	81.00±3.78
LKHT 12–18	49.83±3.39*	105.08±1.89
Citicoline(500 mg/kg)	35.73±3.65*	89.14±2.50*

Note: * – p<0.05 in comparison with the control group of rats

Injection of LHT 4–15 and LHT 12–18 with simulated TBI resulted in a marked decrease in the severity of neurological deficit in comparison with the control. Statistically significant (p<0.05) improvement in the severity of neurological deficit was registered in the group of animals injected by the LHT 12–18 at the dose of 50 mg/kg in comparison with the control group, the data obtained presented in table 3.

On the 2nd day, the rats injected by the LKHT 4–15, LKHT 10–18, LKHT 11–18, LKHT 12–18 at the dose of 50 mg/kg had more mildsymptoms of neurological deficit in accordance with mNSS scale in comparison with the control group of animals. But statistically significant differences (p<0.05) was observed only in groups LKHT 4–15and LKHT 12–18 at the dose of 50 mg/kg, data obtained presented in table 4. Rats of these groups had more pronounced motor skills compared with control group.

Besides the results of neurological deficit on two scales of pronounced neuroprotective activity in LKHT 4–15 and LKHT 12–18 at the dose of 50 mg/kg was revealed. In rodents of these groups, the lowest scores of neurological deficits and a higher level of motor activity of the limbs were recorded. The statistically significant differences in the two scales and the level of lethality LKHT 12–18 was identified as a leader.

The effect of new ethylthiadiazole derivatives on the indicators of the behavioral status in simulated TBI

The Open Field Test, the motor and exploratory ac-

tivity of intact animals was evaluated, as well as animals with simulated TBI without pharmacological treatment and administered with ethylthiadiazole derivatives. The data obtained presented in table 3.

Among the Open Field testing 72 hours after injury simulation showed that pharmacological treatment, motor and exploratory activity in comparison with the intact group was lower by 2 times and 3 times, respectively; movement in the field was chaotic with extremely rare peeks into holes and rearing behaviors, the rats did not investigated the entire area of the field.

Motor activity significantly decreased in all groups relative to intact animals. The LKHT 12–18 at the dose of 50 mg/kgcould significantly hinder the reduction of motor activity in the animals on the background of a traumatic brain injury, $p<0.05$ in comparison with the indicators of the control group from all groups.

The evaluation of exploratory activity was also decreased in all groups in comparison with intact animals. In the control group, the largest decrease in this indicator was still observed; the data obtained presented in table 5. Hole exploratory behavior in the groups injected by the LKHT 4–15, LKHT 10–18, LKHT 11–18, LKHT 12–18 did not decrease significantly, relative to the control group of animals. The number of rearing behaviors in the injected group by the LKHT 12–18 at the dose of 50 mg/kg increased by 1.5 times, statistically significant ($p<0.05$) relative to the control group. The number of defecations and urinations decreased not statistically significant. Grooming was statistically significantly different from the control in all groups ($p<0.05$).

The Rota-rod Test, the control group recorded a decrease in the retention time of animals on a rotating rod for 1 attempt (latent period) and 3 attempts, in comparison with the intact group, the data obtained presented in table 6. TBI simulation of the control group caused significant violations of strength and coordination. Regression of the total retention time relative to the intact group was determined. The injecttion of all ethylthiadiazole derivatives LKHT 4–15, LKHT 10–18, LKHT 11–18, LKHT 12–18 at the dose of 50 mg/kg significantly increased the retention time for 1 attempt on the rod ($p<0.05$) in comparison with the control. The highest indicators in this test for 1 attempt of retention were registered in groups of LKHT 4–15 – 5.5 times, LKHT 12–18 – 7 times higher than in the control. The lowest rates were observed in the group with TBI and LKHT 11–18 – only 4 times.

The total retention time of animals on the rod for 3 attempts statistically significant differences in groups LKHT derivatives 4–15 (1.4 times longer), LKHT 11–18 (1.25 times longer), LKHT 12–18 (1.5 times longer) at the dose of 50 mg/kg compared with the control ($p<0.05$).

Based on the results of the Rota-rod Test, the most promising compound for pharmacological treatment of the consequences of traumatic brain injury is LKHT 12–18 at the dose of 50 mg/kg.

DISCUSSION

For identification a promising neuroprotective compound among new ethylthiadiazole derivatives, the most significant and reliable indicators were found in the compound LKHT 12–18 at the dose of 50 mg/kg: the lowest percentage of mortality among groups with pharmacological treatment of pathology (8%); LKHT 12–18 administration to rats with simulated TBI led to a significant decrease in the severity of neurological deficit in comparison with the control, the decrease of the severity of neurological deficit in the group of animals administered with LKHT 12–18 at the dose of 50 mg/kg was statistically significant ($p<0.05$) in comparison with the control group; the evaluation of neurological deficit indicators using mNSS scale showed a pronounced neuroprotective activity of LKHT 12–18 at the dose of 50 mg/kg. In rodents of this group, the lowest scores of neurological deficits and a higher level of motor activity of the limbswere recorded. The number of rearing behaviors in the group of animals injected by LKHT 12–18 at the dose of 50 mg/kg increased by 1.5 times, statistically significant ($p<0.05$) relative to the control group. Based on the results of the Rota-rod Test, the total retention time of animals on the rod for 3 attempts significantly differed in the groups LKHT 4–15 (1.4 times longer), LHT 11–18 (1.25 times longer), LHT 12–18 (1.5 times longer) at the dose of 50 mg/kg compared with the control ($p<0.05$).

Based on the results obtained in this study, it is planned to study in more detail the LKHT 12–18 at the dose of 50 mg/kg using a complex of biochemical and morphometric research methods that will suggest a potential mechanism of action of this leader compound.

CONCLUSION

LHT 12–18 – an ethylthiadiazole derivative at a dose of 50/mg has the most pronounced neuroprotective effect in an experimental model of traumatic brain injury. Based on the results obtained in this study, a more detailed study of LHT 12–18 is planned using a complex of biochemical and morphometric research methods, which will suggest a potential mechanism of action of this leader compound.

