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АРТЛЕГИА

Олокизумаб

Одобренное показание — патогенетическая терапия синдрома высвобождения цитокинов при новой коронавирусной инфекции (COVID-19) среднетяжелого и тяжелого течения [1]

Артлегиа включена в рекомендованные схемы терапии госпитализированных пациентов с COVID-19 [2]:

легкого течения
(пациенты с высоким индексом коморбидности)

среднетяжелого течения

тяжелого и крайне тяжелого течения

[1] Инструкция по медицинскому применению лекарственного препарата Артлегиа, регистрационное удостоверение ЛП-006218 от 21.05.2020.
[2] Временные методические рекомендации МЗ РФ «Профилактика, диагностика и лечение новой коронавирусной инфекции (COVID-19)». Версия 16 от 18.08.2022

Краткая инструкция по медицинскому применению лекарственного препарата АРТЛЕГИА

АРТЛЕГИА (олокизумаб), 160 мг/мл, 0.4 мл, раствор для подкожного введения
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Фармакотерапевтическая группа: антитела моноклональные
Показания к применению:
Терапия пациентов старше 18 лет с ревматоидным артритом средней или высокой степени активности в комбинации с метотрексатом, при недостаточной эффективности монотерапии метотрексатом или ингибиторами фактора некроза опухоли (иФНО). Патогенетическая терапия синдрома высвобождения цитокинов при новой коронавирусной инфекции (COVID-19) среднетяжелого и тяжелого течения.
Противопоказания:
Гиперчувствительность к олокизумабу, любому компоненту препарата в анамнезе. Активные инфекционные заболевания (в том числе и туберкулез). Детский возраст до 18 лет. Наследственная непереносимость фруктозы (препарат содержит сорбитол). Период грудного вскармливания.
С осторожностью:
У пациентов с серьезными или оппортунистическими инфекциями в анамнезе; с сопутствующими заболеваниями и состояниями, являющимися факторами риска развития инфекций (сахарный диабет, почечная недостаточность, прием иммуносупрессивных препаратов, пожилой возраст и др.). У пациентов, контактировавших с больными туберкулезом; с дивертикулитом или перфорациями кишечника в анамнезе и другими факторами риска перфорации кишечника; с нарушениями функции печени и печеночной недостаточностью. Применение у беременных систематически не изучалось. Если пациентка, получающая олокизумаб, забеременеет, она должна немедленно прекратить применение и обратиться к врачу. Предполагается, что ИЛ-6 играет важную роль в раскрытии шейки матки, поэтому применение олокизумаба может нарушать родовую деятельность. Не следует применять олокизумаб во время беременности за исключением тех случаев, когда имеется очевидная клиническая необходимость. Проникновение олокизумаба в грудное молоко не изучалось. Клинические данные о влиянии олокизумаба на фертильность у человека отсутствуют. В исследованиях на животных отрицательного воздействия олокизумаба на фертильность самцов и самок яванских макак не обнаружено.
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Нежелательные реакции, отмечавшиеся при терапии олокизумабом: очень часто: повышение активности АЛТ; часто: латентный туберкулез, фарингит, конъюнктивит, лейкопения, нейтропения, тромбоцитопения; повышение содержания липидов в крови; гипертензия; диарея, боль в животе; повышенная концентрация прямого и непрямого билирубина; повышенная активность печеночных ферментов, печеночных трансаминаз, АСТ; сыпь, дерматит; скелетно-мышечная боль; реакции в месте инъекции; повышение уровня ГГТ. нечасто: сепсис; грибковая инфекция кожи; лекарственная гиперчувствительность; гипотиреоз; сахарный диабет; мигрень; стенокардия; фибрилляция предсердий; тромбоз глубоких вен; интерстициальное заболевание легких; гастрит; миозит; почечная колика; маточное кровотечение.
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REVIEWS

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Models of neuroinflammation for the assessment of kappa-opioid receptor ligands

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The development of new drugs to combat neuroinflammation is highly relevant as it opens up possibilities for the treatment of a wide range of diseases, including Alzheimer's disease, Parkinson's disease, epilepsy, schizophrenia, depression, and others. Kappa-opioid agonists represent a promising class of compounds with a high potential to be used in the treatment of neurological conditions accompanied by neuroinflammation.

The aim of the study was to provide a summary of the current strategies employed to evaluate the neurotropic anti-inflammatory effects of kappa-opioid ligands in laboratory animals with induced neuroinflammation.

Materials and methods. The databases, such as Google Scholar, PubMed, ScienceDirect, Scopus, e-Library were used as search tools. The search comprised the following keywords and phrases in Russian and English: kappa opioids + neuroinflammation; kappa opioid receptors + neuroinflammation; neuroinflammation models; neuroinflammation models in rats, neuroinflammation models in mice. 148 relevant articles were found, 122 were included in this review.

Results. Various experimental models of neuroinflammation, including chemically-induced and bacterial endotoxin-induced neuroinflammation, as well as traumatic and genetic models in mice and rats were evaluated. In addition, the strengths and limitations of each model were critically assessed to identify the most appropriate and reliable approach for investigating the relationship between neuroinflammation and signaling pathways associated with kappa-opioid receptors.

Conclusion. The neurotropic anti-inflammatory activity of kappa-opioid ligands have been comprehensively described. The review discusses both experimental models where the effects of kappa-opioid agonists have been investigated, as well as the models where the anti-inflammatory properties of kappa-opioid agonists have not been studied yet.

Keywords: neuroinflammation; experimental models; neuroimmune processes; microglia; kappa-opioid receptors; kappa-opioid agonists; lipopolysaccharide

Abbreviations: Aβ – amyloid beta; AKT (PKB) – protein kinase B; CD – cluster of differentiation; CDK5 – cyclin dependent kinase 5; ERK – extracellular signal-regulated kinases; GFAP – glial fibrillary acidic protein; GluT3 – glucose transporter 3; GluT4- glucose transporter 4; GPCR – G-protein-coupled receptors; GSK3β – glycogen synthase kinase-3 beta; IFN – interferon; IL – interleukin; iNOS – Inducible nitric oxide synthase; IRF2 – interferon regulatory factor 2; JNK – c-Jun N-terminal kinase; LPS – lipopolysaccharides; MDA5 – melanoma differentiation-associated protein 5; MIP – macrophage inflammatory protein; mPGES-1 – microsomal prostaglandin E synthase-1; NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells; NGF – nerve growth factor; NLRP3 – nod-like-receptor family pyrin domain containing 3; NO – nitric oxide (II); nor-BNI – norbinaltorphimine; MAPK – mitogen-activated protein kinase; poly(I:C) – polyinosinic:polycytidylic acid; STAT3 – signal transducer and activator of transcription 3; PI3K – phosphoinositide 3-kinases; TLR3 – toll-like receptor 3; TLR4 – toll-like receptor 4; TNF-α – tumor necrosis factor alpha; TGF-β – transforming growth factor beta; ATP – adenosine triphosphate; ROS – reactive oxygen species; AD – Alzheimer's disease; PD – Parkinson's disease; GKS – glucocorticosteroids; BBB – blood-brain barrier; DNA – deoxyribonucleic acid; KOR – kappa opioid receptors; OPC – oligodendrocyte progenitor cell; OA – okadaic acid; ADEM – acute disseminated encephalomyelitis; RNA – ribonucleic acid; MS – multiple sclerosis; cAMP – cyclic adenosine monophosphate; TBI – traumatic brain injury; CNS – central nervous system; COX-2 – cyclooxygenase-2; EAE – experimental autoimmune encephalomyelitis.

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ОБЗОРЫ

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Подходы к изучению каппа-опиоидных лигандов на моделях нейровоспаления

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Разработка новых препаратов для коррекции нейровоспаления вызывает повышенный интерес, поскольку открывает возможности лечения широкого ряда заболеваний, включая болезнь Альцгеймера, болезнь Паркинсона, эпилепсию, шизофрению, депрессию и др. Каппа-опиоидные агонисты представляют собой перспективный класс соединений, обладающих высоким потенциалом применения при патологических состояниях, сопровождающихся развитием нейровоспаления.

Цель. Резюмировать информацию о текущих стратегиях, используемых для оценки нейротропных противовоспалительных эффектов каппа-опиоидных лигандов у лабораторных животных с индуцированным нейровоспалением.

Материалы и методы. В качестве средств поиска использовались поисковые системы и базы данных Google Scholar, PubMed, ScienceDirect, Scopus, e-Library. Поиск проводился по следующим ключевым словам и словосочетаниям: kappa opioids + neuroinflammation; kappa opioid receptors + neuroinflammation; neuroinflammation models; neuroinflammation models in rat; neuroinflammation models in mice, а также по их русскоязычным аналогам. Были найдены 148 релевантных статей, из которых 122 были включены в настоящий обзор.

Результаты. В настоящем обзоре были рассмотрены различные экспериментальные модели нейровоспаления, индуцированного химическими агентами и бактериальным эндотоксином, а также травматические и генетические модели на мышах и крысах. Кроме того, были критически оценены сильные стороны и ограничения каждой модели для определения наиболее подходящей стратегии исследования взаимосвязей между нейровоспалением и сигнальными путями каппа-опиоидной рецепторной системы.

Заключение. Рассмотрены особенности нейротропной противовоспалительной активности каппа-опиоидных лигандов. В обзоре обсуждаются как экспериментальные модели, в которых изучались эффекты агонистов каппа-опиоидных рецепторов, так и модели, в которых противовоспалительные свойства агонистов каппа-опиоидов еще не изучены.

Ключевые слова: нейровоспаление; экспериментальная фармакология; экспериментальные модели; нейроиммунные процессы; микроглия; каппа-опиоидные рецепторы; каппа-опиоидные агонисты; липополисахарид

Список сокращений: Aβ – бета-амилоид; AKT (PKB) – протеинкиназа B; CD – кластер дифференцировки; CDK5 – циклинзависимая киназа 5; ERK – киназы, регулируемые внеклеточными сигналами; GFAP – глиальный фибриллярный кислый белок; GluT3 – глюкозный транспортер тип 3; GluT4 – глюкозный транспортер тип 4; GPCR – рецепторы, сопряженные с G-белком; GSK3β – киназа гликогенсинтазы-3 бета; IFN – интерферон; IL – интерлейкин; iNOS – индуцибельная синтаза оксида азота; IRF2 – регуляторный фактор интерферона 2; JNK – c-Jun N-концевая киназа; LPS – липополисахарид; MDA5 – белок, ассоциированный с дифференцировкой меланомы 5; MIP – воспалительный белок макрофагов; mPGES-1 – микросомальная простагландин E-синтаза-1; NF-κB – ядерный фактор каппа-би; NGF – фактор роста нервов; NLRP3 – белок семейства Nod-подобных рецепторов с пириновым доменом 3; NO – оксид азота (II); nor-BNI – норбиналторфимин; APK – митоген-активируемая протеинкиназа; poly(I:C) – полиинозиновая-полицитидиловая кислота; STAT3 – сигнальный белок и активатор транскрипции 3; PI3K – фосфоинозитид-3-киназы; TLR3 – толл-подобный рецептор 3; TLR4 – толл-подобный рецептор 4; TNF-α – фактор некроза опухоли-альфа; TGF-β – трансформирующий фактор роста бета; АТФ – аденозинтрифосфат; АФК – активные формы кислорода; БА – болезнь Альцгеймера; БП – болезнь Паркинсона; ГКС – глюкокортикостероиды; ГЭБ – гематоэнцефалический барьер; ДНК – дезоксирибонуклеиновая кислота; КОР – каппа-опиоидные рецепторы; КПО – клетка-предшественник олигодендроцитов; ОК – окадаевая кислота; ОРЭМ – острый рассеянный энцефаломиелит; РНК – рибонуклеиновая кислота; РС – рассеянный склероз; цАМФ – циклический аденозинмонофосфат; ЧМТ – черепно-мозговая травма; ЦНС – центральная нервная система; ЦОГ-2 – циклооксигеназа 2; ЭАЭ – экспериментальный аутоиммунный энцефаломиелит.

INTRODUCTION

Neuroinflammation is defined as a common reaction in the brain and spinal cord that occurs in response to various provoking factors, such as infectious agents, a traumatic brain injury, a stroke, exposure to toxins, and others. While an inflammation is a necessary component of the recovery process after a traumatic injury, it can become pathological due to dysregulation of the immune response [1].

Neuroinflammation is a complex process that involves biochemical, histological, and systemic changes [2]. It is characterized by the production of cytokines,

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chemokines, and reactive oxygen species (ROS) by astrocytes and microglia, leading to an increased blood-brain barrier permeability, an immune cell infiltration into the nervous tissue, cerebral edema, and neuronal cell death. The clinical features of acute neuroinflammation are diverse and vary depending on the location of the affected tissue, but typically include chronic neuropathic pain [3], cognitive deficits, and apathy [4].

The primary role in the development of neuroinflammation is attributed to microglia cells, which are resident macrophages of the brain. In normal conditions, microglia cells participate in the synaptic function, cell apoptosis, and a neuronal activity. There are two primary phenotypes of microglia cells in the nervous system, M_1 and M_2. M_1, or pro-inflammatory microglia, produces pro-inflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), STAT3, IL-6, IL-12, IL-23, and ROS. In contrast, M_2 mediates anti-inflammatory and reparative processes by releasing IL-10, IL-4, IL-13, and transforming growth factor beta (TGF-β) [5].

Neuroinflammation is not only driven by microglia-produced pro-inflammatory mediators, but is also associated with the migration of B-lymphocytes, which release antibodies that target the myelin sheath and promote demyelination of the nervous tissue [6]. Additionally, cytokines can cross the blood-brain barrier from the systemic circulation, linking peripheral and central immune responses [7].

From a clinical perspective, neuroinflammation is a critical pathological component of various neuropsychiatric and neurodegenerative diseases. It significantly contributes to the development of neuropsychiatric conditions such as schizophrenia, multiple sclerosis, and Alzheimer's disease (AD) [8]. In AD, the aggregation of β-amyloid in the intercellular space leads to the formation of senile plaques and neurofibrillary tangles, which activate pro-inflammatory microglia [9]. In the postmortem analysis of patients with Alzheimer's disease (AD), as well as in experimental AD models, morphological changes are detected that indicate a microglial activation and inflammation in the tissues surrounding amyloid plaques.

In Parkinson's disease (PD), which is characterized by the degeneration of dopaminergic neurons in the substantia nigra and the presence of aggregated α-synuclein in Lewy bodies, there is evidence of a microglial activation and the release of pro-inflammatory cytokines. An increase in the concentration of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, as well as the activation of their corresponding receptors, has been observed [10].

The involvement of the immune system in psychiatric diseases has been well established through a large body of research. There are genetic associations between a major depressive disorder, a dysregulation of several immune cascades, and cytokine imbalances [11-13]. Patients with chronic inflammatory diseases, such as autoimmune diseases, have an increased risk of a developing depression. This risk is due not only to the psychological burden of the disease itself but also to the elevated levels of pro-inflammatory cytokines [14].

In the literature, there is evidence for increased risk of depression following immunotherapy, but the exact mechanism underlying this phenomenon is not fully understood. The administration of pro-inflammatory cytokines has been shown to induce a depressive-like state in the animal models, and depression is a common side effect of the interferon treatment [15]. In other cases, it is believed that peripheral immune cells and cytokines can cross the blood-brain barrier and impact the primary neurotransmitter systems involved in the pathogenesis of depression, including monoaminergic, glutamatergic, and GABAergic transmission [16].

Neuroinflammation also plays a role in epilepsy. Epileptogenesis is believed to result in the damage to the CNS tissue, creating foci of inflammation characterized by a predominance of the pro-inflammatory phenotype of microglia. This phenotype is characterized by an increased gene expression of various proteins, including inducible nitric oxide synthase and CD16/32 antibodies, as well as an increase in the release of certain inflammatory factors such as IL-1β, IL-6, and TNF-α [17].

Currently, the treatment of neuroinflammatory-associated CNS diseases with available pharmacological agents has been largely unsuccessful due to the limited efficacy or adverse side effects. Conventional therapy typically involves non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticosteroids (GCS), and other immunosuppressants [18].

Although the prior research has demonstrated the effectiveness of NSAIDs in managing various neurodegenerative processes, their usage has not been recommended yet due to the potential harm and lack of efficacy in treating or preventing dementia and cognitive decline. Despite a high efficiency in suppressing neuroinflammatory processes, a prolonged usage of corticosteroids is accompanied by various side effects, including mood disorders, gastrointestinal problems, headaches, and an increased risk of osteoporosis, diabetes, and infectious diseases.

Hence, the investigation of new preventive and therapeutic agents that target neuroinflammation is critical. The class of kappa agonists appears to be a promising option that can significantly decrease neuroinflammatory processes and mitigate the severity of associated diseases [19].

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THE AIM of the study was to provide a summary of the current strategies employed to evaluate the neurotropic anti-inflammatory effects of kappa-opioid ligands in laboratory animals with induced neuroinflammation.

MATERIALS AND METHODS

The databases, such as Google Scholar, PubMed, ScienceDirect, Scopus, e-Library were used as search tools. The search comprised the relevant keywords and phrases shown in Fig. 1. The preference was given to the studies that used rats and mice as experimental animals, owing to the widespread availability and thorough knowledge of their metabolic characteristics.

A total of 148 articles published between 1975 and 2022 were initially identified. After an initial screening of the titles and abstracts, 26 duplicate and irrelevant articles were excluded, as their design did not include descriptions of aspects or patterns of the neuroinflammation process, the effects of kappa-opioid receptors, and the experimental animal models applicable to modeling the similar pathological conditions. A total of 122 publications were included in the review.

RESULTS AND DISCUSSION

1. General characteristics of kappa-opioid receptors

Opioid receptors are members of the G protein-coupled receptor family and are predominantly distributed in the nervous system. There are four main subtypes of opioid receptors: delta (δ), kappa (κ), mu (μ), and nociceptive receptors [20]. Opioid receptors play a significant role in mediating various pharmacological effects, including analgesia [21], sedation [22], anticonvulsant [23], neuroprotection, and some others [24]. The recent evidence has established that kappa-opioid receptors are also present on immune cells [25], and their activation leads to the secretion inhibition of IL-6, TNF-α, and IL-1β. This mechanism contributes to the attenuation of immune responses [26].

Upon the activation, G protein-coupled receptors, including kappa-opioid receptors, trigger a cascade of intracellular signaling pathways. Specifically, the receptor causes the α subunit of the G-protein to dissociate from the β and γ subunits, and these subunits subsequently affect downstream intracellular signaling proteins or target functional proteins. $G\alpha_i$ inhibits adenylate cyclase, which is involved in the synthesis of cAMP, and activates potassium channels in the postsynaptic neuron. At the same time, $G\beta_\gamma$ causes the closure of voltage-gated calcium channels in the presynaptic neuron. Overall, this process results in a reduction of the neuron's responsiveness to excitatory neurotransmitters and a decrease in their secretion [27].

A number of kappa-opioid agonists can exhibit biased agonism [28], which is considered as a ligand-dependent selectivity for certain signal transduction pathways relative to a reference ligand at the same receptor. When the kappa receptor is activated, two intracellular pathways are involved: adenylate cyclase mediated and β-arrestin mediated. The second pathway leads to the phosphorylation and activation of the mitogen-activated protein kinase p38 (p38 MAPK) and its activation, causing the translocation of the SERT gene and dysphoria.

The activation of p-STAT3 and suppression of caspase-3 are among the mechanisms through which kappa-opioid receptor (KOR) agonists can modulate neurodegeneration and neuroinflammation [29, 30]. KOR agonists may also reduce glutamate excitotoxicity by inhibiting the level of free Ca^{2+} in synaptosomes and the release of presynaptic glutamate by closing N-type Ca^{2+} channels [31-33].