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AUTHORS' CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ANALYSIS OF THE IMPLEMENTATION OF THE FEDERAL ASSURANCE PROGRAM OF SUPPORTING BENEFICIARIES WITH INDISPENSABLE MEDICINAL PREPARATIONS IN THE SUBJECTS OF THE RUSSIAN FEDERATION

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The aim of the research was to study the main indicative indicators of the implementation of The Federal Program "Provision of Essential Medicines" in 20 constituent entities of the Russian Federation based on the results of 2018 and 2019.

Materials and methods. The analyzed data were provided on the basis of the request cards specially designed by the health authorities of 20 subjects of the Russian Federation located in seven federal districts.

Results. It has been established that the funds allocated to the constituent entities of the Russian Federation, directly depend on the number of beneficiaries who retained the right to receive state social assistance in the form of a set of social services. These funds also correlate with the indicator "Population of the subject of the Russian Federation". In all the studied constituent entities of the Russian Federation, more than 50% of the total number of people who retained the right to preferential drug provision in 2018–2019, asked for medical help as part of the program "Provision of Essential Medicines". Herein, in the constituent entities of the Russian Federation, the average cost of one prescription amounted to 1,107.2 rubles in 2018 and 1,297.2 rubles in 2019. The estimated indicator "The average actual expenditures per 1 citizen entitled to state social assistance in the form of a set of social services, amounted to 1,723.0±90.2 rubles in 2018 and 1,526.8±80.5 rubles in 2019, which is higher than the approved input normative (823.3 rubles and 861.8 rubles in 2018 and 2019, respectively).

Conclusion. Thus, an excess of average actual expenditures per citizen entitled to state social assistance in the form of a set of social services, was notified over the standards established by the decrees of the Government of the Russian Federation. The revealed discrepancy between the normative and actual expenditures can also be an indirect confirmation of the fact that the most needy beneficiaries with chronic diseases remained in the program "Provision of Essential Medicines".

Keywords: assurance program of supporting beneficiaries with indispensable medicinal preparations, federal beneficiaries, medicine assistance

Abbreviations: GSA – government social assistance; MA – medicine assistance; MP – medicinal preparation; SSS – a set of social services; NIA – No information available; IA – inapplicable; SIMP – supporting with indispensable medicinal preparations.

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АНАЛИЗ РЕАЛИЗАЦИИ ФЕДЕРАЛЬНОЙ ПРОГРАММЫ ОБЕСПЕЧЕНИЯ НЕОБХОДИМЫМИ ЛЕКАРСТВЕННЫМИ ПРЕПАРАТАМИ В СУБЪЕКТАХ РОССИЙСКОЙ ФЕДЕРАЦИИ

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Цель. Изучить основные индикативные показатели реализации федеральной программы ОНЛП в 20 субъектах РФ по итогам 2018 и 2019 гг.

Материалы и методы. В исследовании анализировались данные, предоставленные на основании специально разработанных карт-запросов органами управления здравоохранением 20 субъектов РФ семи федеральных округов.

Результаты. Установлено, что объем выделенных субъектам РФ финансовых средств напрямую зависит от количества льготополучателей, сохранивших право на получение государственной социальной помощи (ГСП) в виде набора социальных услуг (НСУ), а также коррелирует с показателем «Численность населения субъекта РФ». Во всех исследуемых субъектах РФ более 50% граждан из общего числа лиц, сохранивших право на льготное лекарственное обеспечение, в 2018–2019 гг. обращались за лекарственной помощью в рамках программы ОНЛП, при этом в целом по субъектам РФ средняя стоимость одного рецепта составила 1107,2 руб. в 2018 г. и 1297,2 руб. в 2019 г. Расчетный показатель «Средняя сумма фактических затрат на 1 одного обратившегося гражданина, имеющего право на ГСП в виде НСУ» составила 1723,0±90,2 руб. в 2018 г. и 1526,8±80,5 руб. в 2019 г., что выше утвержденных нормативов (823,3 руб. и 861,8 руб. в 2018 и 2019 гг.).

Заключение. Таким образом, отмечено превышение средних фактических затрат на одного гражданина, имеющего право на ГСП в виде НСУ, над установленными постановлениями Правительства РФ нормативами. Выявленное несоответствие может являться косвенным подтверждением того, что в программе ОНЛП в основном остались самые нуждающиеся льготополучатели, имеющие хронические заболевания.

Ключевые слова: программа обеспечения необходимыми лекарственными препаратами, федеральные льготополучатели, льготное лекарственное обеспечение

Сокращения: ГСП – государственная социальная помощь, ЛЛО – льготное лекарственное обеспечение, ЛП – лекарственный препарат, НСУ – набор социальных услуг, нд – нет данных, нп – не применимо, ОНЛП – обеспечение необходимыми лекарственными препаратами.

INTRODUCTION

One of the main federal programs of medicine assistance (MA) is the assurance program of supporting beneficiaries with indispensable medicinal preparations (SIMP) in accordance with the standards of medical care through federal budget subsidies to the constituent entities of the Russian Federation (RF) [1–4]. The main aim of the program is to ensure a high-quality and timely provision of federal beneficiaries with medicinal preparations. The Program of supporting with indispensable medicinal preparations (SIMP), is implemented on the principle of social insurance, which involves the consumption of a set of social services (SSS) including drug provision. This Program concerns not all the citizens assigned to the persons entitled to benefits, but only those who have a real need for MPs on account of their diseases [2]. At the same time, since 2006, federal beneficiaries have been legally provided with the possibility of refusing the SSS and receiving a monthly cash payment [2, 4, 5].

The functioning of the medicine assistance (MA) system is of high social significance, as it helps to maintain beneficiaries' health's and increases the level and quality of their lives. However, at present, no more than 20% of residents of the Russian Federation (mostly the citizens with chronic diseases) have retained the right to receive

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benefits in kind under the SIMP program. Consequently, in the regions of the Russian Federation, there is a shortage of financial resources that can be used to purchase the necessary drugs for federal beneficiaries [1, 4, 5].