KOR agonists have been reported to have a positive effect on remyelination and oligodendrocyte maturation in autoimmune diseases, in addition to their anti-inflammatory properties. This effect is believed to be due to the activation of the GPCR kinase, the MAPK family (ERK 1/2, p38, and JNK), as well as the JAK2/STAT3 and IRF2 cascade [27]. The ERK 1/2 and JAK2/STAT3 pathways seem to be the most promising in terms of both clinical efficacy and safety. Oligodendrocyte progenitor cells (OPCs) increase a pSTAT3 expression in the areas of enhanced oligodendrogenesis after the nerve tissue injury, while the deletion of STAT3 in OPCs reduces the oligodendrocyte differentiation during the cell growth and development [31]. Thus, STAT3 phosphorylation in the oligodendrocyte lineage cells may be one way in which KOR agonists mediate their promyelinating effects in synergy with ERK1/2 signaling [34].

2. Mechanisms of neuroinflammation in different experimental models

2.1. Models based on immune response

2.1.1 LPS-induced neuroinflammation

This is one of the most widely used models suitable for both *in vivo* and *in vitro* studies. The administration of lipopolysaccharide (LPS) results in the formation of the LPS-CD14 complex, which activates microglia and causes the release of pro-inflammatory mediators: cytokines, chemokines, and proteins of the complement system. The manifestations of the LPS-induced inflammation can differ considerably depending on various factors such as

the mode and duration of the administration, as well as the age of the animals under study.

Microglia cells play a key role in the pathogenesis of neuroinflammation. Microglia can polarize into M_1 (a pro-inflammatory phenotype) or M_2 (an anti-inflammatory phenotype) after the activation. The M_1 phenotype is associated with the production of pro-inflammatory cytokines, which can lead to the nerve cells damage, astrocyte apoptosis, and the blood-brain barrier disruption, while the M_2 phenotype exerts the opposite effect [35].

The relationship between the LPS-induced neuroinflammation and the kappa opioid receptor system has been well studied. LPS has been observed to decrease the expression of KOR. It is hypothesized that the reduction in the KOR function triggers the activation of microglia, leading to the production of pro-inflammatory cytokines within the brain [36].

Dynorphins, the main KOR ligands, are known to induce the M_2 polarization of microglia *via* the suppression of the TLR4/NF-κB pathway. This effect has been demonstrated in the LPS-stimulated BV-2 microglial cells. Conversely, the treatment with the KOR inhibitor GNTI resulted in an opposing effect [19].

It is noteworthy that the administration of dynorphin-A (an endogenous KOR agonist) led to the inhibition of the LPS-induced CD16/32 expression and the suppression of M_1 cytokine production, including IL-1β and IL-6. A comparable outcome following the treatment with the selective kappa agonist U50,488 was observed, and the effect was attenuated by the KOR antagonist nor-binaltorphimine (nor-BNI) [37]. Moreover, dynorphin-A increased the CD206 expression and stimulated the production of M_2-associated cytokines (IL-4 and IL-10) in the LPS-stimulated BV-2 cells. In contrast to dynorphin-A, the selective kappa-opioid receptor antagonist GNTI produced opposing effects and prevented dynorphin-A-mediated polarization of BV-2 microglia into the M_2 phenotype [19].

In the central nervous system, microglia cells are distributed unevenly [38], with the highest expression of the mRNA encoding KOR found in the striatal region [39]. Further research is needed to investigate whether there are regional differences in the response of microglia to the stimulation or blockade of KOR. The role of microglial kappa-opioid receptors in the regulation of neuroinflammation remains unclear, as the influence of circulating pro-inflammatory cytokines released by other immune cells also plays a role [36]. It is important to consider the age and sex of the individuals exposed to LPS, since the administration of LPS at an early age can provoke (depending on sex) different responses in behavior and relative expression levels of pro- and anti-inflammatory factors [40, 41]. It has been reported that in the young rats, in contrast to more mature ones, the level of IL-1β increased to a greater extent during the development of neuroinflammation, but the level of interferon-γ increased to a lesser extent [42].

It has been demonstrated that LPS increases the levels of TNF-α and IL-1β by interacting with Toll-like receptor 4 (TLR4) [43]. TNF-α and IL-1β levels are regulated by a nuclear factor κB (NF-κB). Although these cytokines are essential components of the innate immune response, their excessive expression can lead to endotoxemia. The studies have shown that the kappa-opioid agonists U50,488 and salvinorin A reduce the levels of TNF-α and IL-10 (but not IL-1β) in the LPS-stimulated peritoneal macrophages [44, 45].

In summary, despite the significant insights gained from the current model, further investigation is required to uncover the complete network of interactions between the KOR and the neuroinflammation pathways induced by LPS.

2.1.2. Neuroinflammation induced by polyriboinosinic polyribocytidylic acid

Polyinosinic-polycytidylic acid (poly(I:C) is a synthetic analogue of double-stranded RNA that, when administered to experimental animals, triggers systemic inflammation, ultimately leading to the development of neuroinflammation [46].

Based on this, a model wherein a single intravenous injection of poly(I:C) was proposed. It is given to pregnant female rats on the 17 days of gestation (GD17). The exposure of the mouse offspring to a systemic immune challenge during a specific time window in late gestation (GD17) increases their susceptibility to the age-related brain pathology and cognitive impairment [47].

In this case, the mechanism of neuroinflammation is mediated by the TLR3-induced microglial activation [48], followed by the NF-kB-dependent induction of pro-inflammatory cytokines, including IL-1β, IL-6, IL-8, TNF-α, and types I and II interferons [49]. In turn, kappa-opioid agonists are able to suppress the development of inflammation by reducing the level of these cytokines, primarily IL-1α, IL-1β, IL-2, and IL-17 [50].

It is reported that poly(I:C) enhances the synthesis of the enzymes associated with the prostaglandin E2 production through the activation of various microglial signaling pathways. In addition, TLR4-associated signaling, which plays an important role in the synthesis of COX-2 and mPGES-1, is also enhanced. Thus, it is proposed that these two TLR3 mechanisms independently mediate the neuropathological effects of poly(I:C) [51, 52].

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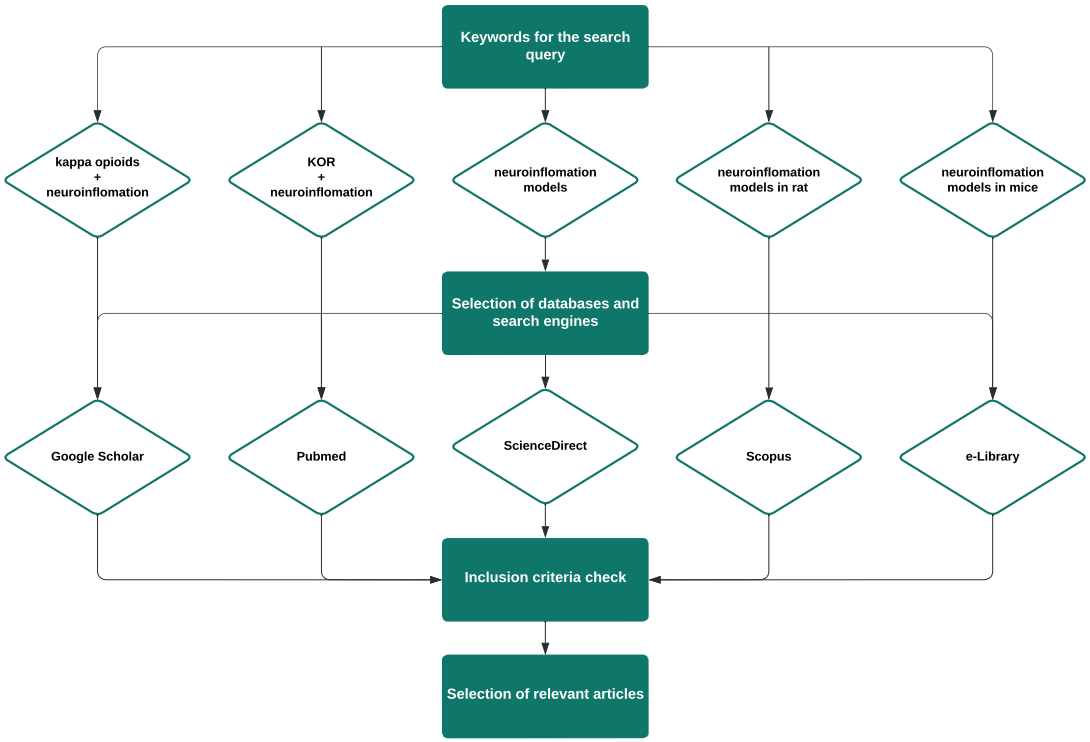

Figure 1 – Flowchart describing methodology for searching and processing literature data

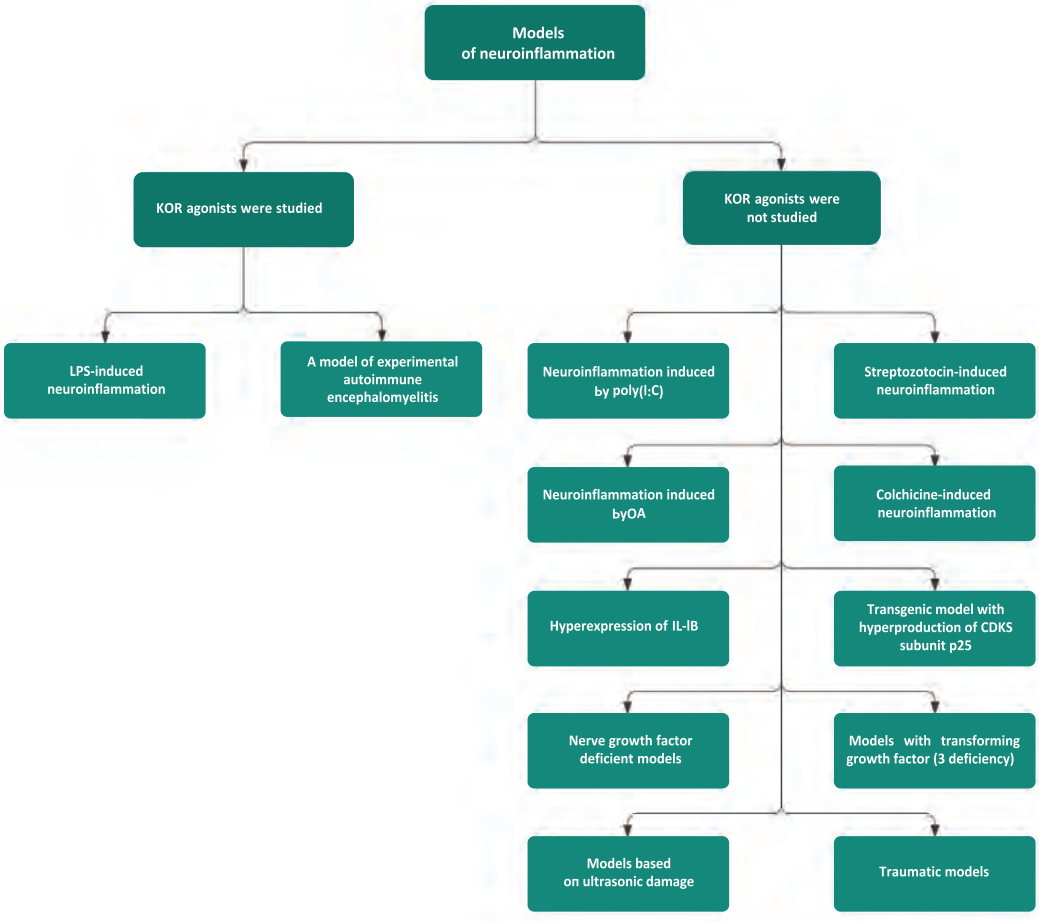

Figure 2 – Experimental models of neuroinflammation for assessing the efficacy of kappa-opioid receptor agonists

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Poly(I:C) can engage other mechanisms. In the mice with an inactivated MDA5 gene (MDA5$^{-/-}$) the administration of poly(I:C) did not result in an elevation of serum IFN-γ levels compared to wild-type mice. The production of IL-6 and IL-12p40 was also impaired in MDA5$^{-/-}$ mice [51]. Moreover, poly(I:C) can also trigger the formation of NLRP3 inflammasomes *via* TLR3- and MDA5-independent pathways [54]. At the same time, the recent studies have shown that the NLRP3-mediated neuroinflammation is closely associated with the secondary brain damage after intracerebral hemorrhage [55].

Poly(I:C) activates MAPKs, which are important regulators of the expression of the inflammatory mediators [52]. For example, the activation of ERK promotes an increase in the production of nitric oxide and IL-1β in macrophages [56].

2.1.3. Model of experimental autoimmune encephalomyelitis

The experimental autoimmune encephalomyelitis (EAE) model is mainly used as an animal model of autoimmune inflammatory diseases of the CNS, usually in the studies of multiple sclerosis (MS). This model is instrumental in studying the pathogenesis of MS and evaluating new therapeutic interventions for its prevention and treatment. Additionally, certain variations of the EAE model can reproduce other, less prevalent CNS inflammatory diseases like monophasic acute disseminated encephalomyelitis (ADEM) or optomyelitis (Devic's disease).

The EAE consists of two phases: the induction phase and the effector phase. The induction phase involves the priming of myelin epitope-specific CD4$^+$ T cells by the myelin or myelin antigen injection. During the effector phase, these activated myelin-specific T cells migrate into the CNS and produce chemokines and cytokines that trigger an influx of peripheral mononuclear phagocytes into the CNS. The cytokines produced by T cells also activate peripheral monocytes and CNS-resident microglial cells. This activation leads to the axonal demyelination, the process mediated by the phagocytic activity of the activated mononuclear cells, along with the inflammatory and cytotoxic effects of cytokines such as IFN-γ, TNF-β, IL-17, TNF-α, and NO, which are released from the activated CD4$^+$ T cells and monocytes [57]. Typically, between 7 to 12 days following immunization, the inflammatory cells infiltrate the CNS and cause the destruction of the myelin sheath, leading to the movement impairments and a gradual onset of the hind limb paralysis [58]. A passive or adoptive-transfer EAE (AT-EAE) can be induced in the recipient animals by transfer of the pathogenic myelin-specific CD4$^+$ T cells that were generated in the donor animals *via* an active immunization. This process involves only the effector phase of the immune response [59].

The previous studies suggest that the activation of KOR prevents the progression of multiple sclerosis. For example, the administration of nalfurafine and U50,488 contributed to the restoration and remyelination of the nervous tissue after EAE. This effect was blocked by the KOR antagonist nor-BNI, indicating that nalfurafine mediates the recovery from EAE in a KOR-dependent fashion [2]. In addition, the administration of nalfurafine resulted in a reduction of the CNS infiltration with the CD4$^+$ and CD8$^+$ T cells, and also improved the immune homeostasis by suppressing Th17 responses [2].

2.2 Neurotoxic models

2.2.1 Streptozotocin-induced neuroinflammation

Streptozotocin is currently the most basically used agent in the diabetes mellitus research [60]. As the molecule is unable to cross the blood-brain barrier, its systemic administration does not result in a direct impact on the brain cells [61]. Nonetheless, several studies have suggested that laboratory animals with diabetes mellitus develop neuronal degeneration in the frontal lobes and hippocampal atrophy [62]. It is believed that these effects are linked to the pathological mechanisms such as an oxidative stress, which arises from the production of hydrogen peroxide and nitric oxide, as well as the DNA damage. [63, 64].

Neuroinflammation is induced by an intravenous or intracerebroventricular streptozotocin injection. The intracerebroventricular administration of streptozotocin (1–3 mg/kg) provokes neurodegenerative symptoms that resemble those found in Alzheimer's disease (AD) [65].

The manifestation of neurodegeneration following a streptozotocin injection can vary between 1-6 weeks depending on the laboratory animal species and their unique traits [66, 67]. It should be emphasized that in this model not only neuroinflammation but also other manifestations of AD are observed.

Several authors believe that a decreased PI3K/AKT signaling activity and the intraneuronal glucose metabolism are key mechanisms for the development of AD, both in natural conditions and in the experimental model [68]. The studies on epilepsy have demonstrated that dynorphin acting on the KOR can engage the PI3K/AKT pathway, leading to neuroprotection [69].

In the periphery, the insulin's primary role is to lower a blood glucose concentration *via* GluT4. Although GluT4 is mostly found in the adipose and muscle tissues, it has also been discovered in the hippocampus. Researchers

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suggest that under the conditions of the increased energy demand, GluT4 can transfer glucose to the brain cells, performing an auxiliary function along with GluT3, the main neuronal transporter. GluT4 plays an important role in memory processes, and therefore, a decrease in the activity of this transporter may underlie cognitive impairments associated with the insulin resistance [70]. The kappa-opioid receptor agonists can reduce diabetes mellitus symptoms (including neuroinflammatory processes caused by diabetes mellitus) through the GluT4 translocation and adiponectin phosphorylation, including neuroinflammatory processes caused by diabetes mellitus [71].

The neuroprotective effect of kappa-opioid agonists is linked to the reduction of the ROS production and an oxidative stress, a key component of neuroinflammation in the streptozotocin-induced model [72]. However, at least one study found out that KOR agonists can stimulate the ROS production by activating JNK. It remains unclear how the KOR-mediated ROS production contributes to neurotoxicity [73].

2.2.2 Neuroinflammation induced by okadaic acid

Okadaic acid (OA) selectively inhibits the activity of protein phosphatase 2A [74], which is one of the factors involved in the pathogenesis of AD and has been linked to neuroinflammatory diseases [75]. A neurotoxic effect of OA includes hyperphosphorylation of the tau protein, cell apoptosis, beta-amyloid deposition, an oxidative stress, neuroinflammation [76-78], which are accompanied by cognitive impairments, in particular, memory disorders [79, 80].

Some publications [81, 82] point out to the fact that the drugs designed to treat dementia may be also effective in treating the symptoms caused by the administration of OA. This makes it possible to suppose that neuroinflammation is the secondary consequence of neurodegeneration. In this regard, an experimental model involving the intrahippocampal administration of OA was proposed to induce an oxidative stress [79].

The studies found out that the kappa-opioid agonist U50,488H reduces serum levels of TNF-α, IL-1β and IL-6, suppresses the expression of NF-κB in the hippocampus, and decreases the rate of apoptosis of hippocampal neurons in the rats, indicating its effectiveness in combating an oxidative stress and neuroinflammation [83]. These cytokines can be used as markers when studying kappa-opioid ligands in the model of neuroinflammation induced by OA.

2.2.3 Colchicine-induced neuroinflammation

Colchicine is a known cytotoxic agent that disrupts the axoplasmic transport, leading to the neuronal death [84]. Recent investigations have revealed that a systemic administration of colchicine can elevate the concentration of COX-2 in the cytoplasm of cells [85], thereby inducing neuroinflammation and specific cognitive and behavioral symptoms [82].

In the model of colchicine-induced neuroinflammation, KORs can mediate neuroprotective effects by reducing the excessive production of iNOS, COX-2, TNF-α, and IL-1β, as demonstrated in the study of the LPS-induced inflammation in alveolar macrophages [86]. Furthermore, KORs have been found out to impact an oxidative stress. For instance, the endogenous KOR agonist dynorphin-A has been shown to decrease an oxidative stress during epileptiform discharges in hippocampus [69].

2.3. Genetically determined models

2.3.1. Hyperexpression of IL-1ß

Neuroinflammation is characterized by an increased production of IL-1β [87]. This led to the creation of a transgenic mouse line overexpressing interleukin-1β [88]. The elevated levels of IL-1β lead to the development of microgliosis, astrogliosis, and a chronic increase in pro-inflammatory agents. However, this model shows no significant change in the synthesis of the beta-amyloid precursor protein, and the number of amyloid plaques may even decrease, setting it apart from other models [89]. Furthermore, despite significant cognitive impairments, neuronal apoptosis is not histologically detected in this model [90]. Therefore, this model can be used to reproduce neuroinflammation without neurodegenerative changes.

Kappa-opioid receptor agonists may act in this model by reducing the levels of IL-1α, IL-1β, IL-2, and IL-17 [91] and inhibiting the Nod-like receptor of the NALP family (NLRP3), thus suppressing the inflammatory process.

2.3.2. Transgenic model with hyperproduction of cyclin-dependent kinase 5 (CDK5) subunit p25

Based on the available literature data, the changes in the expression of some cell cycle proteins can serve as a biomarker and a cause of neuroinflammatory processes. Cyclins and cyclin-dependent kinases, such as CDK5, have received a particular attention [92]. Normally, the p35 subunit forms a complex with CDK5 and regulates corticogenesis, a synaptic vesicle metabolism, a neurotransmitter release, and a signal transduction in brain cells [93]. However, under neurotoxic conditions, an aincreased cleavage of p35 into p25 by calcium-dependent kinase leads to the CDK5 dysregulation and neurotoxic effects [94, 95].

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Similar phenomena have been observed in patients with neurodegenerative diseases [92], and it is believed that the p25-CDK5 complex induces hyperphosphorylation of the tau protein [96]. In the animal models, this reaction develops in response to neuroinflammation, which is characterized by astrocytosis and elevated levels of pro-inflammatory cytokines, including TNF-α, IL-1β, and MIP-1α [97].

In mice, the pathological changes sequentially appear in the order as below: the onset of neuroinflammation (at the end of the 1st week), hyperphosphorylation of the tau protein (at the 5th week), cognitive deficits (at the 7th week), and finally, accumulation of amyloid plaques (at the 9th week) [98, 99].

2.3.3 Nerve growth factor deficient models

This model is based on the use of transgenic laboratory animals that express antibodies against a nerve growth factor (NGF). In such animals, the development of neurodegenerative processes is evident, which is characterized by visual recognition and spatial memory deficits, neuronal degeneration, cholinergic insufficiency, hyperphosphorylation of the tau protein, and the formation of β-amyloid plaques [100, 101]. At the biochemical level, the expression of various pro-inflammatory cytokines, such as IL-1β, TNF-α, and IFN-γ-induced ATPase, is also observed, which occurs due to the development of an autoimmune reaction [102].

Agonists of KOR can attenuate the progression of this process *via* the inhibition of the TLR4-dependent signaling pathway in neurons of the peripheral nervous system, resulting in the reduced production of pro-inflammatory interleukins and an immunosuppressive effect [103].

2.3.4. Models with transforming growth factor β deficiency

A transforming growth factor-β (TGF-β) is a cytokine involved in various parts of the inflammatory process. Its effects depend on the type of the target cells, the cellular environment, as well as the degree and duration of exposure [104]. In the CNS, TGF-β is produced by both neurons and glial cells [105]. In particular, TGF-β1 has a protective effect in neuropathies by preventing the glia activation, reducing the release of pro-inflammatory cytokines, and reducing the infiltration of T-lymphocytes and macrophages into the peripheral nervous system. A moderate upregulation of the TGF-β1 synthesis in astroglia increased Aβ clearance in the old transgenic mice expressing a human β-amyloid precursor protein gene [106], thereby confirming a neuroprotective role of TGF-β. On the contrary, in the laboratory animals with the TGF-β gene knockout, the neuroprotective effects are completely absent and a pronounced neurodegeneration is detected [107].

Nevertheless, upon the examination of autopsy samples taken from the patients with AD, an elevated level of TGF-β was discovered in cerebral vessels, which might contribute to the release of pro-inflammatory cytokines such as TNF-β and IL-1β, from the brain endothelial cells [108]. This finding is consistent with the experimental data obtained from the transgenic mice in which a prolonged overexpression of TGF-β correlates with an increased perivascular amyloidogenesis [109]. As a result, these data raise questions about a neuroprotective role of TGF-β and the representativeness of the model.

2.4 Physical models

2.4.1. Models based on ultrasonic damage

The aforementioned animal models affect the whole body systemically, involving various organs and tissues in the pathological process, including those outside the nervous system. To achieve an isolated effect on the brain, Kovacs ZI et al. proposed a technique based on focused ultrasonic pulses [110]. The exposure to a high-frequency sound led to an acoustic cavitation and a sterile damage to the BBB with the development of an inflammatory reaction in the brain parenchyma. This was confirmed biochemically by an increase in the levels of the heat shock protein 70, IL-1, IL-18, TNFα, and the expression of pAKT and pGSK3β. However, the activation of other signaling pathways such as p38-MAPK, pERK, and pJNK, has not been confirmed.

This approach has been proposed for the treatment of tumor diseases [111] and as a method that facilitates a drug penetration through the BBB [112]. Nevertheless, it is reasonable to speculate that the localized neuroinflammation resulting from the BBB damage could serve as a valuable tool for investigating the properties of putative neuroprotective drugs, such as KOR.

2.4.2. Traumatic models

An alternative form of a physical impact on the brain is through a mechanical damage or traumatic brain injury (TBI). Neuroinflammation is initiated during the acute phase of TBI and persists throughout the chronic phase [113]. It is important to note that inflammation can take place during the recovery process after TBI, which often leads to the development of secondary undesirable effects associated with hyperproduction of pro-inflammatory cytokines [114].

TBI results in the activation of glial cells, a release of pro-inflammatory mediators, and a recruitment of leukocytes (migration of peripheral immune cells to the

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affected area is accelerated) [115, 116]. The activation of microglia causes an imbalance between M_1 and M_2 phenotypes [117], leading to the increased cytotoxicity and long-term neurodegenerative processes.

In addition to TBI, neuroinflammation can develop as a result of other injuries. For example, a model of a tibial fracture in rats was proposed [118]. The study indicates that such an injury leads to an increase in the level of IL-1β and a marker of astrogliosis GFAP, as well as microglial and astrocytic responses in the hippocampus. However, the exact mechanism underlying this phenomenon remains unclear.

The earlier studies have also established a relationship between a tibial fracture and the severity of the consequences of a TBI [119]. The authors observed more pronounced neuroinflammation and cerebral edema in the animals exposed to combined traumatic effects. It is hypothesized that a hyperproduction of inflammatory mediators in the brain may be linked to the development of systemic inflammation that occurs as a result of fractures of large bones [120], resulting in more severe consequences of TBI.

Taking into account the fact that TBI-induced inflammation occurs through typical signaling pathways it can be assumed that KOR agonists could have a significant neuroprotective effect. A KOR activation suppresses the production of pro-inflammatory cytokines IL-1β and IL-6, resulting in a shift towards the neuroprotective M_2 phenotype. Given a high incidence of TBI both in Russia [121] and worldwide [122], the evaluation of the therapeutic potential of kappa-opioid agonists in this model appears to be particularly promising.

CONCLUSION

Neuroinflammatory processes in the brain are associated with an increase in the density of immunoreactive microglia, dystrophic, apoptotic and necrotic changes in oligodendroglia, demyelination and degeneration of axons, and an imbalance of cytokines. Given the difficulties in treating neuroinflammation-related diseases, there is a need to search for and develop new drugs.

To date, numerous studies indicate protective effects of kappa-opioid agonists in neuroinflammation-related diseases. Several mechanisms may be involved in these effects, including a direct influence on neurons, glia (especially microglia), and cells of the immune system (both within and outside of the CNS). However, the clinical potential of using kappa-opioid agonists for the conditions associated with neuroinflammation has not been fully explored.

The model of neuroinflammation induced by LPS is the most extensively studied, particularly regarding the investigation of the anti-inflammatory effects of KOR agonists. Transgenic and knockout models, as well as various physical and traumatic models are currently actively used, however, their representativeness (the ability to most fully and adequately reproduce a specific pathology) may be questionable due to their reductionistic nature and limitations, since neuroinflammation is characterized by extremely complex pathogenesis and etiology (Fig. 2).

There are a number of models where the activity of KOR ligands has not been previously evaluated, which opens up a pool of opportunities for exploration of the anti-inflammatory properties of this class of compounds.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Konstantin Yu. Kalitin – tasks setting, concept development, scientific and methodical literature analysis, article writing and editing; Alexander A. Spasov – draft manuscript critical revision, making intellectual comments, final manuscript approval; Olga Yu. Mukha – data collection, manuscript drafting and writing, text editing and formatting.

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Physiology, pharmacology and prospects for dipeptidilpeptidase-4 inhibitors use

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Modern requirements for the treatment of type 2 diabetes mellitus (DM2) include not only achieving a glycemic control, but also reducing the risk of developing cardiovascular complications. Dipeptidyl peptidase 4 (DPP-4) inhibitors are inferior in the effectiveness to some other actively developing groups of hypoglycemic drugs (SGLT2 inhibitors and GLP-1 receptor agonists); however, they seem relevant at the present time.

The aim of the study is to analyze the literature data on the therapeutic potential and results of the of DPP-4 inhibitors research.

Materials and methods. When searching for the review article materials, the abstracting databases of PubMed, Google Scholar and e-Library were used. The search was carried out on the publications for the period from 2006 to 2022, using the following keywords: DPP-4 inhibitors; glucagonlike peptide-1 (GLP-1); glucose-dependent insulinotropic peptide (GIP); sitagliptin, and other drugs.

Results. DPP-4 belongs to the serine proteases family and is involved in the degradation of various chemokines and peptide hormones, including incretins secreted by intestinal L- and K-cells – GLP-1 and GIP. They regulate a postprandial insulin secretion and a β-cell function, modulate a fasting and postprandial glucagon secretion, regulate the eating behavior and have many pleiotropic (immunomodulatory, anti-inflammatory, antifibrotic, etc.) effects. DPP-4 inhibitors reduce an enzyme activity by 70–90%, increasing plasma incretin levels by 2–4 times and have been used to treat DM2 since 2006. Now there are 13 DPP-4 inhibitors on the market in different countries, differing primarily in pharmacokinetic parameters. They are actively used in the combination therapy for type 2 diabetes, increasing the glycemic control effectiveness without increasing the risk of hypoglycemia. The evidence is emerging about the therapeutic potential of DPP-4 inhibitors in COVID-19.

Conclusion. A peroral form, an ability to create effective combinations with other hypoglycemic drugs without increasing the risk of hypoglycemia, the pleiotropic effects of DPP-4 inhibitors, make this group relevant at the present time.

Keywords: diabetes mellitus; dipeptidyl peptidase 4; glucagonlike peptide-1; glucose-dependent insulinotropic peptide; sitagliptin; COVID-19

Abbreviations: FAP-α – fibroblast activator protein-α; FDA – Federal Food and Drug Administration of the USA; bFGF2 – basic fibroblast growth factor; GRP – gastrin-releasing peptide; MCP-1 – monocytic chemotactic protein-1; MDC – macrophage-derived chemokine; MIP-1α – macrophage inflammatory protein 1α; NHE3 – subtype 3 sodium-hydrogen exchanger; NHE3 NPY – neuropeptide Y; PAI-1 – type 1 plasminogen activation inhibitor; PYY – peptide YY; SDF-1a – Stromal Derived Factor-1α; TGFβ – transforming growth factor beta; ATE2 – angiotensine transforming enzyme 2; AD – Alzheimer's disease; GIP – glucose-dependent insulinotropic peptide; GM-CSF – granulocyte-macrophage colony-stimulating factor; GLP-1 – glu1cagonlike peptide-1; BBB – blood-brain barrier; DPP-4 – dipeptidyl peptidase 4; iDPP-4 – dipeptidyl peptidase-4 inhibitor; CTs – clinical trials; NAFLD – non-alcoholic fatty liver disease; ACS – acute coronary syndrome; DM – Diabetes mellitus; GFR – glomerular filtration rate; COPD – chronic obstructive pulmonary disease; CRF – chronic renal failure.

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Физиология, фармакология и перспективы применения ингибиторов дипептидилпептидазы-4

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Современные требования к терапии сахарного диабета 2 типа (СД 2) включают не только достижение гликемического контроля, но и снижение риска развития сердечно-сосудистых осложнений. Ингибиторы дипептидилпептидазы 4 (ДПП-4) уступают по эффективности некоторым другим активно развивающимся группам гипогликемических препаратов (ингибиторы SGLT2 и агонисты рецепторов ГПП-1), однако представляются актуальными и в настоящее время.

Цель. Проанализировать данные литературы о терапевтическом потенциале и результатах исследований ингибиторов ДПП-4.

Материалы и методы. При поиске материала для написания обзорной статьи использовали реферативные базы PubMed, Google Scholar и e-Library. Поиск осуществлялся по публикациям за период с 2006 по 2022 год, с использованием следующих ключевых слов: ингибиторы ДПП-4; глюкагоноподобный пептид-1 (ГПП-1); глюкозозависимый инсулинотропный пептид (ГИП); ситаглиптин и другие препараты.

Результаты. ДПП-4 принадлежит к семейству сериновых протеаз и участвует в деградации некоторого количества хемокинов и пептидных гормонов, в том числе и инкретинов, секретируемых L- и K-клетками кишечника: ГПП-1 и ГИП, которые регулируют постпрандиальную секрецию инсулина и функцию β-клеток, модулируют тощаковую и постпрандиальную секрецию глюкагона, регулируют пищевое поведение и оказывают множество плейотропных эффектов (иммуномодулирующее, противовоспалительное, антифибротическое действие и др.). Ингибиторы ДПП-4 снижают активность фермента на 70–90%, повышая уровень инкретинов в плазме в 2–4 раза и применяются для лечения СД 2 с 2006 года. Сейчас на рынке разных стран присутствуют 13 ингибиторов ДПП-4, различающихся прежде всего фармакокинетическими параметрами. Они активно используются в комбинированной терапии СД2, повышая эффективность гликемического контроля без увеличения риска развития гипогликемии. Появляются данные о терапевтическом потенциале ингибиторов ДПП-4 при COVID-19.

Заключение. Пероральная форма, возможность создавать эффективные комбинации с другими гипогликемическими препаратами без увеличения риска гипогликемии, плейотропные эффекты ингибиторов ДПП-4 делают данную группу актуальной и в настоящее время.

Ключевые слова: сахарный диабет; дипептидилпептидаза 4; глюкагоноподобный пептид-1; глюкозозависимый инсулинотропный пептид; ситаглиптин; COVID-19

Список сокращений: FAP-α – фибробласт-активирующий белок альфа; FDA – Управление по санитарному надзору за качеством пищевых продуктов и медикаментов США; FGF2 – основной фактор роста фибробластов; GRP – гастрин-рилизинг пептид; MCP-1 – моноцитарный хемотаксический протеин-1; MDC – макрофагальный хемокин; MIP-1α – макрофагальный воспалительный протеин 1α; NHE3 – натрий-водородный обменник 3 подтипа; NPY – нейропептид Y; PAI-1 – ингибитор активации плазминогена 1 типа; PYY – пептид YY; SDF-1a – фактор стромальных клеток 1 альфа; TGFβ – трансформирующий фактор роста бета; АПФ2 – ангиотензинпревращающий фермент 2; БА – болезнь Альцгеймера; ГИП – глюкозозависимый инсулинотропный пептид; ГМ-КСФ – гранулоцитарно-макрофагальный колониестимулирующий фактор; ГПП-1 – глюкагоноподобный пептид-1; ГЭБ – гематоэнцефалический барьер; ДПП-4 – дипептидилпептидаза 4; иДПП-4 – ингибитор дипептидилпептидазы-4; КИ – клинические исследования; НАЖБП – неалкогольная жировая болезнь печени; ОКС – острый коронарный синдром; СД – сахарный диабет; СКФ – скорость клубочковой фильтрации; ХОБЛ – хроническая обструктивная болезнь легких; ХПН – хроническая почечная недостаточность.

INTRODUCTION

Diabetes mellitus (DM) and related diseases will obviously remain a serious threat to the life and health of the population in almost all countries for many decades to come. In 2021, according to the estimates of the International Diabetes Federation, the number of diabetes patients in the world exceeded 536 million, and in 2045, it will amount to 783.2 million people. Modern guidelines for the treatment of DM indicate the feasibility of the early treatment using rational

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combinations of drugs with a high safety profile, and notify the importance of preventing vascular DM complications [1, 2].

Enzyme dipeptidyl peptidase-4 inhibitors (iDPP-4s) have been developed all over the world for more than 30 years and remain in demand nowadays. In 2019, the global market for DPP-4 inhibitors and their combinations exceeded $12 billion [3]. In Russia, this group is actively used in the treatment of diabetes. The characteristics of the domestic market for iDPP-4 are summarized in Fig. 1.

The DPP-4 enzyme was identified in 1966 by Hopsu-Havu and Glenner as glycylproline naptylamidase. DPP-4 was first obtained from the rat liver in 1967, and from the pig kidney – in 1968. DPP-4 is an intramembrane glycoprotein and serine exopeptidase of the S9B subfamily, consisting of 766 amino acids. The active enzyme in rats, mice and humans was found out in epithelial cells of the intestine, kidneys, liver, lungs, thymus, and spleen. In addition to DPP-4, the representatives of the S9B protease subfamily are a fibroblast activator protein-α (FAP-α), DPP-6, DPP-8, DPP-9. However, DPP-4 is the main enzyme responsible for the physiological degradation of incretin hormones [4].

The functions of all isoenzymes have not been *ad finem* understood; it is assumed that FAP is responsible for the cell growth, and their inhibition has a toxic effect, causing thrombocytopenia, splenomegaly, reticulocytopenia, pathology of various organs, which makes the selectivity of the inhibitory action important for the representatives of the drugs with a similar mechanism of action [5]. DPP-4 is a tetramer in which each subunit consists of two domains – an N-terminal β-helical (β-propeller) domain and a C-terminal catalytic domain, which enclose an internal cavity with the active site. This cavity is connected to the main part of the active site by means of an “open screw/propeller” and a “side hole”. Substrates and inhibitors of DPP-4 enter and leave the active site through this side opening [6]. The main parts for binding to DPP-4 ligands are the S1 hydrophobic pocket, which determines the substrate specificity of DPP-4, the S2 hydrophobic pocket with ionic interaction sites, and the S3 pocket. The S1 region in DPP-4, DPP-8, and DPP-9 is almost identical, while S2 in DPP-4 is smaller. The S1 and S1’ regions slightly differ in their composition and conformation: in contrast to DPP-8 and DPP-9, relatively negatively charged groups are attached to S1’ in DPP-4. The S3 region is the most variable for each isoenzyme; in DPP-4, the groups of ligands of a smaller size compared to DPP-8 and DPP-9, are attached to it [7].

The DPP-4 enzyme cleaves many physiologically active substances, including hormones secreted by L- and K-cells of the intestine – glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), which regulate a postprandial insulin secretion and are involved in maintaining carbohydrate homeostasis. Besides, these incretins regulate insulin biosynthesis in a glucose-dependent manner, suppress a glucagon secretion, suppress glucogenesis in the liver, promote the regeneration and differentiation of islet β-cells, and play an important role in the regulation of the eating behavior: the formation of a satiety feeling and slowing gastric emptying [5].

Like GLP-1 receptor agonists, DPP-4 inhibitors are well tolerated and do not cause hypoglycemia. However, a few cases of acute pancreatitis were reported after incretin-based drugs had been introduced to the market, and in 2013, the Federal Food and Drug Administration of the USA (FDA) reported an increased risk of pancreatitis and precancerous cellular changes (metaplasia) of pancreatic ducts against the background of their application. In addition, the FDA warns that patients with a history of pancreatitis are at an increased risk of pancreatitis recurrence when treated with these drugs, so, they should be used with caution. Numerous subsequent studies over the past time have not been able to unambiguously prove a relationship between the use of incretin mimetics and the development of the notified pathologies. At the same time, all authors point out to the need for longer follow-up and additional studies to form conclusions [8–10].

It should be notified that the DPP-4 inhibition also affects the elimination of a large number of substrates: incretins (GLP-1, GLP-2, GIP, gastrin-releasing peptide (GRP), peptide YY (PYY)); cytokines (interleukin-3, a granulocyte-macrophage colony-stimulating factor (GM-CSF), erythropoietin, a basic fibroblast growth factor (bFGF2), etc.); chemokines (monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein 1α. (MIP-1α), Stromal Derived Factor-1α, (SDF-1 α), macrophage-derived chemokine (MDC)), and others); neuropeptides (neuropeptide Y (NPY), substance P) [11].

DPP-4 (also referred to as CD26) is also expressed on the surface cells of the immune system (T- and B-lymphocytes, NK cells, dendritic cells and macrophages [11]. However, in relation to these cells, the regulatory function of DPP-4 (CD26) has not been *ad finem* disclosed. 8, 11 Thus, the pleiotropic potential of DPP-4 inhibitors requires a further evaluation.