An important indicator of the SIMP program implementation in the constituent entities of the Russian Federation, is the amount of allocated funding; the proportion of the patients who have retained the right to receive the government social assistance (GSA) in the form of a set of social services; the proportion of the citizens who have applied for medical care under the SIMP program, actual costs per one beneficiary who have applied (taking into account pharmaceutical services); the number of prescriptions written; the average cost of one prescription. The indicators of the SIMP program implementation in various constituent entities of the Russian Federation have some differences, which are due to the structure of the beneficiaries' morbidity, the amount of funding, the range of purchased drugs, etc. [3].

THE AIM of the research was to study the main indicative indicators of the federal SIMP program implementation in 20 constituent entities of the Russian Federation based on the results of 2018 and 2019.

MATERIALS AND METHODS

A multicenter study was carried out on the territory of 20 constituent entities of the Russian Federation. The choice for the analysis of the Russian Federation constituent entities was due to the fact that they have different demographic, socio-economic and infrastructural indicators. Taking into account the fact that administrative and territorial structure of the Russian Federation is represented by 85 constituent entities, the sample of our study included about 24% of the country's regions. Based on the analysis of regional characteristics, the situation in the medicine assistance (MA) sector of federal beneficiaries of various federal districts of the Russian Federation, has been characterized.

The data analysis was carried out on the basis of request cards, specially developed by the government health agencies of 20 constituent entities of the Russian Federation in seven federal districts, including:

- 5 constituent entities of the Russian Federation of the Central Federal District: Belgorod, Voronezh, Smolensk, Tula regions; the city of federal significance Moscow;
- 4 constituent entities of the Russian Federation of the Volga Federal District (VFD): Kirovskaya, Samaraskaya oblasts, Chuvashskaya republic, Republic of Tatarstan;
- 4 constituent entities the Far Eastern Federal District (FEFD) of the Russian Federation: the Republic of Sakha (Yakutia), Sakhalin Region, Khabarovsk Territory, Zabaikalsky Territory;
- 2 constituent entities of the Russian Federation in the Southern Federal District (SFD): Astrakhan Region, Krasnodar Territory;
- 2 constituent entities of the Russian Federation of the Ural Federal District: Chelyabinsk, Kurgan regions;
- 2 constituent entities of the Russian Federation of the Siberian Federal District (SFD): Omsk Region, Altai Territory;
- 1 constituent entity of the Russian Federation of the Northwestern Federal District (NWFD): The Republic of Karelia.

The request cards were developed at the Department of Management and Economics of Pharmacy at Samara State Medical University (the Ministry of Health of Russia) and in structure, they corresponded to the objectives of the study.

The research program included a comparative assessment of the following data:

- the total amount of financing in the analyzed constituent entities of the Russian Federation (including the cost of pharmaceutical services);
- the number of beneficiaries who have retained the right to receive the government social assistance (GSA) in the form of a set of social services (SSS);
- the total amount of costs associated with medicinal products delivered up to beneficiaries;
- the average proportion of beneficiaries in the total number of the Russian Federation constituent entities;
- the proportion of beneficiaries who have retained the right to receive the government social assistance (GSA) in the form of a set of social services (SSS);
- the indicator of the appealability of beneficiaries for medical care under the SIMP program;
- the number of prescriptions under the SIMP program;
- the actual costs per one beneficiary who have applied (taking into account pharmaceutical services);
- the average cost of one prescription in the Russian Federation constituent entities.

In the analysis, the following kinds of methods were used: comparative, structural, logical and content. The statistical processing of the numerical material was carried out by methods of descriptive statistics, using the statistical software package IBM SPSS Advanced Statistics 24.0 No. 5725-A54 (IBM, USA). The statistical patterns were revealed in the generalized data. As for methods of generalization, they were represented by grouping and calculating of the summary indicators for the population as a whole, and for the selected groups. For all quantitative features, the arithmetic mean and root-mean-square (standard) errors of the mean were estimated, as well as the median, the determination of 10% and 90% percentiles. In the text, the descriptive statistics are presented as M ± SD, where M is the mean, and SD is the standard deviation for a normal distribu-

tion of a trait, or Med for an abnormal distribution of a trait. To determine the nature of the distribution of the obtained data, the Kolmogorov-Smirnov test with the Lilliefors normality test, and the Shapiro-Wilk test were used. The Lilliefors test is a normality test. To assess the relationship between the indicators, the Pearson correlation coefficient (r-Pearson) was used. The differences were considered significant if the probability was more than 95% ($p<0.05$)

RESULTS

To study the features of the federal SIMP program implementation in the Russian Federation, a comparative analysis of the main indicative indicators of the program has been carried out. Herewith, the results of studying 20 Russian Federation constituent entities based on 2018 and 2019, have been used. The analysis showed that the amount of the funds allocated to the Russian Federation constituent entities, depends on the number of the beneficiaries who have retained the right to receive the government social assistance (GSA) in the form of a set of social services (SSS) (r-Pearson in 2018 and 2019 – 0.95 and 0.94, respectively, $p <0.05$), and also correlated with the indicators "Population of the constituent entity of the Russian Federation" (r-Pearson in 2018 and 2019 was 0.98, $p <0.05$) (Tables 1, 2).

Table 1 shows that the amount of funding for the SIMP program implementation in the studied regions of the Russian Federation, varied from 204.8 to 7651.6 million rubles in 2018 and from 241.8 to 7744.8 million rubles in 2019, while the maximum amount of funding (more than 1 billion rubles) was in Moscow (the city of federal significance), Krasnodar Territory, the Republic of Tatarstan. In total, in 2019, the volume of MA financing, including pharmaceutical services, in the studied constituent entities of the Russian Federation amounted to 18.57 billion rubles, which was 0.5% more than in 2018 (18.48 billion rubles). The average change in the volume of financing in the constituent entities of the Russian Federation in 2019 relative to 2018, was (mean ± root-mean-square error) 3.13±1.10%.