THE AIM of the study is to analyze the literature data on the therapeutic potential and results of the of DPP-4 inhibitors research.

MATERIALS AND METHODS

When searching for the review article materials, the abstracting databases of PubMed, Google Scholar and

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e-Library were used. The search was carried out on the publications for the period from 2006 to 2022, using the following keywords: DPP-4 inhibitors (DPP-4 inhibitors); GLP-1 (GLP-1); glucose-dependent insulinotropic peptide (GIP); sitagliptin (sitagliptin); vildagliptin (vildagliptin); dutogliptin (dutogliptin); saxagliptin (saxagliptin); linagliptin (linagliptin); alogliptin (alogliptin); gemigliptin (gemigliptin); teneligliptin (teneligliptin); anagliptin (anagliptin); omarigliptin (omarigliptin); gosogliptin (gosogliptin); denagliptin (denagliptin); melogliptin (melogliptin); trelagliptin (trelagliptin); retagliptin (retagliptin); evogliptin (evogliptin); carmegliptin (carmegliptin). 522 sources were analyzed; after the systematization, the literary sources with similar theoretical information, were removed.

RESULTS AND DISCUSSION

1. Physiology of DPP-4

Native GLP-1 has a short half-life (about 1-2 min) due to its destruction by the DPP-4 enzyme or excretion from the bloodstream by the kidneys. DPP-4 cleaves GLP-1 (7-36 amide) and GLP-1 (7-37) at the N-terminal dipeptide with the formation of the corresponding inactive metabolites: GLP-1 (9-36 amide) or GLP-1 (9-37), which are also excreted by the kidneys. The clearance of GLP-1 and its metabolites is slowed down in the patients with renal insufficiency [12, 13].

DPP-4 exists in two forms – a transmembrane protein and a soluble form that circulates in the blood. In the intestine, DPP-4 is highly expressed in the brush border of enterocytes and in endothelial cells, so most of the secreted GLP-1 is already degraded in the capillaries of the distal intestine. At the same time, approximately, only 25% of active GLP-1 reaches the liver and about 10–15% is distributed in plasma [13].

The DPP-4 activity can change under the influence of various stimuli. Thus, dexamethasone-induced hyperglycemia is accompanied by hyperacetylation of histones in the promoter region of the DPP-4 gene with an increase in its expression, which may be an addition to the knowledge about the already known mechanisms for the development of steroid diabetes, as well as a new goal of pharmacotherapy [14].

2. iDPP-4 pharmacology

iDPP-4 inhibitors improve a glucose control in patients with type 2 diabetes. Thus, iDPP-4s have a large number of biological effects and, unlike other antidiabetic agents, do not cause such undesirable effects as a weight gain and the development of a hypoglycemia state. Therefore, these drugs are in the center of research and development of many pharmaceutical companies and scientific centers, which led to the appearance of such a large number of drugs of the DPP-4 group on the pharmaceutical market. 13 of these, are currently approved and used for the treatment of type 2 diabetes, while 6 others (carmegliptin, retagliptin, melogliptin, denagliptin and dutagliptin) are in the pre-registration/phase 2,3 and/or awaiting the approval.

Based on international non-proprietary names, the common fragment of which is “gliptin”, the entire group of DPP-4 inhibitors is commonly called gliptins. The drugs of this group reduce the activity of the enzyme by 70–90%, do not have a direct effect on the satiety feeling or on the rate of gastric emptying. In the absence of the data on the passage of DPP-4 inhibitors through the blood-brain barrier (BBB), they are able to enhance the central effect of GLP-1 increasing its plasma level by 2–4 times [15, 16]. Despite the same action, various gliptins differ in their pharmacodynamic and pharmacokinetic properties, which may be clinically significant for certain categories of patients (with renal or hepatic insufficiency, pancreatitis, cardiovascular diseases, etc.).

The main advantages of the drugs over other hypoglycemic drugs with the DPP-4 inhibitory activity are a moderate efficacy, a higher safety: a low risk of hypoglycemia, cardiovascular complications; it does not cause edema and a weight gain. The drugs from the DPP-4 group have a number of class-specific properties, which consist in a dual action mechanism (on the function of α- and β-cells). That leads to an improvement in the postprandial profile of glucagon and insulin secretion patterns. The inhibition of the GLP-1 degradation has a positive effect on glucose homeostasis by increasing insulin levels and suppressing a glucagon secretion, slowing gastric emptying and reducing the appetite. DPP-4 inhibitors are characterized by a neutral effect on the body weight and do not provoke hypoglycemia [17, 18]. In patients with type 2 diabetes, their use leads to a steady decrease in the concentration of HbA1c, blood glucose levels on an empty stomach and after a meal. In the work by Korbut A.I. and Klimontov V.V. [19], the data on the effect of GLP-1 and DPP-4 iD analogues on structural and functional changes in the kidneys in DM, have been summarized. In experimental and clinical nephropathy of a diabetic and non-diabetic origin, GLP-1 and DPP-4 iP analogues slow down the development of fibrosis and a decrease in the kidney function. Their nephroprotective effect is due to a decrease in hyperglycemia, an increase in sodium excretion, a suppression of inflammatory and fibrogenic signaling pathways, an oxidative stress and apoptosis in the kidneys.

It is important to note that the effect of incretins (GLP-1 and GIP) on the insulin and glucagon secretion depends on the level of glycemia. Under the conditions

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of normoglycemia, an increase in GLP-1 and GIP does not affect the insulin secretion, while GIP stimulates a glucagon secretion during fasting glycemia and hypoglycemia [20]. An increase in the glucose levels above the physiological values leads to the stimulation of insulin secretion (GLP-1 and GIP) and suppression of the glucagon production (GLP-1). At the same time, under the conditions of hypoglycemia, GIP significantly increases a glucagon secretion, helps maintain glucose homeostasis and prevents a further development of hypoglycemia. By increasing the level of GLP-1 and GIP, which have a glucose-dependent mechanism of action, DPP-4 inhibitors can help normalize the insulin/glucagon balance and improve glucose homeostasis in the patients with type 2 diabetes without increasing the risk of hypoglycemia [18, 21]. Based on the foregoing, and taking into account the recommendations accepted in the world regarding the use of the combination therapy for type 2 diabetes, it can be assumed that the addition of iDPP-4 to the drugs that tend to cause hypoglycemia (sulfonylurea derivatives and thiazolidinediones) will reduce the likelihood of its development, which is often notified in clinical trials. (CTs).

2.1. Classification of iDPP-4s

Kushwaha R.N. et al. [22] divide gliptins into several groups based on their chemical skeleton:

– sitagliptin and related gliptins include retagliptin, gemigliptin, omarigliptin and evogliptin, which had been developed from triazolopiperazine derivatives. Sitagliptin is the first DPP-4 gliptin approved for the treatment of type 2 diabetes;

– cyanopyrrolidine gliptins include vildagliptin, saxagliptin, anagliptin, denagliptin and melogliptin. Vildagliptin is the first inhibitor of this class on the market;

– teneligliptin and gosogliptin are gliptins based on diprolyl;

– linagliptin belongs to the xanthine-based class of gliptins, while alogliptin and trelagliptin belong to the pyrimidinedione class;

– dutagliptin and carmegliptin are boric and tricyclic gliptins, respectively.

Nabeno M. et al. [6] classify iiDPP-4s nto three classes depending on their binding modes in the DPP-4 active site:

Class 1 contains wilda- and saxagliptin, which bind to the S1 and S2 subsites and form a covalent bond with the nitrile group of their cyanopyrrolidine moiety to the Ser630 site of DPP-4. Saxagliptin is 5 times more potent than vildagliptin at inhibiting DPP-4.

Class 2 contains alo- and linagliptin, which interact with “daughter” S1’ subsites, and in the case of linagliptin with S1’ and S2’, in addition to S1 and S2. The uracil rings of both gliptins induce a conformational change in Tyr-547 in the daughter subsites at S1’. Due to the additional interaction of linagliptin with the daughter subsites S2’, it is 8 times more active than alogliptin.

Class 3 has the highest inhibitory activity against DPP-4, since sita- and teneligliptin interact not only with the S1 and S2 regions of DPP-4 (like Class 1), but also with the extensive S2 subsite. Teneligliptin has a unique J-shaped structure with an anchor lock domain, which explains its strong inhibitory activity and low IC value (0.37 nM). Binding to the extensive S2 subsite of some inhibitors also determines their high specificity for DPP-4, since other related peptidases (DPP-8, DPP-9, and FAP) lack this site.

DPP-4i can be also divided according to the effect duration, highlighting the drugs with a prolonged action for the oral administration once a week: omarigliptin (MK-3102, Marizev®, Merck) and trelagliptin (SYR-472, Zafatec®, Takeda/Furiex), which had been approved for use in Japan [23, 24].

In 2006, the FDA approved the first DPP-4 inhibitor, sitagliptin. After that, the development of drugs in this group was continued, and today they are 17 in this group [22].

2.2. Pleiotropic properties of DPP-4 inhibitors

DPP-4 (CD26) is expressed by vascular endothelial cells, lungs, kidneys, liver, small intestine, and heart, as well as the cells of the immune system [11]. In the review article by Zou H. et al. [25], biological functions, key molecular pathways, interactions, and associations of DPP-4 in the context of developing new treatments for lung diseases such as asthma, chronic obstructive pulmonary disease (COPD), and cancer, are reviewed. The authors note out that DPP-4 may regulate the immune response through the T-cell activation and modulation of chemotaxis, and can be also involved in the development of asthma and COPD. The DPP-4 inhibition can slow down a smooth muscle cell proliferation and facilitate a pulmonary artery remodeling, as well as improve the overall survival of the lung cancer patients.

DPP-4 is constitutively expressed on lung fibroblasts and is involved in the regulation of their functional activity (collagen synthesis and a secretion of inflammatory cytokines). Under conditions of pulmonary hypertension, the inhibition of DPP-4 slows down the transition of vascular remodeling from the reversible to the irreversible stage due to the complex antioxidant, anti-inflammatory and antifibrotic effects, as well as slowing down the proliferation and migration of fibroblasts [26].

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In the study by Zhang S. et al. [27], anagliptin was shown to reduce a lung injury in the mice exposed to the chronic alternating stress for 2 weeks. The chronic stress induced the inflammation and an oxidative stress leading to the lung damage. This was accompanied by an increase in the plasma DPP-4 activity, an increase in the gene expression of several pro-inflammatory cytokines, the adhesion molecules, and a plasminogen activator inhibitor-1 (MCP-1, Vcam-1, Icam-1 and PAI-1), as well as a decrease in the expression of eNOS proteins, Sirt1 and Bcl-2. The inhibition of DPP-4 both by the administration of anagliptin and by generating genetic knockouts (DPP-4$^{-/-}$) prevented a stress-induced lung injury associated with the inflammation, oxidative stress and apoptosis.

In the work by Patel P.M. et al. [28], a DPP-4 role in the development of various skin diseases is discussed. Herewith, the following factors are taken into consideration: its expression, influence on the function of melanocytes, keratinocytes, and fibroblasts, as well as the participation in the formation of a balance between regulatory T-lymphocytes (Treg) and effector T-lymphocytes. In skin diseases, a DPP-4-mediated disruption of Treg immunosuppression is possible, contributing to the development of inflammation. The therapeutic potential of DPP-4 inhibitors in various inflammatory skin diseases (psoriasis, atopic dermatitis, fibrosing diseases, etc.) is being investigated.

The study published in 2011, noted an increase in the DPP-4/CD26 expression in the patients with atopic dermatitis. In the experimental part of the work, a skin inflammation was induced in the knockout animals (DPP-4$^{-/-}$) with a predominance of T-helper 1 (Th1) or type 2 (Th2), respectively, with a predominance of a cellular or humoral immune response. In the animals without DPP-4, a reduced cutaneous inflammatory response was found out in the Th2 inflammation model, which underlies the diseases such as atopic dermatitis, bronchial asthma, etc. On the other hand, in the animals without DPP-4 in the Th1-dominated model, a cutaneous inflammatory response was increased [29]. It can be assumed that the DPP-4 inhibition in inflammatory skin diseases can have both potential benefits and harms, which requires taking into account the individual characteristics of the patient. At the time of writing this review, few publications have been published on the association of the use of DPP-4 inhibitors with inflammatory skin diseases. However, one study showed that the use of DPP-4 inhibitors (vildagliptin and linagliptin) was associated with a 3-fold increase in the risk of bullous pemphigoid, and a therapy discontinuation was associated with improved clinical outcomes [30].

A significant number of cytokines among the substrates of degraded DPP-4 indicates the study prospects of iDPP-4 for the correction of the chronic inflammation in autoimmune rheumatic diseases [31]. However, currently, the information is insufficient and there are conflicting data on both positive [32] and negative [33] effects of DPP-4 inhibitors on the course of rheumatoid arthritis in the experiment.

To reduce potential negative DPP-4i effects associated with a slowdown in the elimination of certain cytokines, the possibility of using antagonists of the corresponding receptors, is being considered [34].

The possibility of using DPP-4i in the immune regulation and therapy of autoimmune rheumatic diseases is discussed.

In the progression of a renal failure, DPP-4 inhibitors can have a protective effect, including antifibrotic effects in diabetic nephropathy [35]. DPP-4 affects a sodium transport in the kidneys, since a 3-subtype sodium-hydrogen exchanger (NHE3) in the brush border membranes of the epithelium of the proximal tubule exists in combination with DPP-4, and the decrease/suppression of the expression/signaling and/or activity of DPP-4 can lead to an increased excretion of sodium and water [36]. Investigating gemigliptin effects in the mice in the ureteral obstruction model, Min H.S. et al. [37] found out that in the animals receiving gemigliptin at the dose of 150 mg/kg p.s. with food, a decrease in proteinuria and kidneys structural changes was observed within 14 days. Against the background of the administration of the drug, a urinary excretion of 8-isoprostane (a marker of the oxidative stress level) in the mice decreased. The authors note that the nephroprotective effect of gemigliptin is realized through several mechanisms associated with fibrosis, inflammation, and an oxidative damage, regardless of its hypoglycemic effects.

As mentioned above, colony-stimulating factors and various cytokines are also DPP-4 targets, which can have a significant potential in the organ and tissue transplantation (islet cells of the pancreas, lungs, skin, hematopoietic stem cells, etc.) [11, 38].

An increased DPP-4 expression in the liver contributes to the development of a non-alcoholic fatty liver disease (NAFLD) and insulin resistance. This is associated with a reduced level of GLP-1, as well as auto- and paracrine DPP-4effects. In the experimental studies, gemigliptin reduced the inflammation severity, an oxidative stress and alleviated the course of liver fibrosis. DPP-4 is considered as a promising target for the NAFLD treatment [39].

Jung E et al. [40] studied the effect of gemigliptin on retinal pericytes and the process of neovascularization in a model of ischemic proliferative retinopathy in the

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mice prone to DM type 2 (db/db). The administration of gemigliptin for 12 weeks led to a significant decrease in the intensity of retinal pericyte apoptosis and improved the retinal neovascularization. The authors note a pronounced retinoprotective effect of gemigliptin due to the DPP-4 suppression and the suppression of the plasminogen activator-1 (PAI-1) expression.

The review paper [41] summarizes the research results studies that indicate the ability of DPP-4i to prevent the onset and progression of diabetic microangiopathy.

Some well-known drugs have an inhibitory activity against DPP-4, mitoxantrone has a significant inhibitory activity against DPP-4 both *in vitro* and *in vivo* [42]; being inhibitors of metalloaminopeptidases and bacterial proteases, bestatin and bacitracin also inhibit a DPP-4 activity and, therefore, are considered as a structural element for creating new compounds [43]. Oxytocin is considered as a peptide endogenous inhibitor of the DPP-4 activity [44]. The inhibitory activity against DPP-4 was found out in bovine α-lactalbumin hydrolysates [45].

Curcumin, syringic acid, resveratrol [46], berberine [47], garlic extract [48] have a high affinity for the DPP-4 enzyme, which increases interest in these natural products.

Currently, despite the discovery of a large number of substances that exhibit an inhibitory activity against DPP-4, medical chemistry continues to develop new compounds.

2.3. Potential for use of DPP-4 inhibitors in COVID-19

A potential use of DPP-4 inhibitors in the complex treatment of COVID-19 is of particular interest. It is hypothesized that DPP-4 inhibitors can play a role in reducing the COVID-19 severity by preventing the virus from entering the cells. This led to the hypothesis that the use of DPP-4 inhibitors can be the optimal strategy for the COVID-19 treatment in the diabetic patients, who are at a double risk of a severe infection [49, 50].

In silico modeling of the SARS-CoV-2 spike protein predicted its potential interaction with DPP-4 in addition to the angiotensine transforming enzyme 2 (ATE2, ACE2) [51]. These models suggest that DPP-4 can be a co-receptor for the SARS-CoV-2 virus entry. DPP-4 (a membrane and soluble form) is a target not only for SARS-CoV-2, but also for MERS-CoV [52]. In the literature, the possibility of using a monoclonal antibody to CD26 (Begelomab) to block the interaction of SARS-CoV-2 with DPP-4 has been considered, but there are no data on any clinical studies of this approach [53].

The fact that DPP-4 exists in soluble and membrane forms complicates understanding of the iDPP-4 potential in COVID-19. The previous studies have shown that a soluble DPP-4 form acts as a decoy molecule for MERS-CoV, as it does for SARS-CoV-2, blocking a viral S-protein binding to the cell surface [54, 55]. The research by Schlicht K. et al. [56] in the severe COVID-19 patients showed a reduced level of soluble DPP-4, which correlated with the severity of the disease. However, it is not clear whether a decrease in the soluble DPP-4 was a consequence of the disease or an individual initial condition that causes an increased susceptibility to MERS-CoV or SARS-CoV-2. The level of soluble DPP-4 in serum can also be reduced in various clinical diseases, such as diabetes, obesity and metabolic syndrome, which can lead to a severe course of an infectious disease [4]. Herewith, the administration of DPP-4 inhibitors can increase the level of soluble DPP-4 [57]. Thus, there is a hypothesis that DPP-4 inhibitors can help retain viral particles in the bloodstream by increasing the level of soluble DPP-4, which, in turn, can limit the reproduction of the virus in the human body.

DPP-4 is expressed by the immune system cells and is involved in the regulation of inflammatory processes. The anti-inflammatory effects of DPP-4 inhibitors can be useful in COVID-19 patients to prevent a “cytokine storm” in order to reduce the disease severity [49, 58]. DPP-4 also enhances a fibroblast activation by increasing transforming growth factor β (TGFβ), which indicates the antifibrotic potential of DPP-4 inhibitors, confirmed by experimental models of pulmonary and skin fibrosis [59].

3. Representatives of DPP-4 inhibitors group

Summarized information about the representatives of DPP-4 inhibitors is given in Table 1.

3.1. Sitagliptin (MK-0431, Januvia®, Merck)

Sitagliptin was developed by the pharmaceutical company Merck (Germany) based on triazolopiperazine. Since 2006, it has been approved by the FDA for use in type 2 diabetes. It is highly active against DPP-4 (IC_{50}=18 nM) and selective (for DPP-8 – 48000 nM, for DPP-9 – >100000 nM); it improves the function of pancreatic β-cells, as well as a glycemic control on an empty stomach and after a food intake in patients with type 2 diabetes. The presence of a trifluoromethyl group in the triazole ring improves its bioavailability [22]. Sitagliptin dose-dependently inhibits a DPP-4 plasma activity up to 80 and 47% when measuring the enzyme activity at the 2nd and 24th h, respectively, after a single dose of 25 mg (in the type 2 diabetes patients) [60]. Only a small part of the drug is metabolized with the participation of CYP3A4 and CYP2C8 enzymes.

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The metabolites are conjugates of N-sulfate and N-carbamoylglucuronic acid of the parent drug, a mixture of hydroxylated derivatives, a glucuronide ester of the hydroxylated metabolite, and two metabolites formed by an oxidative desaturation of the piperazine ring followed by the cyclization. All 6 metabolites lack a DPP-4 inhibitory activity. Sitagliptin is the most studied DPP-4 inhibitor and the effectiveness of its combinations with hypoglycemic drugs of other groups is being actively studied.