Based on the data presented in Table 2, it can be concluded that the amount of MPs costs under the SIMP program, was directly related to the total amount of funding for the program (r-Pearson 0.94 in 2018 and 0.95 in 2019, $p<0.05$), the indicators "The number of beneficiaries who have retained the right to receive the government social assistance (GSA) in the form of a set of social services" and "The population of the constituent entity of the Russian Federation". In total, under the SIMP program, federal beneficiaries received MPs at the amount of 17.35 billion rubles in 2018 and 17.57 billion rubles in 2019. In 2018, the median of MPs costs amounted to RUB 487.0 million rubles (the range within 159.8–6,181.8 million rubles), in 2019 – 452.95 million rubles (the range within 151.5–4,912.1 million rubles). The average change in this indicator in 2019 relative to 2018 was +7.33±3.01%. The maximum values were also reported in Moscow, Krasnodar Territory and the Republic of Tatarstan.

The maximum number of federal beneficiaries (over 100,000 people), live in the same constituent entities of the Russian Federation that had the largest amount of funding for the program. The minimum number of federal beneficiaries (no more than 20 thousand people), is registered in the following constituent entities of the Russian Federation: Sakhalin and Astrakhan regions, Republic of Karelia (these regions had the smallest amounts of funding). In total, the number of federal beneficiaries in 2019 compared to 2018, practically did not change (–0.03%), the average change in the studied constituent entities of the Russian Federation was –1.29±0.95%. The maximum reduction in the number of federal beneficiaries was recorded in the Kurgan region (–14.41%), the largest increase in the number of beneficiaries was in the Voronezh region (+ 6.65%).

When analyzing the proportion of federal beneficiaries in the total population of the constituent entity of the Russian Federation, it was determined that the median value of this indicator was 2.6% (10th and 90th percentiles 2.2 and 3.1%, respectively) in 2018 and 2.5% (10th and 90th percentiles 2.2 and 3.0%, respectively) in 2019 (Table 3). The largest proportion of federal beneficiaries in the structure of the population of the Russian Federation constituent entity (more than 3.0%) is reported in the Republic of Sakha / Yakutia, Moscow and the Republic of Karelia.

Table 3 shows that the proportion of the persons who have retained the right to receive the government social assistance (GSA) in the form of a full set of social services (SSS), differs significantly in separate constituent entities of the Russian Federation, with the median being 27.3% (10^{th} and 90^{th} percentiles 18.6 and 62.8%, respectively) in 2018 and 25.2% (10^{th} and 90^{th} percentiles 17.4 and 59.8%, respectively) in 2019. More than 50% of citizens eligible for medicine assistance under the SIMP program, have retained the right to receive benefits in kind in the Republic of Sakha/Yakutia, Smolensk and Sakhalin regions. The lowest value of this indicator was reported in the Belgorod Region (13.6% in 2019). In general, in the analyzed period, the proportion of beneficiaries in the total population and the proportion of the persons who have retained the right to receive the government social assistance (GSA) in the form of a set of social services (SSS), has not undergone significant changes in the studied constituent entities of the Russian Federation.

More than 50% of the citizens from the total number of persons, who have retained the right to medicine assistance in 2018–2019, appealed to a medical organization, they were prescribed MPs under the SIMP program (Table 3). According to the indicator "Appealability of federal beneficiaries for receiving medicine assistance under the SIMP program", several typological groups of the constituent entities of the Russian Federation can be distinguished:

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Table 1 – Comparative analysis of the quantitative characteristics of the federal SIMP program implementation in the constituent entities of the Russian Federation in 2018–2019

No.	Constituent entity of the Russian Federation	Allocated funds for a year, mln. RUB			Medicinal preparations distributed at the amount of mln. RUB (excluding pharmaceutical services)			Number of beneficiaries who have retained the right to receive medicine assistance according to SIMP program (at end of reporting period), ths. people		
		2018	2019	Change compared to 2018	2018	2019	Change compared to 2018	2018	2019	Change compared to 2018
1	Mocow	7,651.60	7,744.78	1.22%	6,181.8	4,912.12	–20.54%	491.18	472.41	–3.82%
2	Krasnodar Territory	1,840.00	1,800.00	–2.17%	NIA	NIA	IA	NIA	NIA	IA
3	Republic of Tatarstan;	1,668.22	1,671.25	0.18%	1,298.54	1,562.88	20.36%	115.93	117.54	1.39%
4	Chelyabinsk region	981.60	992.30	1.09%	722.8	885.7	22.54%	76.28	76.65	0.48%
5	Altai Territory	784.60	786.10	0.19%	633.4	689.5	8.86%	59.12	58.80	–0.53%
6	Omsk region	686.70	675.20	–1.67%	572	612.9	7.15%	51.39	51.39	0.00%
7	Voronezh region	564.76	583.21	3.27%	524.45	526.42	0.38%	44.60	47.57	6.65%
8	The Republic of Sakha (Yakutia)	553.20	505.57	–8.61%	487.01	424.5	–12.84%	38.57	39.66	2.80%
9	Belgorod region	530.54	516.66	–2.62%	520.764	506.807	–2.68%	39.62	39.39	–0.59%
10	Tula region	495.20	478.20	–3.43%	418.2	481.4	15.11%	36.90	36.40	–1.36%
11	Khabarovsk Territory	434.70	375.30	–13.66%	298.98	309.35	3.47%	27.89	26.87	–3.66%
12	Zabaikalsky Territory	423.20	432.50	2.20%	319.2	348.2	9.09%	32.00	30.80	–3.75%
13	Kirovskaya oblast	410.80	402.69	–1.97%	398.04	378.99	–4.79%	31.86	31.37	–1.53%
14	Kurgan region	349.98	320.07	–8.55%	290.8	326.4	12.24%	25.39	21.74	–14.41%
15	Chuvash-skaya republic	344.21	309.65	–10.04%	207.3	243.9	17.66%	24.54	23.52	–4.17%
16	The Republic of Karelia	287.68	267.30	–7.08%	178.06	224.91	26.31%	20.13	19.96	–0.85%
17	Smolensk region	269.60	265.30	–1.59%	197.7	243.5	23.17%	20.82	20.70	–0.58%
18	Sakhalin region	204.80	198.2	–3.22%	159.8	151.5	–5.19%	14.30	14.60	2.10%
19	Astrakhan region	NIA	241.76	IA	NIA	203.52	IA	NIA	19.00	IA
20	Samaraskaya oblast	NIA	NIA	IA	923.2	910.31	–1.40%	70.44	69.48	–1.35%
	Total	18,481.39	18,566.04	+0.46%	17,346.34	17,574.10	+1.31%	1,762.07	1,761.54	–0.03%
	Mean change			–3.13 ±1.10%			+7.33 ±3.01%*			–1.29 ±0.95%

Note: NIA – No information available; IA – inapplicable; * – taking into account the actual volume of distribution in the Krasnodar Territory.

Table 2 – Correlation between indicators of the SIMP program implementation in 2018–2019

Indicator		Funds allocated for a year	Costs of dispensed MPs	Number of beneficiaries	Population	Proportion of patients who have retained the MA rights*	Appealability by results of the year**	Actual costs per month***	Number of prescriptions	Average cost of one prescription***
Funds allocated for a year	2018	1.00	0.98	0.95	0.98	0.86	0.44	0.90	0.85	0.75
	2019	1.00	0.96	0.94	0.98	0.86	0.61	0.90	0.86	0.56
Costs of distributed medicinal preparations	2018	0.98	1.00	0.99	0.95	0.93	0.53	0.97	0.80	0.74
	2019	0.96	1.00	1.00	0.95	0.93	0.64	0.98	0.81	0.59
Number of beneficiaries*	2018	0.95	0.99	1.00	0.93	0.95	0.58	0.99	0.77	0.71
	2019	0.94	1.00	1.00	0.93	0.95	0.63	0.99	0.77	0.59
Population	2018	0.98	0.95	0.93	1.00	0.79	0.42	0.88	0.84	0.73
	2019	0.98	0.95	0.93	1.00	0.79	0.51	0.89	0.82	0.60
Proportion of patients who have retained the MA rights*	2018	0.86	0.93	0.95	0.79	1.00	0.60	0.96	0.67	0.66
	2019	0.86	0.93	0.95	0.79	1.00	0.74	0.95	0.69	0.52
Appealability by results of the year**	2018	0.44	0.53	0.58	0.42	0.60	1.00	0.61	0.51	0.14
	2019	0.61	0.64	0.63	0.51	0.74	1.00	0.61	0.53	0.44
Actual costs per month***	2018	0.90	0.97	0.99	0.88	0.96	0.61	1.00	0.71	0.69
	2019	0.90	0.98	0.99	0.89	0.95	0.61	1.00	0.73	0.58
Number of prescriptions	2018	0.85	0.80	0.77	0.84	0.67	0.51	0.71	1.00	0.35
	2019	0.86	0.81	0.77	0.82	0.69	0.53	0.73	1.00	0.15
Average cost of one prescription***	2018	0.75	0.74	0.71	0.73	0.66	0.14	0.69	0.35	1.00
	2019	0.56	0.59	0.59	0.60	0.52	0.44	0.58	0.15	1.00

Note: * – The number of beneficiaries who have retained the right to receive the government social assistance (GSA) in the form of a set of social services (SSS); ** – Appealability by results of the year (proportion of the citizens who have been prescribed MPs, out of the total number of persons who have retained the MA rights); *** – Taking into account pharmaceutical services

Table 3 – Selected indicators of the SIMP implementation program in constituent entities of the Russian Federation in 2018–2019

No.	Constituent entity of the Russian Federation	Percentage of beneficiaries in the total population, %		Proportion of patients who have retained the MA rights, %		Appealability by results of the year (proportion of the citizens out of the total number of persons who were prescribed MPs, and have retained the MA rights), %	
		2018	2019	2018	2019	2018	2019
1	The Republic of Sakha (Yakutia)	4.0%	4.1%	63.1	63.1	57.0	98.8
2	Moscow	3.9%	3.7%	NIA	NIA	70.5	72.3
3	The Republic of Karelia	3.2%	3.2%	32.5	32.8	68.0	66.3
4	Kurgan Region	3.0%	2.6%	25.3	25.2	74.0	81.4
5	Zabaikalsky Territory	3.0%	2.9%	34.8	32.8	60.0	60.0
6	Republic of Tatarstan	3.0%	3.0%	34.4	35.2	80.6	80.2
7	Sakhalin Region	2.9%	3.0%	62.4	56.4	54.2	54.4
8	Omsk Region	2.6%	2.7%	28.8	28.6	60.1	56.7
9	Belgorod Region	2.6%	2.5%	NIA	13.6	63.8	61.6
10	Altai Territory	2.5%	2.5%	23.9	24.3	68.0	68.0
11	Kirovskaya Oblast	2.5%	2.5%	19.3	19.1	72.8	70.2
12	Tula Region	2.5%	2.5%	18.5	18.5	64.2	65.2
13	Samaraskaya Oblast	2.2%	2.2%	27.3	27.4	60.4	59.7
14	Smolensk Region	2.2%	2.2%	75.5	76.4	61.0	63.0
15	Chelyabinsk Region	2.2%	2.2%	22.0	22.0	100	100
16	Khabarovsk Territory	2.1%	2.0%	32.1	31.1	50.6	51.6
17	Chuvashskaya Republic	2.0%	1.9%	18.7	18.0	74.0	61.7
18	Voronezh Region	1.9%	2.0%	15.8	16.5	53.1	54.2
19	Astrakhan Region	NIA	1.9%	NIA	23.6	NIA	57.7
20	Krasnodar Territory	NIA	NIA	21,4	21,7	NIA	NIA
Mediana (10th and 90th percentiles)*		2.5 (2.1–3,5)	2.5 (2.0–3.4)	27.3 (18.6–62.8)	25.2 (17.4–59.8)	66.1 (60.1–74.0)	64.1 (58.7–76.3)