Hou L. et al. [61] conducted a meta-analysis of the studies published up to 2012, which evaluated the efficacy and safety of the combined therapy with metformin + sitagliptin and a combination of metformin (≥1500 mg) and sulfonylurea derivatives (glipizide, glibepiride, glibenclamide) in patients with type 2 diabetes and an inadequate glycemic control. The authors showed that sitagliptin and sulfonylurea drugs are comparable in effectiveness (in reducing HbA1c) when the baseline metformin therapy is added. However, in the combination therapy with metformin and sulfonylurea drugs, the risk of developing hypoglycemia remained high, while the addition of sitagliptin to metformin did not increase the risk of developing a hypoglycemic state.

Hayes J. et al. [62] evaluated the efficacy and safety of the sitagliptin+metformin combination in the treatment of type 2 diabetes patients. The authors compared the results of 11 studies lasting from 24 to 104 weeks. This research included the studies where the following drugs had been used: sitagliptin and metformin in fixed doses separately or in the dual therapy; sitagliptin and metformin compared with other hypoglycemic drugs and metformin; sitagliptin and metformin as a part of a triple combination therapy (sitagliptin + metformin + sulfonylurea or insulin). The authors have found out that the combination of sitagliptin and metformin reduced HbA1c and other glycemic parameters better than either drug separately. This combination had a high safety profile and was well tolerated by patients. The risk of hypoglycemia was lower with the combination of metformin and sitagliptin than with the combination of metformin, glipizide, or gliperimide.

Fonseca V. et al. [63] evaluated the efficacy and safety of sitagliptin in the triple combination therapy with metformin (≥1500 mg per day) and pioglitazone (≥30 mg per day) in type 2 diabetes patients (HbA1c=7.5–11%) during a placebo controlled, double-blind study for 26 weeks. The addition of sitagliptin resulted in significant (p <0.001) changes from the baseline compared to placebo in HbA1c (-0.7%), fasting plasma glucose (-1.0 mmol/L) and 2 h *postprandial* (-2.2 mmol/l). In patients with baseline HbA1c ≥9.0%, the mean changes from the baseline in HbA1c were -1.6 and -0.8% for the sitagliptin and placebo groups, respectively (between the groups the difference was -0.8%; p <0.001). The frequency of adverse events was generally comparable between the treatment groups, the episodes of hypoglycemia were observed in 4.5 and 3.8% in the sitagliptin and placebo groups, respectively (p=0.786). The authors conclude that the addition of sitagliptin to the combination therapy with metformin and pioglitazone resulted in the improved glycemic control and was generally well tolerated.

In the domestic multicenter observational program "Dia-Da", in type 2 diabetes patients and the HbA1c concentration of 7–8%, who had received the sitagliptin therapy at the dosage of 100 mg per day in combination with metformin for 6 months, there was a decrease in HbA1c levels by 1.1%. In patients with a more pronounced violation of carbohydrate metabolism (HbA1c >10%), the decrease in this indicator was 4.1%. On average, over 6 months of treatment, the level of HbA1c decreased by 1.7%. In the combination of sitagliptin + metformin, the level of fasting plasma glucose also decreased from the initial level of 8.8 to 6.1 mmol/l after 6 months [64].

Sitagliptin helps to reduce the visceral fat depot in type 2 diabetes patients when added to metformin, which was noted in the study by Ametov A.S. et al. [65]. After 6 months of treatment, in addition to improving the glycemic parameters (glucose levels measured on an empty stomach and *postprandial*, as well as HbA1c), there was a decrease in the body mass index (BMI) by an average of 5.29% in the sitagliptin+metformin group, and in the group metformin monotherapy – by 1.96%. The area of the visceral fat decreased by an average of 7.52% in the combination therapy group (p <0.001), while in the metformin monotherapy group it decreased by an average of 1.76%

The presented above results show that the inclusion of sitagliptin in the hypoglycemic therapy composition leads to a significant increase in the effectiveness of the glucose metabolism control and the safety of treatment in general. It should be notified that sitagliptin is the most studied representative of this pharmacotherapeutic group, and at the same time, the interest of researchers and doctors in it does not decrease.

3.2. Vildagliptin (LAF-237, Galvus®, Novartis)

Vildagliptin is a representative of the first generation of inhibitors and the first gliptin of the cyanopyrrolidine class, developed by Novartis (Switzerland), approved for the treatment of type 2 diabetes. This is active (IC_{50}=3.5 nM) and moderately selective for DPP-4 against DPP-8 (>250-fold) and DPP-9 (>23-fold), but much more selective for DPP-2 and FAP. The vildagliptin half-life is

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1.5 h, its bioavailability is 85%; it improves a glycemic control (reduces HbA1c levels by 0.7%), causes the DPP-4 inhibition by 80% within 7 h and is maintained by 40% for 24 h after a single dose of 100 mg. 69% of the received drug dose undergoes a biotransformation, the main metabolite – LAY151 (57% of the dose) – is pharmacologically inactive; it is a hydrolysis product of the cyanocomponent. It improves a β-cell function and an insulin sensitivity. It is used in monotherapy and in combination with other antidiabetic drugs. The drug was approved by the European Medicines Agency in 2008 for use in the European Union [22].

Numerous trials have shown the efficacy of adding vildagliptin to metformin, insulin, sulfonylurea derivatives, and thiazolidinediones. The level of glycated hemoglobin decreased by an average of 0.6–1.1%. In most patients, the body weight remained stable, and in some cases, there was a tendency to decrease it, especially when the drugs had been combined with metformin [66].

Azuma K. et al. [67] investigated the effect of vildagliptin (100 mg per day) on the β-cell function in type 2 diabetes patients. Against the background of the vildagliptin use, the concentration of *postprandial* GLP-1 and GIP increased by 3 times and bid, respectively. The insulin secretion increased by 50% (p <0.01), the concentration of glucose in the blood plasma measured on an empty stomach and after a meal, decreased by 1.3±0.3 and 1.6±0.3 mmol/l (p <0.01), respectively, and glucagon *postprandially* – by 16% (p <0.01). The authors found out that against the background of the vildagliptin use, the *postprandial* concentration of glucagon was 41% lower than in the placebo group. It was also found out that under the conditions of hypoglycemia, the difference between the level of glucagon and insulin was 38%, indicating an increase in the α-cells function. The authors conclude that vildagliptin enhanced the response of α-cells to both the inhibitory effect of glucagon under the conditions of hyperglycemia and its stimulatory effect under the conditions of hypoglycemia, indicating the efficacy and safety in DM 2.

Odawara M. et al. [68] reviewed two open-label studies in patients with a poorly controlled type 2 DM who were taking one of the oral hypoglycemic agents – sulfonylurea, metformin, thiazolidinedione, an α-glucosidase inhibitor, and glinide. After 52 weeks of treatment, the addition of vildagliptin (50 mg per day) to these drugs reduced HbA1c compared to monotherapy by -0.64, -0.75, -0.92, -0.94 and -0.64%, respectively. The episodes of hypoglycemia were rare, with a slight advantage in the sulfonylurea group. The decrease in the HbA1c concentration in the combined use of vildagliptin with insulin secretogens (sulfonylureas or glinides) was less compared with its combination with other drugs. In all combination therapy groups, mean fasting glucose concentrations decreased, as did triglyceride and cholesterol levels. The HOMA-β index increased only in the patients treated with vildagliptin/sulfonylurea, in the rest ones, this indicator decreased. The authors conclude that vildagliptin has a good tolerability profile in DM 2 patients.

Ametov A.S. [69] reported the results of several vildagliptin studies, one of which examined the efficacy and safety of adding vildagliptin to the basic therapy. The study identified three groups of patients who had received various types of therapy: group 1 – metformin at the dose of ≥1500 mg per day; group 2 – gliclazide MB at the dose of 90–120 mg per day; group 3 – a combined therapy with metformin + gliclazide MB at the maximum therapeutic doses. After 24 weeks of therapy in groups 1, 2 and 3, the reduction in HbA1c was -1.2, -1.32 and -1.26%, respectively, and the target values of HbA1c ≤7.0% were achieved in 54, 60 and 32%. Even in the patients treated with gliclazide, the risk of hypoglycemia did not increase with vildagliptin. There was also a significant decrease in the glycemic variability in all three groups, which improves the long-term prognosis of the disease.

Kosaraju J. et al. [70] studied the effect of vildagliptin on the rats with streptozotocin-modeled Alzheimer's disease (AD): 3 months after the AD induction, vildagliptin was administered *p.o.* at the doses of 2.5, 5, and 10 mg/kg/day for 30 days. The treatment of the animals with vildagliptin resulted in an increase in the concentration of GLP-1, a decrease in the severity of cognitive deficits, and a dose-dependent decrease in the tau-phosphorylation, Aß, and inflammatory markers. Based on the foregoing, the authors conclude that vildagliptin has pronounced neuroprotective properties.

Arruda-Junior D.F. et al. [71] investigated the effects of vildagliptin in the rats with a simulated heart failure. Six weeks after the surgery, vildagliptin (120 mg/kg/day) was administered *p.o.* to the rats for 28 days. As evidenced by a fluid retention, the untreated rats had an impaired renal function, a low glomerular filtration rate (GFR), and a high urinary protein excretion. The treatment with vildagliptin restored the GFR, protein excretion, and Na^+. A restoration of the kidney function in the rats was associated with increased levels of active GLP-1, suppression of the DPP-4 activity, and an increase in protein kinase A in the renal cortex. Based on this, the authors concluded that vildagliptin has a reno- and cardioprotective effect.

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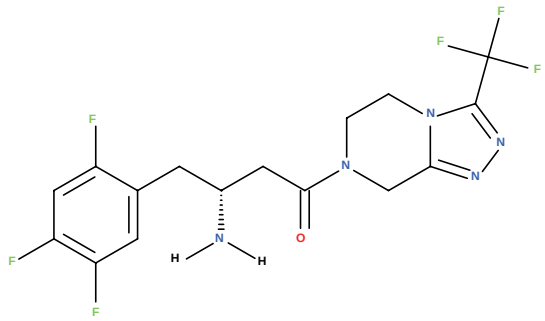
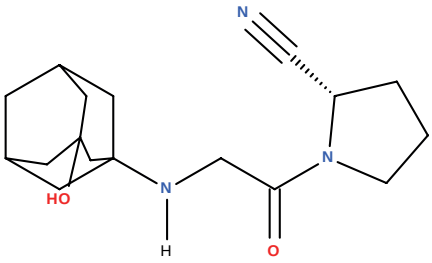
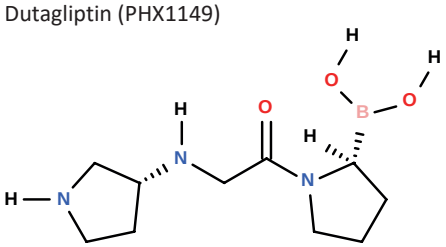
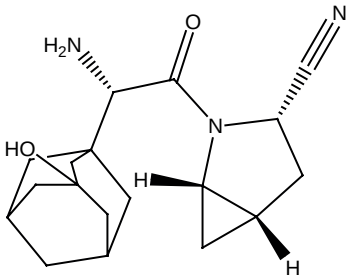
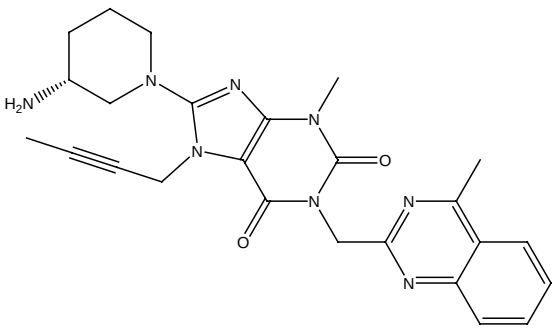
Table 1 – DPP-4 inhibitors, general information

Drugs	General information
Sitagliptin (MK-0431, Januvia®) 	IC_{50}=18 nM; manufactured by Merck, registered in more than 40 countries, including the US and EU countries; bioavailability is 87%; after a single dose of 25 mg its DPP-4 enzyme activity is inhibited by 80% and 47% at 2 and 24 h, respectively; selectivity for related enzymes: DPP-8 and DPP-9 >2 600 times; $T_{1/2}$ – 12 h; it reduces the content of glycated hemoglobin at the dose of 100 mg per day 0.8%; a small part of the drug is metabolized; the enzymes CYP3A4 and CYP2C8 are involved in the process. There are six metabolites found out, they do not have any DPP-4 inhibitory activity [22, 60].
Vildagliptin (LAF-237, Galvus®) 	IC_{50}=3.5 nM; manufactured by Novartis, registered in more than 78 countries, including the US and EU countries; bioavailability is 85%; after taking a single dose of 100 mg, the activity of the DPP-4 enzyme is inhibited by 80% within 7 h and retains 40% after 24 h; selectivity for natural enzymes: DPP-8 >250 times and DPP-9 by 23 times; $T_{1/2}$ – 3 h; it reduces the content of glycated hemoglobin at the dose of 25 mg per day – 0.6%; 69% of the drug dose undergoes biotransformation, the main metabolite, LAY151 (57% of the dose), is pharmacologically inactive and is hydrolysis product of the cyanocomponent [22].
Dutagliptin (PHX1149) 	IC_{50}=25 nM; manufactured by Phenomix Corp, passes the 3rd stage of CTs; the activity of the DPP-4 enzyme is inhibited by 90% when using the drug at the dose of 400 mg for 24 h, and by 50% – within 24 h if the dose is 100 mg; its selectivity for related enzymes: DPP-8 and DPP-9 >400 times; $T_{1/2}$ – 10–13 h; reduces the content of glycated hemoglobin at the dose of 400 mg after 12 weeks – 0.52%, at the dose of 200 mg – -0.35%; it is excreted unchanged through the kidneys [72, 73].
Saxagliptin (BMS-477118, Onglyza®) 	IC_{50}=26 nM; manufactured by Bristol-Myers Squibb, registered in 56 countries including the US, Canada, Mexico, 30 EU countries, Chile, India, Brazil, Argentina and Switzerland; bioavailability is 67%; it inhibits DPP-4 activity by 80 and 57% for up to 90 min and 24 h, respectively, at a single dose of 10 mg. At a single dose of 100 mg, it inhibits the DPP-4 activity by more than 95%; selectivity for related enzymes: DPP-8 >390 times and DPP-9 >77 times; it reduces the content of glycated hemoglobin at the dose of 2.5–10 mg ~ 1%; metabolized to the active metabolite M_2. $T_{1/2}$ – 2–4 h for saxagliptin and 3–7 h for the M_2 metabolite [13].
Linagliptin (BI-1356, Tradjenta®) 	IC_{50}=1.0 nM; manufactured by Boehringer Ingelheim, registered in Austria, Australia, Brazil, Great Britain, Greece, Spain, India, Canada, Korea, Mexico, USA, Singapore, Japan, Russia; bioavailability is 30%; at a single dose of 10.0 mg/kg, the inhibition of plasma DPP-4 is ≥80% within 24 h; selectivity for related enzymes: DPP-8 and DPP-9 >10 000 times; $T_{1/2}$ – 113–131 h; it reduces the content of glycated hemoglobin at the dose of 5 mg after 24 weeks 0.69%; it is practically not metabolized in the body, one main metabolite of linagliptin which does not have a pharmacological activity, is known [83].

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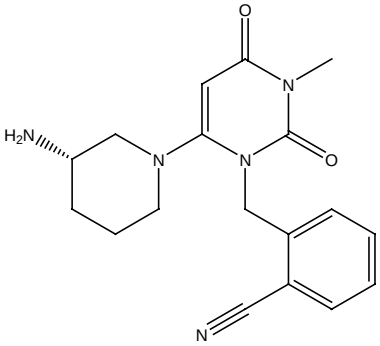
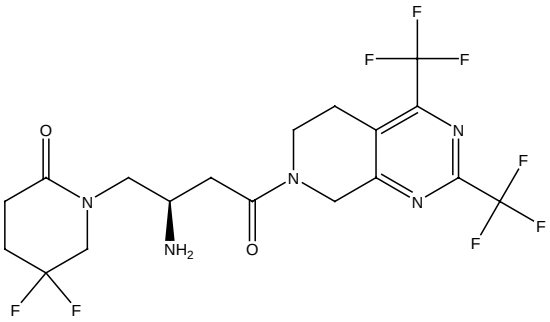
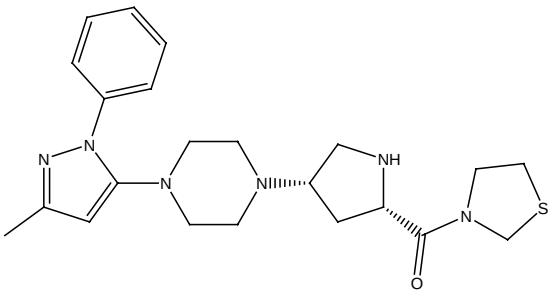
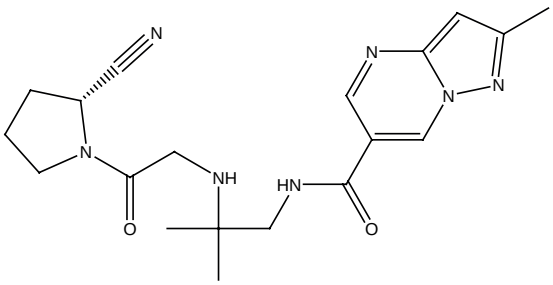
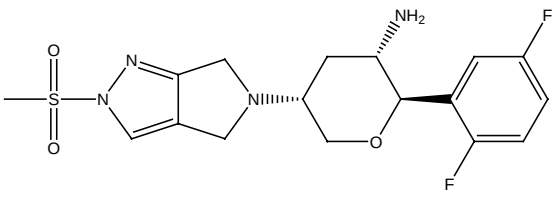
Drugs	General information
Alogliptin (SYR-322, Nesina® in the US and Vipidia® in Europe) 	IC_{50}=7.0 nM; manufactured by Takeda, registered in the USA, EU countries, Russia, China, Japan, Korea; bioavailability is 100%; its application causes more than 90% inhibition of DPP-4 for 24 h at the dose of 25 mg per day; highly selective (>10 000 times) against DPP-4 compared with other isoenzymes (DPP-2, DPP-8, DPP-9, etc.; $T_{1/2}$ – 21 h; at the dose of 25 mg after 26 weeks – 0.6%; it is not extensively metabolized, 60–71% of alogliptin is excreted unchanged by the kidneys. There are two minor metabolites – N-demethylated alogliptin (less than 1% of the original compound) and N-acetylated alogliptin (less than 6% of the original compound). N-demethylated metabolite is active, it is an inhibitor of DPP-4. About 10-20% of the dose of the drug is metabolized in the liver under the influence of cytochromes CYP3A4 and CYP2D6 [89].
Gemigliptin (LC15-0444, Zemiglo®) 	IC_{50}=6.3 nM; manufactured by LG Life Sciences, registered in Korea, India, Colombia, Costa Rica, Panama and Ecuador; its bioavailability is more than 63%; at the dose of 200 mg it inhibits the activity of DPP-4 in plasma by more than 80% within 24 h, at the dose of 400 mg – for 36 h, 600 mg for 48 h; a selectivity for related enzymes: DPP-8 >27 000 times, DPP-9 > by 23 000 times, FAP-α >41 000 times; $T_{1/2}$ – 17 h, for the active metabolite – 24 h; at the dose of 50 mg per day after 24 weeks, the decrease in glycated hemoglobin is 0.71%; about 10% of the dose is metabolized with the participation of cytochrome CYP3A4 to LC15-0636, hydroxylated gemigliptin [97].
Teneligliptin (MP-0513, Tenelia®) 	IC_{50}=1.8 nM; manufactured by Mitsubishi Tanabe, registered in Japan, Korea, India; bioavailability is 63–85%; inhibits the activity of plasma DPP-4 by more than 50% within 24 h after a single dose of 1 mg/kg; the selectivity for related enzymes: DPP-8 >703 times and DPP-9 >1 460 times; $T_{1/2}$ – 8–16 h; the decrease in glycated hemoglobin is 0.9 at the doses of 10 and 20 mg, by 1% at 40 mg after 12 weeks; about 65.6% of the dose is metabolized [100].
Anagliptin (SK-0403, Suiny®) 	IC_{50}=3.8 nM; manufactured by Sanwa Kagaku Kenkyusho, registered in Japan, Korea; bioavailability is 73%; inhibits DPP-4 activity by 95% at the dose of 3 mg/kg; the selectivity for related enzymes: DPP-8 and DPP-9 >10 000 times; at the dose of 100 mg after 24 weeks it causes a decrease in glycated hemoglobin by 0.5%; metabolite M_1 (carboxylate) is 29.2% of the dose, the proportion of other metabolites is about 1%. The half-life of anagliptin is 4.37 h, M_1 is 9.88 h [108].
Omarigliptin (MK-3102, Marizev®) 	IC_{50}=1.6 nM; manufactured by Merck, approved for use in Japan; bioavailability is 74%; causes inhibition of plasma DPP-4 by 77–89% up to 168 h; highly specific for other proteases including DPP-8, DPP-9, QPP, FAP, PEP; $T_{1/2}$ – 68 h; at the dose of 25 mg/week after 54 weeks it causes a decrease in glycated hemoglobin by 0.3%; not metabolized, excreted unchanged mainly through the kidneys, through the intestine – about 3% [113].