Note: * – for constituent entities of the Russian Federation with available information; NIA – No information available; IA – inapplicable

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Table 4 – Indicators of actual costs, the number of prescriptions and the average cost of one prescription under the SIMP program in the constituent entities of the Russian Federation in 2018–2019

No.	Constituent entity of the Russian Federation	The money actually spent on one beneficiary who appealed for MA per month, rubles (including pharmaceutical services)			Number of prescriptions, thousand pieces			Average cost of one prescription, rub. (including pharmaceutical services)		
		2018	2019	Change relativeto 2018, %	2018	2019	Change relativeto 2018, %	2018	2019	Change relativeto 2018, %
1	Moscow	2,960.8	2,769.4	–6.5%	4,898.9	3,176	–35.2%	1,261.9	1,546.6	22.6%
2	Republic of Tatarstan	1,105.8	1,327.5	20.1%	2,029.9	2,200.8	8.4%	729.4	794.4	8.9%
3	Krasnodar Territory	NIA	NIA	IA	980.8	1,178.2	20.1%	NIA	NIA	IA
4	Samaraskaya Oblast	NIA	NIA	IA	796	704,5	–11,5%	NIA	NIA	IA
5	Altai Territory	1,313.8	1,443.0	9.8%	740.7	789	6.5%	855.2	873	2.1%
6	Omsk Region	1,723.0	1,943.0	12.8%	616.5	657.6	6.7%	1,036.5	1,034.1	–0.2%
7	Kurgan Region	1,293.1	1,293.1	0.0%	551.7	559.6	1.4%	527.1	597.8	13.4%
8	Kirovskaya Oblast	1,523.7	1,526.8	0.2%	499.3	426.1	–14.7%	797	889	11.5%
9	Chelyabinsk Region	946.0	1,118.6	18.2%	492.2	409.7	–16.8%	1759.4	2,511.3	42.7%
10	Tula Region	1,469.3	1,689.6	15.0%	419	563.3	34.4%	998.5	854.7	–14.4%
11	Belgorod Region	1,717.4	1,741.1	1.4%	417.8	350.7	–16.1%	1,246.6	1,445.3	15.9%
12	Zabaikalsky Territory	1,095.0	1,162.0	6.1%	362.7	358.1	–1.3%	1,063	1,178	10.8%
13	Chuvashskaya Republic	1,846.5	1,400.7	–24.1%	329.6	320	–2.9%	628.9	762.2	21.2%
14	Voronezh Region	1,909.4	1,894.2	–0.8%	315.8	311.1	–1.5%	1,790.5	1,827.3	2.1%
15	The Republic of Karelia	1,751.6	1,406.6	–19.7%	314	357.1	13.7%	567.1	629.9	11.1%
16	Sakhalin Region	2,202.0	1,590.0	–27.8%	293	235.1	–19.8%	545	644	18.2%
17	The Republic of Sakha (Yakutia	1,839.8	903.2	–50.9%	232	188	–19.0%	2,099.2	2258	7.6%
18	Smolensk Region	2,285.9	2,380.4	4.1%	212.5	236.3	11.2%	930.2	1,030.4	10.8%
19	Khabarovsk Territory	1,764.5	1,859.8	5.4%	162.9	163.2	0.2%	1,987.8	1,895	–4.7%
20	Astrakhan Region	NIA	1,534.3	IA	NIA	78,9	IA	NIA	2,579.47	IA
Mean change				–1.2 ± 4.4%			–1.9 ± 3.8%			+10.0 ± 2.9%

Note: NIA – No information available; IA – inapplicable

1. regions with a high value of this indicator (over 70%): The Republic of Tatarstan, Chelyabinsk, Kurgan, Kirov regions, Moscow.
2. regions with a value of this indicator in the range from 60 to 70%: Chuvashskaya Republic, Republic of Karelia, Altai Territory, Tula, Belgorod, Smolensk, Samara, Omsk Regions, Zabaikalsky Territory.
3. regions with a value of this indicator in the range from 50 to 60%: the Republic of Sakha / Yakutia, Sakhalin, Voronezh regions, Khabarovsk Territory.

It should be notified that the indicator "Appealability of federal beneficiaries for medicine assistance under the SIMP program" does not depend on the proportion of the persons who retained the right to receive benefits in-kind under the SIMP program (r-Pearson 0.05 in 2018 and -0.04 in 2019, p> 0.05).

In 2018, the standard of financial costs for each federal beneficiary was 823.30 rubles per month, in 2019 it was 861.80 rubles [6]. It was established that the amount of actual costs (taking into account the costs of organizational measures – pharmaceutical services) per month per one beneficiary who appealed for MA, depends on the amount of funding to the program in the constituent entities of the Russian Federation (r-Pearson 0.90 in 2018-2019); from the number of beneficiaries who retained the right to MA under the SIMP program (r-Pearson 0.99 in 2018-2019); from the number of prescriptions (r-Pearson 0.73 in 2018, 0.71 in 2019) and from the average cost of one prescription (r-Pearson 0.58 in 2018, 0.69 in 2019) (all p <0.05).

The medians of actual costs in the studied constituent entities of the Russian Federation (10th and 90th percentiles) amounted to 1,723.0 rubles (10th and 90th percentiles of 1,101.5 and 2,235.6 rubles, respectively) in 2018 and 1,526.8 (1,144.6 and 2,118.0 rubles, respectively) in 2019. In general, in 2019, the actual costs per one beneficiary who appealed for MA (in relation to 2018), changed insignificantly (–1.2±4.4%). The largest relative increase in actual costs (≥15%) was reported in the Republic of Tatarstan, Chelyabinsk and Tula regions, the largest cost reductions (≥15%) – in the Republic of Sakha / Yakutia, Sakhalin region, Chuvash Republic, Republic of Karelia.

In the analyzed constituent entities of the Russian Federation in 2018–2019, more than 18 million prescriptions were issued annually under the SIMP program (Table 4).