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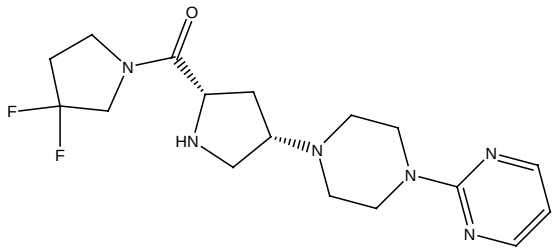
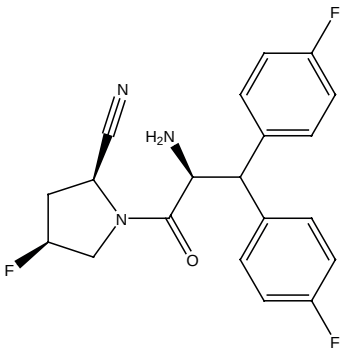
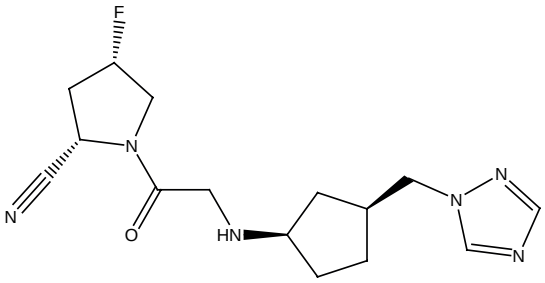
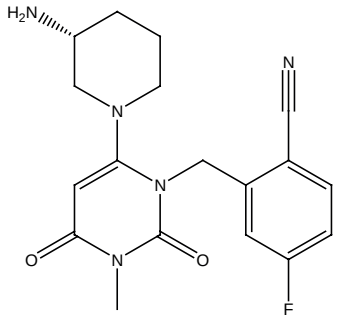
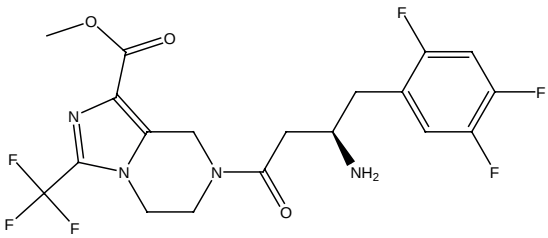
Drugs	General information
Gosogliptin (PF-00734200, Saterex®, SatRx® or Saterex®) 	IC_{50}=13 nM; developed by Pfizer, registered in Russia in 2016; bioavailability is over 99%; it causes inhibition of DPP-4 by 75% after 24 h; selectivity is more than 100-fold for DPP-2, DPP-3, DPP-8 and DPP-9; $T_{1/2}$ – 2.7 h; at the dose of 10 mg per day after 12 weeks it causes a decrease in glycated hemoglobin by 0.7%. The main metabolic pathway of gosogliptin in humans it is associated with hydroxylation of the pyrimidine group (M_5). Other metabolites are associated with amide hydrolysis, carbamoyl glucuronidation, formamide conjugation, glucose conjugation, and creatinine conjugation. Withdrawal: 48.5% – unchanged. It has 8 metabolites, with 17.9% of the dose being metabolite M_5 [117].
Denagliptin (GSK-823093, GW823093) 	IC_{50}=22 nM; manufactured by GlaxoSmithKline, undergoing stage 3 of CTs; a maximum inhibition of DPP-4 is after 30 min and it is more than 85% after 24 h at the dose of 25 mg; at the dose of 45 mg at week 12 of the treatment it causes a decrease in glycated hemoglobin by 0.84%; it has hepatic and extrahepatic metabolism; there are 13 metabolites [22].
Melogliptin (GRC 8200, EMD-675992) 	IC_{50}=1.61 nM; manufactured by Glenmark, passes the 3rd stage of CTs; bioavailability is 60, 90, and 94% in rats, dogs, and monkeys, respectively (5 mg/kg). Data on humans are not published; the drug causes more than 90% inhibition of DPP-4 within 1 h; selectivity for related enzymes: DPP-8 and DPP-9 >10 000 times; it reduces the content of glycated hemoglobin by 0.75 and 0.60% at the dose of 50 mg bid and at the dose of 100 mg per day [22].
Trelagliptin (SYR-472, SYR111472, TAK-472, Zafatec®) 	IC_{50}=4.2 nM; manufactured by Takeda/Furiex, approved for use in Japan and Korea; bioavailability in rats is 50.3%; taking 100 mg causes a 70% inhibition of plasma DPP-4 activity, which persists after 168 h; selectivity for related enzymes: DPP-8 and DPP-9 >10 000 times; $T_{1/2}$ – 72–168 h; at the dose of 100 mg/week after 52 weeks it reduces the content of glycated hemoglobin by 0.57%; metabolized by cytochrome P450 (CYP2D6), excreted mainly through the kidneys [22].
Retagliptin (SP-2086) 	IC_{50}=8 nM; manufactured by Jiangsu Hengrui Medicine, passes the 3rd stage of CTs; selectivity for related enzymes: DPP-8 >3 263 times and DPP-9 >9 438 times; $T_{1/2}$ – 1.5 h [22].

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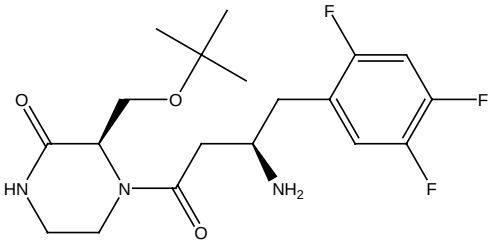
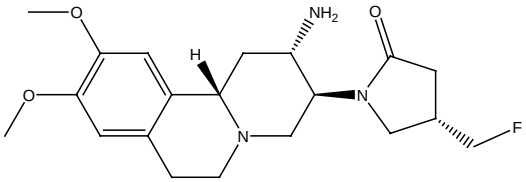
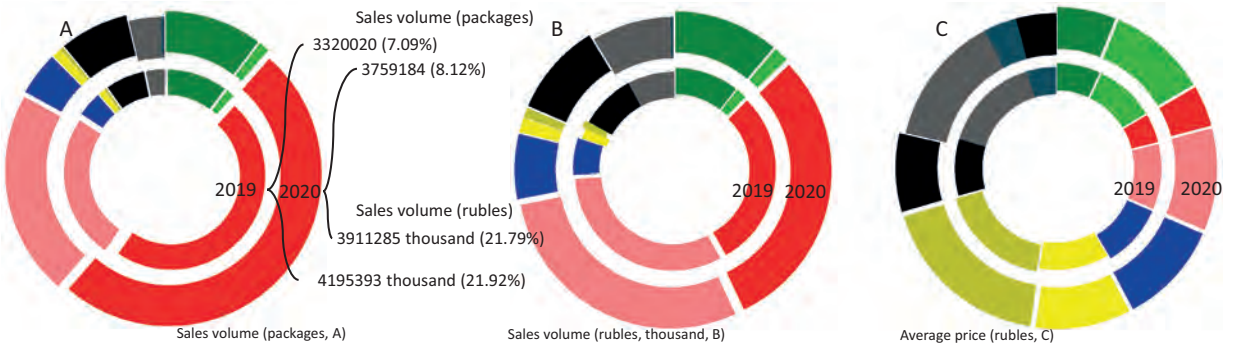
Drugs	General information
Evogliptin (DA-1229, Suganon®, Evodine® or Evodin®) 	IC_{50}=0.98 nM; manufactured by Dong-A Pharmaceutical, registered in South Korea, the drug is sold in Russia; bioavailability is 50.2%; causes inhibition of DPP-4 by more than 80% after a single dose of 5 mg; its selectivity for related enzymes: DPP-8 and DPP-9 >6 000 times; $T_{1/2}$ – 30 h; reduces the level of HbA1c by 0.56% at the dose of 2.5 mg and by 0.61% at the dose of 5 mg. It is metabolized by the processes of oxidation, glucuronization and sulfation. It has four metabolites [22].
Carmegliptin (R-1579) 	IC_{50}=6.8 nM; manufactured by F. Hoffmann-La Roche Ltd, passes the 3rd stage of CTs; bioavailability is 33% at 1 mg/kg in monkeys, 28% in rats, there are no data on humans; it reduces the activity of plasma DPP-4 by 40 and 60% after 24 and 48 h, respectively, after a single oral dose of 3 mg/kg; its selectivity for related enzymes: DPP-8 and DPP-9 >10 000 times, DPP-2 >2 000 times; $T_{1/2}$ – 6.8 h. It is not metabolized; it is excreted unchanged through the liver and kidneys [129].



	Sales volume (packages, A)		Sales volume (rubles, thousand, B)		Average price (rubles, C)	
	2019	2020	2019	2020	2019	2020
Alogliptin	344901 (10.39%)	386596 (10.28%)	415665 (10.63%)	468604 (11.17%)	1090.31 (6.95%)	1027 (6.15%)
Alogliptin + metformin	45844 (1.38%)	43585 (1.16%)	70568 (1.,80%)	67604 (1.61%)	1494.12 (9.53%)	1732.54 (10.38%)
Vildagliptin	1584690 (47.73%)	1876337 (49.91%)	1150815 (29.42%)	1267789 (30.22%)	723.23 (4.61%)	719 (4.31%)
Vildagliptin + metformin	824156 (24.82%)	814582 (21.67%)	1259694 (32.21%)	1214527 (28.95%)	1623.7 (10.36%)	1774.82 (10.64%)
Linagliptin	140976 (4.25%)	174160 (4.63%)	235270 (6.02%)	285992 (6.82%)	1576.83 (10.06%)	1799.83 (10.78%)
Saxagliptin	36965 (1.11%)	37853 (1.01%)	69293 (1.77%)	67458 (1.61%)	1754.28 (11.19%)	1669.22 (10.00%)
Saxagliptin + metformin	14636 (0.44%)	14084 (0.37%)	46093 (1.18%)	43739 (1.04%)	2826.98 (18.03%)	3037.6 (18.20%)
Sitagliptin	222278 (6.7%)	272118 (7.24)	357402 (9.14%)	414779 (9.89%)	1395.99 (8.90%)	1355.08 (8.12%)
Sitagliptin + metformin	104762 (3.16%)	126716 (3.37%)	305884 (7.82%)	354919 (8.46%)	2464.31 (15.72%)	2291.16 (13.73%)
Gosogliptin	812 (0.02%)	11675 (0.31%)	599 (0.02%)	8697 (0.21%)	729.67 (4.65%)	578.31 (3.47%)
Evogliptin	–	1478 (0.04%)	–	1284 (0.03%)	–	703.72 (4.22%)

Figure 1 – Some indicators of iDPP-4 domestic market (according to DSM Group)[1]

Note: the data are presented in Russian rubles, as of 1 Aug 2022, 1 US dollar (USD) corresponded to 61.3 Russian rubles (RUB).

[1] The data was officially purchased from DSM Group. Calculations were made on their basis, diagrams were presented.

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3.3. Dutogliptin (PHX1149, Phenomix Corp)

Dutogliptin is a derivative of boric acid and a representative of the second generation of iDPP-4s. It is active (IC_{50}=25 nM) and highly selective for DPP-4 (unlike DPP-8 and DPP-9 (400 times). It inhibits the enzyme action up to 50% and 80% in dogs and monkeys, respectively, even when measured after 24 h at a single dose of 9 mg/kg. In humans, at the dose of 400 mg, a 90% inhibition of the enzyme is observed within 24 h; at the dose of 100 mg, a 50% inhibition is observed within 24 h [72]. It is excreted unchanged through the kidneys; its half-life is 10–13 h [73]. Currently, it is in Phase III of the clinical trials (CTs).

Pattzi H.M. et al. [74] determined the efficacy and tolerability of dutogliptin in type 2 diabetes patients in a 12-week, multicenter, randomized, double-blind, placebo-controlled study. The patients with a body mass index of 25–48 kg/m^2 and an initial HbA1c level of 7.3–11.0% were randomized to the following groups: dutogliptin – 200 or 400 mg per day, or placebo in addition to taking metformin, thiazolidinedione, or their combinations. After 12 weeks, the use of dutogliptin at the both dosages made it possible to achieve a decrease in the level of HbA1c by 0.52 and 0.35% in the groups treated at the doses of 400 mg (p <0.001) and 200 mg (p=0.006, placebo-corrected values), respectively. The proportion of patients who additionally received 400 and 200 mg of dutogliptin or placebo and achieved the target level of HbA1c <7%, was 27, 21 and 12%, respectively. The fasting plasma glucose levels were significantly lower in both the combination treatment groups compared with placebo: a placebo-adjusted difference of -1.00 mmol/l (p <0.001) for the 400 mg dutogliptin group and -0.88 mmol/ L (p=0.003) for the 200 mg group. Dutogliptin caused a significant decrease in *postprandial* glucose AUC0-120 in both 400 and 200 mg groups (the placebo-corrected values were -2.58 and -1.63 mmol/l/h, respectively). The authors conclude that the treatment with dutogliptin in the combination therapy with metformin and/or thiazolidinedione for 12 weeks improved a glycemic control in the type 2 diabetes patients.

Garcia-Soria G. et al. [75] determined the efficacy and tolerability of dutogliptin (PHX1149) in the type 2 diabetes patients in a multicenter, randomized, double-blind, placebo-controlled 4-week study. The patients with a baseline HbA1c level of 7.3 to 11% were randomized into 4 groups: dutogliptin at the doses of 100, 200, or 400 mg per day, or placebo against the background of a continuous metformin therapy, or metformin+glitazone. In all the groups treated with dutagliptin, there was a significant decrease in glucose AUC0-120 (approximately by 20%). *Postprandialiy,* there was an increase in $AUC_{0–120}$ GLP-1 by 3.90±2.83 pmol/l/h in the placebo group, 11.63±2.86 pmol/l/h in the 100 mg group, 16.42±2,72 pmol/l/h in the 200 mg group and 15.75±2.71 pmol/l/h in the 400 mg dutogliptin group. HbA1c levels were reduced in all the groups treated with dutogliptin; the placebo-corrected change in the 400 mg group was 0.28%. The frequency of adverse events did not differ between the dutogliptin and placebo groups. The authors conclude that the addition of dutogliptin to the chronic metformin or metformin+glitazone therapy in type 2 diabetes patients is well tolerated and improves a glycemic control.

Schenk R. and Nix D. [76] studied the effect of dutogliptin separately and in combination with a granulocyte colony-stimulating factor (G-CSF), which mobilizes stem cells from the bone marrow into the peripheral circulation. According to the authors, dutogliptin prevents the cleavage of the SDF-1 factor of stem cells. The administration of a high/low dose of dutogliptin (the exact doses of dutogliptin are not specified by the authors) in combination with G-CSF for 28 days after a simulated myocardial infarction significantly improved the animal survival and a myocardial remodeling reduced an infarct size compared with dutogliptin and G-CSF used separately. The authors report a planned CT to evaluate the effect of dutogliptin in combination with G-CSF in patients with myocardial infarction.

3.4. Saxagliptin (BMS-477118, Onglyza®, Bristol-Myers Squibb)

Saxagliptin is the first methanopyrrolidine-based iDPP-4. Compared to DPP-8 and DPP-9, saxagliptin is selective and highly active (IC_{50}=26 nM). Saxagliptin inhibits a DPP-4 activity by 80% and 57% for up to 90 min and 24 h, respectively, when taken once at the dose of 10 mg, and at a single dose of 100 mg, it reduces the DPP-4 activity by more than 95%. A bioavailability is about 67% [13]. It is metabolized to active metabolite (M_2). The apparent elimination half-life ($T_{1/2}$) for saxagliptin is 2–4 h, and $T_{1/2}$ for the M_2 metabolite is 3–7 h. The use of the drug significantly reduces the concentration of HbA1c. The drug was approved by the US FDA in 2009.

Rosenstock J. et al. [77] conducted a 12-week, multicenter, randomized, double-blind, placebo-controlled study in type 2 diabetes patients (HbA1c=6.8–9.7%). The patients received saxagliptin at the doses of 2.5, 5, 10, 20, or 40 mg once-daily for 12 weeks (a low dose group). In the second group, the patients received saxagliptin at the dose of 100 mg once-daily for 6 weeks (a high dose group). In all the treatment groups, saxaglipin significantly reduced HbA1c by 0.7–0.9% from the mean baseline compared with placebo (-0.3%). The effect did not depend on the dose. Saxagliptin significantly reduced the concentration of glucose measured on an empty stomach (0.8–1.4 mmol/l). 60 minutes after a meal, the glucose

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levels were lower than in the placebo group by 1.33–1.28 mmol/l. Saxagliptin improved a β-cell function (HOMA) at all the doses. The side effects (hypoglycemia, headache, dyspepsia) were similar to placebo in all the treatment groups.

Matthaei S. et al. [78] studied the efficacy of the saxagliptin administration at the dose of 5 mg per day (compared with placebo) in type 2 diabetes patients (the mean HbA1c was 7.9%) treated with dapagliflozin 10 mg per day and metformin for 52 weeks. The adjusted mean change in HbA1c from the baseline at week 52 was greater in the saxagliptin group than in the placebo one (-0.38% *vs.* 0.05%). The number of patients who achieved the target HbA1c <7% in the group treated with saxagliptin compared with placebo (29% *vs.* 13%), was also higher. The weight loss (≤1.5 kg) was observed in the both groups. A comparable number of patients reported one or more adverse events (58%). The authors conclude that a triple therapy with saxagliptin in addition to dapagliflozin and metformin for 52 weeks improved a glycemic control without any weight gain or an increased risk of adverse events.

Chacra A.R. et al. [79] evaluated the efficacy and safety of saxagliptin in combination with glyburide versus monotherapy in type 2 diabetes patients. The patients received saxagliptin at the doses of 2.5 or 5 mg in combination with glyburide (7.5 mg), while the control group received only glyburide (10 mg) for 24 weeks. In the saxagliptin groups, a more pronounced decrease in the HbA1c concentration was observed (-0.54% in the 2.5 mg group, -0.64% in the 5 mg group *vs.* +0.08% in the glyburide group; p <0.0001) and glucose measured on an empty stomach (-0.389, -0.556 *vs.* +0.056 mmol/L). The number of patients who achieved the target level of HbA1c <7% in the saxagliptin group (2.5 and 5 mg) was greater compared with the glyburide one (22.4 and 22.8% *vs.* 9.1%; p <0.0001).

In a safety study of saxagliptin, in the patients who had taken it at the dose of 5 mg per day for 2 years, the number of patients who were hospitalized for a heart failure in the saxagliptin group was more than in the placebo group (3.5 *vs.* 2.8%). In the absence of an effect on the incidence of ishemia, the rate of hospitalization for a heart failure was not significantly higher. In 2016, the FDA, referring to this study, reported that saxagliptin may increase the risk of developing a heart failure, especially in the patients who had already had heart or kidney diseases. Therefore, the FDA recommends that healthcare professionals consider discontinuing saxagliptin-containing products in the patients who have developrd or are developing a heart failure [80].

In the review research on saxagliptin [81] by Petunina N.A. and Brashchenkova A.V., a number of foreign studies on this drug were summarized. The authors report not only the effectiveness of saxagliptin, but also its high safety. Thus, the subscription of saxagliptin to the patients with type 2 diabetes and a chronic kidney disorder is possible at any stage of the disease, including terminal. The use of saxagliptin is also justified in case of an impaired liver function, including any degree of a liver failure. A very important advantage of saxagliptin can be also considered its cardiovascular safety, confirmed by the results of a meta-analysis of 8 clinical trials.

Kosaraju J. et al. [82] studied the effect of saxagliptin in rats with streptozocin-induced Alzheimer's disease (AD). Three months after the induction of AD, the animals were administered with saxagliptin *p.o.* (0.25, 0.5 and 1 mg/kg) for 60 days. Saxagliptin minimized cognitive deficits, which can be associated with a decrease in the amyloid concentration, tau protein phosphorylation and neuroinflammation, and also showed neuroprotective properties.

3.5. Linagliptin (BI-1356, Tradjenta®, Boehringer Ingelheim)

Linagliptin is a second-generation xanthine-based DPP-4 inhibitor. One of the most highly active (IC_{50}=1.0 nM) and selective for DPP-4 (compared to DPP-8 and DPP-9 by >10 000 times). A bioavailability is approximately 30%, a half-life is 113–131 h. At a single dose of 10 mg/kg, the inhibition of plasma DPP-4 is ≥80% and it persists for 24 h. It was approved by the FDA in the USA in 2011. Unlike other inhibitors, it actively binds to plasma proteins (>80%) and is practically not metabolized. Linagliptin is excreted mainly in the bile (84.7% after the oral administration and 58.2% after the intravenous administration) and less through the kidneys (5.4% after the administration *p.o.* and 30.8% after the intravenous administration). Thus, a dose adjustment for a renal insufficiency is practically not required, which can be an important advantage for the patients with type 2 diabetes and nephropathy [83].

The study by del Prato S. et al. [84] reported the results of a phase 3 multicenter randomized trial of linagliptin. The dosage of the drug was 5 mg per day for the patients with type 2 diabetes who had received the drug for 24 weeks. The average decrease in the concentration of HbA1c, compared with the initial values, was -0.69% (p <0.0001). The severity of the hypoglycemic effect depended on the initial level of HbA1c. So, for the group with the initial level of HbA1c <7.5%, its decrease after the treatment was -0.57%, with HbA1c=7.5–8% – -0.55% (p <0.005), with HbA1c 8–9% – -0.71% (p <0.0001), and with HbA1c ≥9%, the decrease was 1.1% (p <0.0001). In the group treated with linagliptin, there was also a more significant decrease in the glucose concentration measured on an empty stomach (1.3 mmol/l; p <0.0001) and 2 h after a meal (3.2 mmol/l; p <0.0001). The proportion of the patients

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achieving HbA1c <7% after 24 weeks of treatment was 25.2% in the linagliptin group and only 11.6% in the placebo group (p=0.0006).

Taskinen M.R. et al. [85] studied the effect of lingaliptin (5 mg per day) in patients with the uncompensated type 2 diabetes treated with metformin at the dose of ≥1500 mg per day for 24 weeks. In the patients receiving linagliptin in addition to metformin, there was a greater decrease in HbA1c compared with placebo adjusted mean changes from the baseline (-0.49 *vs.* 0.15% placebo), fasting glucose (-0.59 *vs.* 0.58 mmol/l placebo) and glucose levels 2 h after a meal (-2.7 vs. 1.0 mmol/l in the placebo group); p <0.0001. The episodes of hypoglycemia were observed in 3 patients (0.6%) treated with linagliptin and 5 patients (2.8%) in the placebo group. The authors conclude that the addition of linagliptin 5 mg once-daily to the patients with type 2 diabetes resulted in a clinically significant improvement in the glycemic control without increasing the risk of hypoglycemia.

Forst T. et al. [86] compared the effects of linagliptin at the doses of 1, 5 and 10 mg once-daily, glimepiride (1–3 mg once-daily) and placebo in the type 2 diabetes patients with an inadequate glycemic control (HbA1c ≥7.5–10%) with metformin monotherapy. After 12 weeks of treatment, the placebo-corrected mean change in HbA1c levels in the group treated with linagliptin 1 mg was -0.40%, 5 mg -0.73%, 10 mg -0.67%. For glimepiride, the change in mean placebo-adjusted HbA1c from the baseline was –0.9%. The frequency of adverse events was low and comparable in all groups. There were no episodes of hypoglycemia in the linagliptin or placebo groups, in contrast to the glimepiride group (5%).

Owens D.R. et al. [87] reported the results of a multicenter, 24-week, randomized, double-blind clinical trial conducted in type 2 diabetes patients treated with linagliptin at the dose of 5 mg per day or placebo when added to the main therapy with metformin or a sulfonylurea drug. At week 24, a change in the mean placebo-adjusted HbA1c from the baseline was -0.62% (p <0.0001). More patients with the baseline HbA1c ≥7.0% achieved HbA1c <7.0% in the linagliptin group compared with placebo (29.2% *vs.* 8.1%; p <0.0001). The fasting plasma glucose concentration was lower in the linagliptin group compared with placebo (p <0.0001). In addition to metformin or a sulfonylurea, linagliptin also showed significant improvements in the β-cell function (p <0.001). The proportion of patients with serious adverse effects was low in both groups (linagliptin 2.4%, placebo 1.5%). The episodes of hypoglycemia were observed in 16.7 and 10.3% of patients in the linagliptin and placebo groups, respectively. Hypoglycemia was mostly mild to moderate; severe hypoglycemia was noted in 2.7 and 4.8% of participants in the linagliptin and placebo groups, respectively. The authors note that in type 2 diabetes patients, the addition of linagliptin to the combination therapy with metformin and sulfonylurea drugs significantly improved the glycemic control and was well tolerated.

In the course of a two-year study of the linagliptin efficacy (5 mg per day) and glimepiride (1–4 mg per day) in combination with metformin in patients with the uncompensated type 2 diabetes, the average reduction in HbA1c with linagliptin was -0.16%, and glimepiride -0.36%. HbA1c levels less than 7% at week 104 of the treatment were observed in 30% of patients in the linagliptin group and 35% in the glimepiride group. In the linagliptin group, there were fewer episodes of hypoglycemia compared with glimepiride (7 and 36%, respectively) [88].

Kosaraju J. et al. [15] studied the efficacy of linagliptin in 3xTg-AD mice (a transgenic line of mice with AD). The mice were administered with linagliptin *p.o.* (5, 10 and 20 mg/kg) for 8 weeks. The authors found out that the treatment with linagliptin for 8 weeks dose-dependently reduced cognitive deficits, increased the concentration of incretins in the brain, and reduced the tau-phosphorylation, neuroinflammation, and β-amyloidization processes. The authors noted that linagliptin has nootropic properties, which can be explained by the passage of more GLP-1 and GIP through the BBB and an increase in the concentration of incretins in the brain.

3.6. Alogliptin (SYR-322, Nesina® in the US and Vipidia® in Europe, Takeda)

Alogliptin is a third generation DPP-4 inhibitor based on pyrimidinedione (IC_{50}=7 nM) [89]. It is highly selective (>10 000 times) for DPP-4 (compared to other isoenzymes such as DPP-2, DPP-8, DPP-9, etc.), inhibits DPP-4 by more than 90%. The effect persists for 24 h when used at the dose of 25 mg per day. Alogliptin is not extensively metabolized: 60–71% of it is excreted unchanged by the kidneys. There are two minor metabolites, N-demethylated alogliptin (less than 1% of the parent compound) and N-acetylated alogliptin (less than 6% of the parent compound). The N-demethylated metabolite is active and is an inhibitor of DPP-4. About 10–20% of the drug dose is metabolized in the liver under the influence of cytochromes CYP3A4 and CYP2D6. The bioavailability of alogliptin is approximately 100%. It has been approved by the FDA since 2013.

DeFronzo R.A. et al. [90] conducted a 26-week, double-blind, placebo-controlled study in patients with the uncompensated type 2 diabetes and an average initial level of HbA1c=7.9%. The authors found out that the use alogliptin at the doses of 12.5 mg, 25 mg, or placebo 1 once-daily, led to a significant decrease in the

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concentration of HbA1c and glucose, measured on an empty stomach compared with placebo. In the patients receiving 25 mg of alogliptin, a decrease the in HbA1c concentration by 0.6% was observed. At the same time, at week 26 of the treatment, 44% of patients reached the level of HbA1c ≤7%. Significant changes in the fasting glucose concentration and HbA1c were noted as early as week 1. The incidence of side effects (67.4–70.3%) and hypoglycemia (1.5–3.0%) was similar in all the treatment groups. The authors concluded that monotherapy with alogliptin in patients with type 2 diabetes is well tolerated and significantly improves a glycemic control without increasing the incidence of hypoglycemic conditions.

Rosenstock J. et al. [91] also studied the effects of alogliptin in patients with the uncompensated type 2 diabetes with an HbA1c level of about 8.8% in a 26-week, double-blind study. The patients received alogliptin 25 mg per day, pioglitazone 30 mg per day, alogliptin/pioglitazone 12.5/30 mg, or alogliptin/pioglitazone 25/30 mg per day. A combination therapy with alogliptin/pioglitazone (25/30 mg) caused a more significant decrease in the HbA1c concentration (-1.7±0.1%) compared with other groups (alogliptin 25 mg – -1.0±0.1%; p <0.001, pioglitazone 30 mg – -1.2±0.1%, p <0.001 and fasting glucose (-2.8±0.2 mmol/l) *vs.* alogliptin 25 mg group (-1.4±0.2 mmol/l; p<0.001) or pioglitazone 30 mg (-2.1±0.2 mmol/l; p=0.006). The combination of alogliptin (25 mg) and pioglitazone (30 mg) when taken once a day led to a more significant (than monotherapy) decrease in the plasma HbA1c concentration (1.7%) and fasting glucose (-24 mg/dl, which corresponds to 1.33 mmol/l).

Chen X.W. et al. [92] reported the results of a multicenter, randomized, double-blind, placebo-controlled, 26-week use of alogliptin in patients with type 2 diabetes (the mean baseline HbA1c=8.4%). The patients were randomized to the following groups: placebo; metformin 500 or 1000 mg bid; alogliptin 12.5 mg bid; alogliptin 25 mg once-daily; alogliptin 12.5 mg with metformin 500 mg bid or alogliptin 12.5 mg with metformin 1000 mg bid. Both combination therapy options (alogliptin 12.5 mg and metformin 500 or 1000 mg) produced statistically significant improvements in HbA1c and fasting glucose compared with monotherapy. In the groups receiving a combination therapy, the number of patients who achieved the target levels of HbA1c (compared with monotherapy) – 47 and 59% *vs.* 20–34% – was also higher. The authors concluded that alogliptin in combination with metformin significantly improved a glycemic control in patients with type 2 diabetes.

Pratley R.E. et al. [93] presented the results of a 26-week placebo-controlled study in patients with the uncompensated type 2 diabetes who had received pioglitazone separately or in combination with metformin or sulfonylurea (10 mg) (the baseline HbA1c=8%). The addition of alogliptin 25 mg per day to the pioglitazone therapy resulted in statistically significant improvements from the baseline HbA1c and decreased fasting glucose compared to placebo. A clinically significant decrease in HbA1c levels was observed in combination with alogliptin compared with placebo, regardless of the fact whether the subjects simultaneously received metformin or sulfonylurea (0.2% placebo *vs.* 0.9% alogliptin) or pioglitazone (0% placebo *vs.* 0.52% alogliptin).

The safety of alogliptin was studied in patients with type 2 diabetes associated with an acute coronary syndrome (ACS). The patients received alogliptin or placebo in addition to hypoglycemic therapy for 18 months. Mortality from cardiovascular diseases was 4.1% in the alogliptin group and 4.9% in the placebo group. Hospitalization for a heart failure was required in 3.9% of the patients treated with alogliptin compared with 3.3% in the placebo group [94]. Referring to this study, the FDA reported in 2016 that alogliptin (as well as saxagliptin) can increase the risk of a heart failure, especially in the patients who had already had a heart or kidney disease. As a result, the FDA recommended that healthcare professionals consider discontinuing the use of the drugs containing alogliptin in the patients who have a risk of developing a heart failure.

Mkrtumyan A.M., the Head of the Department of Endocrinology and Diabetology, the Faculty of Medicine, Moscow State Medical and Dental University named after A.I. Evdokimov, published a number of review articles on the efficacy and safety of alogliptin, both in monotherapy and in combination with other antidiabetic drugs [95, 96]. It was concluded that the use of alogliptin in patients at a high risk of a cardiovascular failure is not associated with the development of new events, and after a recent ACS, the risk of death from cardiovascular complications during the treatment with alogliptin is not higher than in the patient's taking placebo.

3.7. Gemigliptin (LC15-0444, Zemiglo®, LG Life Sciences)

Gemigliptin is a structural analog of sitagliptin, it has a long inhibitory effect on DPP-4 (IC_{50}=6.3 nM), with a high selectivity against isoenzymes DPP-8 (more than 27 000 times), DPP-9 (more than 23 000 times), FAP-α (over 41 000 times). After the oral administration, about 10% of the dose is metabolized to the active metabolite LC15-0636, which is twice as potent as gemigliptin. Its absolute bioavailability is more than 63%, it inhibits the activity of DPP-4 by more than 80%, and the effect persists for 24 h. The drug has been approved for the treatment of type 2 diabetes in the South Korea [97].

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Rhee E.J. and co-authors studied the effect of different doses of gemigliptin (50, 100 and 200 mg per day) in a double blind, randomized study for 12 weeks [98]. All the three doses of gemigliptin significantly reduced HbA1c from the baseline (-0.06 in the placebo group *vs.* -0.98, -0.78, and -0.74% in the 50, 100, and 200 mg groups, respectively), with no significant differences between the doses. The patients with higher baseline HbA1c levels (≥8.5%) experienced greater reductions. After 12 weeks of treatment, the insulin sensitivity and secretion improved significantly, and the concentrations of total cholesterol and low-density lipoprotein decreased in the 50 and 200 mg per day groups compared to the placebo group. The authors conclude that the treatment with gemigliptin (50 mg per day) for 12 weeks reduces HbA1c and fasting glucose, improves an insulin sensitivity and a β-cell function, and is well tolerated by patients.

A randomized, double-blind, phase III study evaluated the efficacy of gemigliptin in combination with metformin [99]. The patients had been randomized to receive gemigliptin 50 mg per day, metformin (long-acting) or a combination of the two once-daily. The mean daily dose of metformin at week 24 was 1.7 mg in combination with gemigliptin and 1.9 mg in the metformin monotherapy group, respectively. The mean change in HbA1c from the baseline was -2.1% in the gemigliptin+metfomin group compared to -1.2% in the gemigliptin group and -1.5% in the metformin group, respectively (p <0.0001). The differences in achieving the target HbA1c level of 6–7% were also statistically significant (p <0.0001) between the groups receiving combined and monotherapy. The authors conclude that gemigliptin and metformin are effective treatments for type 2 diabetes.

3.8. Teneligliptin (MP-0513, Tenelia®, Mitsubishi Tanabe)

Teneligliptin is a bicyclic derivative of heteroarylpiperazine. It has high activity (IC_{50}=1.8 nM) and selectivity for DPP-4 in comparison with DPP-8 more than 700 times, and DPP-9 – more than 1460 times. The half-life in rats is 8–16 h, a bioavailability at the dose of 0.1–1.0 mg/kg *p.o.* is 63–85%. 65.6% of the drug dose is metabolized. It inhibits the activity of plasma DPP-4 by more than 50% within 24 h after a single dose of 1 mg/kg and significantly reduces the concentration of glucose in the blood in a dose-dependent manner [100]. Teneligliptin was approved for the treatment of type 2 diabetes in Japan in 2012.

Kadowaki T. and Kondo K. [101] studied various doses of teneligliptin (10, 20 and 40 mg per day) *vs.* placebo in the patients with uncompensated type 2 diabetes in monotherapy for 12 weeks. In all the groups, with the exception of placebo, there was a decrease in the concentration of HbA1c and fasting glucose. The difference in the HbA1c reduction was not significant between the groups receiving different doses of teneligliptin, and it was -0.9% for the doses of 10 and 20 mg, and -1.0% for 40 mg. The difference in fasting glucose declines between placebo and teneligliptin 10, 20, and 40 mg was -17.8 mg/dL (0.9 mmol/l), -16.9 mg/dl (0.9 mmol/l) and -20.0 mg/dl (1.1 mmol/l), respectively (p <0.001).

Otsuki H. et al. [102] studied the effects of teneligliptin at the dosage of 20 mg per day in patients with type 2 diabetes and a terminal stage of the renal disease. After 4 weeks of treatment, the plasma glucose concentration decreased by 36.7 mg/dL (2.0 mmol/l), and at week 24, the difference in HbA1c between the teneligliptin and control groups was -3.1% (p <0.05) and -0.57% (p=0.057), respectively. These parameters were also reduced in the patients who had started teneligliptin instead of voglibose 0.2 mg tid or vildagliptin 50 mg/day due to a poor glycemic control. The authors concluded that teneligliptin (20 mg per day) was well tolerated, safe, significantly improved a glycemic control, and was more effective than either voglibose or vildagliptin.

Hasikata T. et al. [103] studied the effect of teneligliptin on the endothelial and left ventricular function in patients with type 2 diabetes who had been taking the drug at the doses of 20 or 40 mg per day for 3 months. Compared to the baseline levels, HbA1c decreased (from 7.6±1.0 to 6.9±0.7%; p <0.01). 3 months after the end of treatment, there was an improvement in the systolic and diastolic function of the left ventricle, an improvement in the endothelial function: RH-PAT index (Reactive Hyperemia Peripheral Arterial Tonometry) increased from 1.58±0.47 to 2.01±0.72%; p <0.01). In addition, the concentration of circulating adiponectin increased from 27.0±38.5 to 42.7±33.2 pg/mL, which corresponds to 0.09±0.13 and 0.15±0.12 nmol/l, respectively (p <0.01) without changes in patients' body weight. The authors conclude that the teneligliptin treatment improved a left ventricular and endothelial function and also increased serum adiponectin concentrations. These results confirm the cardioprotective effects of teneligliptin in patients with type 2 diabetes.

Kadowaki T. and Kondo K. [104] reported the results of a double-blind, placebo-controlled study in which patients with type 2 diabetes had received teneligliptin at the dose of 20 mg per day in combination with glimepiride (1–4 mg per day). After 12 weeks of treatment in the group receiving combination therapy, the concentration of HbA1c glucose measured on an empty stomach and 2 h after a meal, decreased (the difference with the group receiving placebo and glimepiride was -1.0% HbA1c, -1.5 mmol/l glucose measured on an empty stomach and -2.7 mmol/l after a meal). The entire study lasted 52

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weeks, by the end of this period there was a significant (p <0.001) decrease in HbA1c levels compared to the baseline, and the improvement in the glycemic control (p <0.05).

In another study, Kadowaki T. and Kondo K. [105] investigated the effectiveness of the combined use of teneligliptin 20 mg per day and pioglitazone (15–30 mg per day) in patients with type 2 diabetes for 12 weeks. In the group receiving a combination therapy, there was a decrease in the concentration of HbA1c, fasting glucose and 2 h after a meal (the difference with the placebo and pioglitazone group was -0.7% HbA1c, -16.4 (or 0.911 mmol/l) and -51.3 mg/dl (or 2.85 mmol/l) for fasting and 2 h *postprandial* glucose, respectively).