According to the data presented in Table 4, for one federal beneficiary who appealed for MA (taking into account the proportion of the persons who retained the MA right under the SIMP program, and the appealability), an average of 18 prescriptions are issued per year (the range of 6–37 prescriptions and 5–31 prescriptions for one beneficiary who appealed for MA in 2018 and 2019, respectively). The largest decrease in the number of prescriptions in 2019 compared to 2018, was reported in Moscow (–35.2%), Sakhalin Oblast (-19.8%) and the Republic of Sakha / Yakutia (–19.0%). The largest relative increase in the number of prescriptions was recorded in the Tula region (+ 34.4%) and the Krasnodar Territory (+ 20.1%).

Based on the data in Table 4, it was determined that the average cost of one prescription (± root-mean-square error) under the SIMP program in 20 constituent entities of the Russian Federation, was 1,107.2 (±150.4) rubles in 2018 and 1,297.2 (± 144.2) rubles in 2019.

In 2018, the average cost of one prescription ranged from 527.1 rubles up to 2,099.2 rubles, in 2019 – from 597.8 up to 2,258.0 rubles. In general, there was a significant increase in the cost of one prescription in most constituent entities of the Russian Federation by 10.0 ± 2.9% on average, while the maximum increase was reported in the Chelyabinsk Region (by 42.7%), in Moscow (by 22.6%) and in the Chuvashskaya Republic (by 21.2%).

The indicators "Number of prescriptions" and "Average cost of one prescription" correlated with the total amount of funding for the program and the number of beneficiaries who retained the MA right under the SIMP program (p <0.05).

DISCUSSION

In the Russian Federation, as in many other developed countries of the world, there is a tendency towards centralized controlled prescription of drugs with the active use of digital technologies. The MA programs adopted here, are aimed at improving the quality of healthcare and ensuring control over the costs of drugs [3, 7–9]. At the same time, in foreign countries, the mechanisms for MA programs implementing, have specific features and differences of their own.

For example, in the USA there is no public health system [10, 11]. In this regard, people who do not have health insurance, cannot receive elective care, or they are unable to buy prescribed drugs [12, 13]. At the same time, there are fully funded and government-run health care programs in the United States. In the MA systems, the patients, healthcare providers, large medical groups and integrated supply systems are payers themselves. Some of the oldest government welfare programs are those created in 1965, to fund medical care for the poor (Medicaid) and elderly Americans over 65 (Medicare). Medicare also includes the disabled and the people with certain chronic diseases, such as those on dialysis for chronic kidney disease, and those who have become disabled due to other diseases, such as cancer. Medicare and Medicaid are a form of social security. They are funded from a set of taxes collected by the federal government but administered by the states. Therefore, the eligibility criteria and the amount of aid funded in these programs, may differ from state to state. Medicare is the closest thing to a community system that includes several components (inpatient care, outpatient care, private

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insurance), but all Medicare components have copayments, and those copayments exceed those of health plans in other countries [10, 13].

In Canada, prescription drugs provision is regulated at the government level of each province and territory, in accordance with a list of prescription drugs, which includes drugs for the provision of outpatient health care to selected categories of citizens (for example, the elderly and other benefit categories), as well as at the federal level (the government provides reimbursement of the cost of medicines to privileged categories of citizens belonging to aboriginal peoples and Inuit inhabitants). Despite the creation of the National Pharmaceuticals Strategy and the 10-Year Plan to Strengthen Health Care, adopted at the 2004 ministerial meeting, the efforts made were insufficient to develop an *All*-Canadian program of the population coverage with drugs in case of catastrophic medical expenses [14, 15].

In Great Britain, the established system of public health care and social security, was formally brought into action in 1948 [13, 16]. The main principle of the system was medical care on free-of-charge basis for all people living in the country. The system is funded from the state budget. The citizens pay for medications without a prescription from their own funds. As for prescription drugs, medical devices and services, England has a fixed co-payment; Wales, Scotland and Northern Ireland have no co-payment. More than 90% of medicines are available free of charge: for the citizens over 60 years old, children under 16 years old (or up to 19 years old if they are full-time students), patients with certain categories of diseases (type II diabetes mellitus, hypoparathyroidism, severe hypothyroidism, oncological diseases, epilepsy, myasthenia gravis, etc.), people with low incomes, pregnant women and those who gave birth in the previous 12 months, the disabled. The medicines used in hospitals, day hospitals, and medicines prescribed for the treatment of tuberculosis and sexually transmitted diseases do not require co-payment. For expensive medicines and technologies, the National Institute for Health and Clinical Excellence (NICE) conducts a cost-benefit pharmacoeconomic analysis. The more an intervention can save Quality Adjusted Life Years (QALYs), the more likely it is that NICE will make a positive recommendation on the preferential introduction of this technology into the national health system. This scheme does not allow solving the problem of high costs for expensive technologies [17].

One of the oldest systems for providing state social guarantees, is the German health care system [13]. The system is based on the existence of health insurance funds, which were formed on an industrial or regional basis. The health insurance funds are non-profit organizations that insure the risks associated with the diseases, and negotiate with doctors (or their associations) and drug manufacturers / suppliers regarding the cost of their services / goods. In Germany, social insurance does not include the citizens whose income exceeds certain thresholds. More than 70% of health care costs in Germany, are spent from social insurance funds. To reduce the growth in health care costs, a number of measures had been taken to reduce the cost of care and the demand for it. A negative non-refundable list had been created, and maximum covered prices had been introduced. In Germany, since 2006, there are also co-payments in the amount of 10% of the cost of the drug, if the costs are at least 5 euros, but not more than 10 euros. When spending on medicines exceeds 1% of the total household income for patients with chronic forms of the disease and 2% of the total household income for patients without chronic forms of disease, patients are exempted from co-payments [12].

In the Scandinavian countries, the health care system is characterized by tax (non-insurance) coverage of medical expenses and decentralization [16, 18]. In Finland, health care is financed from the municipalities' general tax revenues and from the social insurance system through the organization responsible for social insurance. In Finland, reimbursement of the drugs costs, is carried out under several schemes. If a drug was referred to the basic category (the drugs which had been planned to be paid for from public funds), then the patient's copayments will be 58% with a fixed component. Two other categories of drugs are special, and to be referred to these categories, it is necessary that they be used to treat certain diseases and have proven effectiveness. Patients' co-payments for the purchase of medicines referred to the first special category (used to treat bronchial asthma, arterial hypertension), are 28%. The medicines referred to the second special category (used to treat severe and / or life-threatening conditions such as malignant neoplasms and diabetes mellitus), are fully covered by the social insurance organization, but a patient has to pay a fixed fee (3 euros per purchase). The complexity of the system is the possibility of having a drug in more than one list [18].

In the People's Republic of China, it is currently planned to implement a program to co-finance the costs of drugs for the treatment of arterial hypertension, diabetes mellitus and some other diseases at the expense of health insurance funds, the share of reimbursable costs can reach 50% [19].

A system of multi-stage assessment of the cost-effectiveness of drugs, has been adopted by European countries. This allows the EU countries to include drugs with proven effectiveness for each therapeutic area and disease in the drug reimbursement systems, in contrast to the United States, where the cost of any purchased drugs can be reimbursed, which, along with lack of a reference price, can be a heavy burden for taxpayers [12, 13, 20, 21]. According to 2015 data, 64% of drugs costs in the United States, were paid directly by consumers or through private insurance. The level of the cost coverage in the EU countries is much higher, for example, in

Germany: at least 80% of the costs are paid by the state [12, 22].

In the Commonwealth of Independent States, there is a rather low level of economic accessibility of medicines for the population, in particular, in privileged categories, which is due to the extremely low level of financing of health care systems, a low solvent level of the population and the prevalence of the population expenditures in the structure of total financing of medicines, as well as irrational spending of funds in the existing systems of privileged drug provision [13, 23].

The experience of the Russian Federation in the implementation of federal MA programs, differs from the experience of foreign countries. The main feature is that in our country federal beneficiaries are not co-payers of their own drug provision, since the costs of drugs under the SIMP program, are fully reimbursed from the federal budget. Another characteristic feature is that in the Russian Federation, the amount of funding for SIMP program depends on the number of federal beneficiaries, while the program budget is formed on the basis of the principle of equal per capita funding [1–3, 23].

The results obtained in the course of the study, indicate a significant variation in the values of the indicative indicators of the SIMP program implementation, as well as their dynamics in various constituent entities of the Russian Federation. However, there is a number of universal objective laws.

The amount of funding for the program, is calculated according to the same criteria, based on the standard of monthly financial costs for each federal beneficiary. In this regard, it is logical that a linear correlation between the amount of funding for the program and the number of beneficiaries in the constituent entity of the Russian Federation, has been obtained. The correlation with the population size in a constituent entity of the Russian Federation, can be also explained by the relatively comparable (in most constituent entities of the Russian Federation) proportion of citizens eligible for privileged drug provision (from 1.9 to 4.1%).

The indicators "Actual costs for beneficiary per month" and "Average cost of one prescription" in the constituent entities of the Russian Federation vary, which may be due to differences in the contingent of beneficiaries, in the structure of their morbidity, as well as in the structure of the assortment of prescribed drugs. At the same time, it should be notified that the amount of actual costs for one beneficiary per month who appealed for MA in the surveyed constituent entities of the Russian Federation (which are to some extent a representative sample for assessing the SIMP program implementation in the Russian Federation as a whole), exceeded the standards of financial costs for one citizen receiving the government social assistance (GSA) in the form of a set of social services (SSS), per month. It should be also notified that this Regulation was established by the RF Government. After the recalculation, the average amount of actual costs per one citizen eligible for GSA in the form of SSS, amounted to 1119.7±90.2 rubles in 2018 and 1081.5±80.5 rubles in 2019, compared to the standards of 823.30 rubles and 861.80, respectively.

The results of the analysis confirm the previously identified problems in the field of medicine assistance (MA), caused by the massive refusal of federal beneficiaries to receive the government social assistance (GSA) in the form of set of social services (SSS). Since the majority of federal beneficiaries (about 80%) prefer to receive monetary compensation, the principle of social insurance is really violated. As a result, patients with severe illnesses, who also need expensive drugs, remain in the SIMP program predominantly [1, 14, 24]. Despite the fact that in this study, there are no obtained statistically significant relationships between the average cost of one prescription, actual costs, and the proportion of the citizens who retained the right for GSA in the form of SSS, this may mean the presence of a more complex relationship between these indicators (taking into account socio-economic and demographic features of the constituent entities of the Russian Federation). The revealed discrepancy between the standard and actual costs, can be also an indirect confirmation of the insufficient funds in the federal program to fully cover the need for drugs and the need to reorganize the existing system for the elimination of the imbalance in financing these social assistance measures [24, 25].

CONCLUSION

Thus, the experience of the Russian Federation in federal MA programs implementation, differs from the experience of foreign countries. The main feature is that in our country, federal beneficiaries are not co-payers of their own drug provision, since the costs of purchasing drugs under the SIMP program, are fully reimbursed from the federal budget. Another characteristic feature is that in the Russian Federation, the amount of funding for the SIMP program depends on the number of federal beneficiaries, while the program budget is formed on the basis of the principle of equal per capita funding.

On the example of 20 constituent entities of the Russian Federation from 7 federal districts, the differences in the indicative indicators of the SIMP program implementation in 2018-2019, have been revealed. It has been notified that the average actual costs per citizen eligible for government social assistance (GSA) in the form of set of social services (SSS), exceeded the standards established by the Regulations established by the RF Government.

Based on the analysis carried out, it can be concluded that the federal MA program for federal beneficiaries, needs further improvement. According to the authors' opinions, when implementing this program, it is advisable to use the lost insurance principle: the patients who need medical care, should be provided with drugs under the total budget of the medical material program.

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AUTHORS' CONTRIBUTION

All authors have equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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