When studying the combination therapy efficacy with teneligliptin (20 mg per day) and metformin (≥1000 mg per day) in patients with type 2 diabetes for 16 weeks, a difference was notified between the teneligliptin and placebo groups in terms of changes in the HbA1c concentration and glucose measured on an empty stomach (-0.78% and -1.24 mmol/l (22.42 mg/dl), respectively [106].

Tanaka K. et al. [107] studied the effects of teneligliptin (20 mg/day) and linagliptin (5 mg per day) in patients with type 2 diabetes and a chronic renal failure (CRF) in a 12-day crossover study. The patients took teneligliptin or linagliptin for 6 days, and then changed the drug. The average amplitude of changes in the glucose concentration was 83.8±34.0 mg/dl (4.7±1.9 mmol/l) in the linagliptin group and 82.6±32.6 mg/dl (4.6±1.8 mmol/l) in the teneligliptin group. The both drugs reduced the average 24-hour glucose concentration comparablely; there was no significant difference in the maximum and minimum glucose concentrations between them. The authors concluded that in the patients with type 2 diabetes and a chronic renal failure, teneligliptin or linagliptin reduce blood glucose concentrations comparable, having the same safety profile.

3.9. Anagliptin (SK-0403, Suiny®, Sanwa Kagaku Kenkyusho)

Anagliptin is a 2-methyl-pyrazolopyrimidine derivative of cyanopyrrolidine, it has a high activity (IC_{50}=3.8 nM) and a selectivity for DPP-4 compared to DPP-8 and DPP-9 (more than 10 000 times), its bioavailability is about 73%. Metabolite M_1 (carboxylate) is 29.2% of the dose, the share of other metabolites is about 1%. The half-life of anagliptin is 4.4 h, for M_1 it is 9.9 h [108]. The drug dose-dependently inhibits the activity of DPP-4 by 95% at the dose of 3 mg/kg, increases the level of GLP-1 insulin and improves the glycemic control. Anagliptin was approved for the treatment of type 2 diabetes in Japan in 2013.

Kaku K. et al. [109] published the data on the results of a multicenter, randomized, double blind, and placebo-controlled study of anagliptin in patients with type 2 diabetes. The patients received anagliptin (25 to 200 mg bid) or placebo for 12 weeks. In the anagliptin groups, the HbA1c concentration was significantly and dose-dependently lower (25–100 mg), and the difference between the 100 and 200 mg groups was only 0.07%. In the subgroup with the initial HbA1c level of 8.4% or more, the decrease in the HbA1c concentration was significantly greater in the 200 mg group than in the 100 mg bid group. However, the authors conclude that the optimal dose is 100 mg bid, and in the patients with high HbA1c levels, a dose of 200 mg bid can be also used.

Yang H.K. et al. [110] reported the results of a double blind, randomized, placebo-controlled trial in which patients took anagliptin 100 or 200 mg bid or placebo for 24 weeks. At the end of the study, the concentration of HbA1c was significantly lower in the groups treated with anagliptin at the dose of 100 mg (-0.50±0.45%) and 200 mg (-0.51±0.55%). In the placebo group, the concentration of HbA1c increased over the same period (0.23±0.62%). Both doses of anagliptin significantly reduced both fasting plasma glucose (-0.53±1.25 and -0.72±1.25 mmol/l, respectively) and the proinsulin/insulin ratio (-0.04±0.15 and -0.07±0.18 mmol/l, respectively) compared with placebo. No significant change in the body weight from the baseline was observed in all 3 groups. After 24 weeks of treatment with anagliptin, the plasma DPP-4 activity was significantly lower and it was >75% for 100 mg and >90% for 200 mg. The authors concluded that anagliptin at the doses of 100 and 200 mg bid, effectively improves a glycemic control in patients with type 2 diabetes.

Kakuda H. et al. [111] studied the effect of anagliptin on glucose and lipid metabolism, as well as the development of the oxidative stress in patients with type 2 DM. The patients received 200 mg of anagliptin per day *p.o.* for 12 weeks; after that, they were observed for another 12 weeks (the total study lasted 24 weeks). At week 12 of the study, an increase in the early phase insulin secretion, a decrease in HOMA-R and fasting glucose concentrations were found out, indicating a positive effect of anagliptin on the insulin resistance and insulin secretion. After 12 weeks of the treatment, anagliptin reduced the concentration of plasma glucose, triglycerides, atherogenic lipoproteins and LDL, which returned to the level at week 24 (after the drug withdrawal). The authors summarize that since postprandial (alimentary) lipidemia promotes the production of pro-inflammatory cytokines, the development of the oxidative stress and, as a result, the occurrence of the endothelial dysfunction, the use of anagliptin can slow down the development of these conditions.

Kaku K. et al. [112] studied the effects of anagliptin in the combination therapy with an α-glucosidase

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inhibitor, metformin, sulfonylurea drugs (glimepiride, glibenclamide) or thiazolidinedione (pioglitazone) in patients with uncompensated type 2 diabetes (HbA1c=6.9–10.4%) for 52 weeks. An additional 200 mg of anagliptin per day (100 mg bid) or placebo was added to the patients' main therapy. The authors noted an improvement in glycemic parameters (HbA1c) comparable between the groups treated with anagliptin and significantly different from placebo as early as the 12th week.

3.10. Omarigliptin (MK-3102, Marizev®, Merck)

Omarigliptin was developed by Merck and approved for use in Japan in 2015. The drug is an analogue of sitagliptin based on aminotetrahydropyran, in which the central basis of sitagliptin is changed to rigid cyclohexylamine. It is highly active (IC_{50}=1.6 nM) and selective for DPP-4 isoenzymes. Omarigliptin has a unique pharmacokinetic profile with a half-life of about 68 h, once-weekly dosing, and a bioavailability of about 74% [113]. During a 12-week study, it was shown that its use at the dose of 25 mg reduces the concentration of blood glucose and HbA1c. It inhibits plasma DPP-4 by 77–89% for up to 168 h after a single dose and increases the concentration of GLP-1 almost twice. The drug is highly specific for other proteases (IC_{50}>67 μM), including DPP-8, DPP-9, QPP, FAP, PEP; it has a biphasic pharmacokinetic profile, phase I α (40–50 h) and phase I β (93-116 h). The drug is excreted mainly through the kidneys unchanged, through the intestines – about 3%. C_{max}=750 nmol/l, the half-life is about 68 h, T_{max}=0.75–4 h [113].

Sheu W. et al. [114] studied the effects of omarigliptin at the doses of 0.25, 1, 3, 10 and 25 mg per week for 78 weeks compared with placebo in patients with type 2 diabetes. 12 weeks after starting the treatment, omarigliptin reduced HbA1c levels in a dose-dependent manner (the dose of 0.25 mg was minimally effective). Omarigliptin also reduced the concentration of glucose measured on an empty stomach (-1.3 mmol/l) and 2 h after a meal (-2.5 mmol/l). All doses of the drug were well tolerated, and the incidence of adverse effects did not depend on the dose. The authors note that the level of an inhibitory activity of omarigliptin at the dose of 25 mg per week differed little from that of sitagliptin taken at the dose of 100 mg (the measurements were made 168 h after taking omarigliptin and 24 h after taking sitagliptin) and amounted to more than 90%.

Evans R. and Bain S. [115] showed that the use of omarigliptin at the doses of 10–100 mg in healthy volunteers led to a more than twofold increase in the level of GLP-1. At the same time, a comparable increase in GLP-1 was observed in individuals with obesity, diabetes or without it. The authors report that in a 24-week study in the patients with poorly controlled type 2 diabetes who received metformin, omarigliptin at the dose of 25 mg per week, HbA1c reduced at the level comparable to sitagliptin (-0.47% omarigliptin and -0.43% sitagliptin). The authors also report a 54-week comparative study of omarigliptin (25 mg/weekly) and glimepiride (6 mg per day) in the patients with uncompensated type 2 diabetes receiving metformin. Glimepiride was more effective at lowering HbA1c levels (omarigliptin -0.30%, glimepiride -0.48%), as well as fasting glucose levels (omarigliptin -0.15 mmol/l, glimepiride -0.46 mmol/l); however, hypoglycemia was significantly more common in patients in the glimepiride group (26.7 and 5%, respectively).

Tan X. [116] reports the result of a 12-week study of omarigliptin at the doses of 0.25, 1, 3, 10, or 25 mg or placebo in patients with type 2 diabetes. The administration of omarigliptin at the dose of 25 mg per week *p.o.* demonstrated a significant reduction in HbA1c compared with placebo (p <0.001) as early as week 12 of treatment. A significantly higher proportion of patients treated with omarigliptin at the dose of 25 mg achieved the target HbA1c levels compared with placebo (<7% 33.6% *vs.* 21.8% placebo and <6.5% 13.6% *vs.* 4.5% placebo), which is due to a decrease in the plasma DPP-4 activity by 80.7% after 12 weeks of treatment.

3.11. Gosogliptin (PF-00734200, Saterex®, SatRx® or Saterex®, Pfizer)

The drug was developed by Pfizer, which subsequently transferred the exclusive molecule rights to the Russian Chemical Diversity Research Institute (CDRI) "Himrar", which is currently registered (at the end of 2016) and approved for use in the Russian Federation as a hypoglycemic drug. Gosogliptin is a diprolyl-derivative of piperazine with a high activity (IC_{50}=13 nM) and selectivity for DPP-4 in contrast to DPP-2 and DPP-8 (100 times), has a half-life of 2.7 h, its bioavailability is more than 99 %. The drug inhibits DPP-4 by 75% after 24 h. The main metabolic pathway of gosogliptin in humans is associated with the hydroxylation of the pyrimidine group, with the formation of the M5 metabolite (17.9% of the dose). The other 8 metabolites [117] are associated with amide hydrolysis, carbamoyl glucuronization, formamide conjugation, glucose conjugation, and creatinine. After the administration *p.o.*, about 77% of the gosogliptin dose is excreted by the kidneys, with 48.5% unchanged, another 10.5% is excreted through the intestines, with a significant proportion coming from gosogliptin metabolites. A half-life after the administration *p.o.* is about 20 h.

According to Muto S. et al. [118], in healthy volunteers, gosogliptin doubled the level of GLP-1 at the dose of 10 mg/kg and inhibited a DPP-4 activity by 75%, even after 24 h.

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Rosenstock J. et al. [119] investigated the effects of gosogliptin at the doses of 20 and 30 mg in patients with uncompensated type 2 diabetes who had already been treated with metformin for 12 weeks (a placebo-controlled, double-blind, randomized, multicenter study). In the patients treated with gosogliptin, a glycemic control improved significantly: compared with placebo, the concentration of HbA1c decreased by -0.79% (corresponding to 8.6 mmol/mol) in the group of the patients taking gosogliptin at the dose of 20 mg, and by -0.92% (corresponding to 10.1 mmol/mol) in the 30 mg group. The positive effects of gosogliptin did not depend on the dose, in contrast to the side ones. The authors conclude that the 20 mg dose of gosogliptin is preferred.

Terra S.G. et al. [120] studied a gosogliptin effect in patients with uncompensated type 2 diabetes (HbA1c=7–11%) in a multicenter, randomized, double-blind, placebo-controlled study. For 12 weeks, patients received metformin and placebo or gosogliptin at the doses of 2, 5, 10, or 20 mg per day. At the dose of 5 mg per day separately, gosogliptin caused a statistically significant decrease in HbA1c compared with placebo. Reductions in HbA1c were observed at -0.31% (2 mg), -0.74% (5 mg), -0.70% (10 mg), and -0.75% (20 mg). The authors note that the 20 mg per day dose provides a better glycemic control compared to the other doses and placebo.

In Russia, the efficacy and safety of gosogliptin was evaluated compared with vildagliptin as monotherapy in patients with type 2 diabetes in 26 clinical centers involving 299 patients [121]. The participants received gosogliptin 20 mg per day (titrated to 30 mg per day) or vildagliptin 50 mg per day (titrated to 100 mg per day) for 36 weeks. After 12 weeks of gosogliptin monotherapy, a mean decrease in HbA1c was 0.93% (p <0.05) and 1.03% (p <0.05) in the vildagliptin group. Side effects and episodes of hypoglycemia were infrequent and differed little between the groups. The authors concluded that gosogliptin has a comparable efficacy and safety profile to vildagliptin.

3.12. Denagliptin (GSK-823093, GW823093C, GlaxoSmithKline)

Since 2010, GlaxoSmithKline (UK) has been conducting denagliptin clinical trials. This compound is a member of the cyanofluoropyrrolidine class, it significantly inhibits DPP-4 (IC_{50}=22 nM), and is more than 100-fold selective to other DPP-4 isoforms. The maximum inhibition of DPP-4 is observed 30 minutes after thhhe administration at the dose of 25 mg and more than 85% persists after 24 h. It increases the levels of GLP-1/insulin and reduces the concentration of glucagon in the blood plasma. It has hepatic and extrahepatic metabolism and 13 metabolites [22].

As reported by Lotfy M. et al. [73], the pharmacokinetic profile, side effects and clinical effects of denagliptin are similar to those of vildagliptin and saxagliptin, but no study data (no reference sources) have been published. The clinical studies are ongoing.

3.13. Melogliptin (GRC 8200, GlenMark)

Melogliptin is a triazole-containing inhibitor of DPP-4 (IC_{50}=1.61 nM), it has a high selectivity for isoenzymes (10 000 times). Its half-life is 1.28, 4.31 and 2.15 h; a bioavailability (at the dose of 5 mg/kg) is 60, 90 and 94% in rats, dogs and monkeys, respectively [22].

According to Kushwaha R.N. et al. [22] when administered to mice db/db, melogliptin causes a decrease in the glucose concentration by 30% and increases insulin levels twice (in a single dose of 3 mg/kg administered *p.o.*). A single dose of 5 mg/kg completely inhibits a DPP-4 activity in dogs within 1 h and more than 90% when analyzed 6 h later. The clinical trials of melogliptin are ongoing.

3.14. Trelagliptin (SYR-472, Zafatec®, Takeda/Furiex)

Trelagliptin is a pyrimidinedione-based DPP-4 inhibitor (IC_{50}=4.2 nM), highly selective for isoenzymes (10 000 times). A bioavailability in rats is 50.3%, in dogs – 29.8%; the data on humans have not been published [22]. Like omarigliptin, trelagliptin is taken once a week and has a similar pharmacological profile. The drug has been approved for the type 2 diabetes treatment in Japan since 2015.

McKeage K. [122] reported that in healthy volunteers, 7 days after a single dose of trelagliptin (100 mg, 30 min before meals), the average maximum plasma concentration (C_{max}) after 1.3 h was 619.4 ng/mL. A mean half-life is 72–168 h; trelagliptin binds to plasma proteins by 22–28%, it is metabolized by cytochrome P450 (CYP2D6) and excreted mainly through the kidneys.

Grimshaw S.E. et al. [123] report that after 168 h of the 100 mg dose administration, the trelagliptin plasma concentration is sufficient to maintain its pharmacodynamic effect, the inhibition of the plasma DPP-4 activity occurs by 70%. The authors found out that trelagliptin has a slower dissociation rate compared to alogliptin (8 times), and also, unlike saxagliptin and vildagliptin (which are covalent inhibitors of DPP-4), trelagliptin binds to DPP-4 non-covalently.

In 2016, Inagaki N. et al. [124] studied the effects of trelagliptin 100 mg/weekly in patients with the uncompensated type 2 diabetes who had previously received hypoglycemic agents *p.o.* (a combination with sulfonylureas, glinide, α-glucosidase inhibitor, biguanide, or thiazolidinedione), and in monotherapy for 52 weeks. At the end of the treatment, the mean change in HbA1c from the baseline was -0.57% in the trelagliptin

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monotherapy group and -0.37, -0.25, -0.67, -0.31, and -0.74% in the combination therapy groups with sulfonylurea, glinide, α-glucosidase inhibitor, biguanide and thiazolidinedione, respectively. The proportion of the patients achieving HbA1c <7.0% at the end of the treatment was 36% for the trelagliptin monotherapy, 22.7, 34.4, 35.0, 46.9 and 44.6% for the combination therapy with sulfonylurea, glinide, α-glucosidase inhibitor, biguanide and thiazolidinedione, respectively. The inhibition of the DPP-4 activity was measured 7 days after the drug administration. It was found out that at the end of treatment, it persisted for 52 weeks and was 76.48–79.6%. The authors conclude that trelagliptin is a highly effective drug for the treatment of type 2 diabetes in monotherapy and in combination with existing hypoglycemic drugs, and once a week the administration is effective and reasonable.

3.15. Retagliptin (SP-2086, Jiangsu Hengrui Medicine)

Retagliptin is a tetrahydroimidazolo derivative [1,5-a]pyrazine (IC_{50}=8 nM), highly selective for DPP-4 compared to DPP-8 (3 263 times) and DPP-9 (9 438 times) [22]. Its half-life is 1.5 h. The drug reduces the concentration of glucose and its change during the oral glucose tolerance test. The clinical studies are ongoing.

Yong X. et al. studied a combined use of retagliptin and metformin in healthy volunteers (retagliptin 100 mg, metformin 1500 mg or retagliptin 100 mg+ metformin 1500 mg). The authors found out that the combination of retagliptin + metformin did not lead to clinically significant changes in the pharmacokinetics of retagliptin or metformin, compared with their use separately. $AUC_{0-\infty}$ and C_{max} of retagliptin used in combination, were 16.49% and 25.88% higher than for retagliptin in monotherapy; $AUC_{0-\infty}$ of metformin in combination with retagliptin was 22.06% more than in the metformin monotherapy. The authors conclude that the combined use of these drugs does not require a dose adjustment of any of them [125].

3.16. Evogliptin (DA-1229, Suganon®, Evodine® or Evodin®, Dong-A Pharmaceutical)

Evogliptin is a β-aminoamide derivative (IC_{50}=0.98 nM) and is highly selective for isoenzymes (6000-fold). The adminisration of the drug inhibits DPP-4 by more than 80% after a single dose of 5 mg, significantly reduces the level of HbA1c by 0.56% at the dose of 2.5 mg and by 0.61% at the dose of 5 mg. Its half-life is about 30 h and it does not depend on food intake, the bioavailability is 50.2% [22]. It is metabolized by the processes of oxidation, glucuronization and sulfation and has 4 metabolites. The drug was approved for the treatment of type 2 diabetes in Korea in 2015. The Russian pharmaceutical company GeroPharm has received a permission to conduct an international multicenter clinical trial (phase III) and is selling this drug in Russia.

Chae Y.N. et al. [126] investigated the effect of evogliptin in the model of diet-induced obesity in mice. After 2 weeks of treatment at the doses of 20, 60 and 200 mg/kg, it caused a dose-dependent decrease in fat mass and reduced the average size of adipocytes. The authors suggest that a part of the evogliptin-induced fat loss may be due to the acecelerated metabolism, which is not only associated with an increase in GLP-1.

Cho J.M. et al. [127] studied the effects of evogliptin with streptozotocin-induced diabetes (100 mg/kg streptozotocin ip) in C57BL/6 mice after 1 week without treatment, the mice received evogliptin at the dose of 300 mg/kg. An intraperitoneal glucose tolerance test (IPGTT) was performed 10 weeks after the treatment with evoligliptin by intraperitoneal (rather than oral, unlike the oral test) administration of 1 g/kg fasting glucose. In contrast to the control group, a significant decrease (p <0.05–0.005) in the blood glucose concentration was observed in the mice treated with evogliptin. Relatively low glucose concentrations were maintained in the animals even 6 weeks after the evogliptin treatment. Plasma insulin levels before (0 min) and 15 min after glucose administration were significantly higher in the mice treated with evogliptin compared to the controls (p <0.005). In addition, in the group treated with evogliptin, the mass of pancreatic β-cells, their proliferation and neogenesis was higher.

Gu N. et al. [128] studied evogliptin in healthy volunteers. At the dose of 5–20 mg, it inhibited a DPP-4 activity by more than 80% for 24 h in all groups, regardless of the dose, and increased postprandial GLP-1 levels by 1.5–2.4 times compared with placebo.

3.17. Carmegliptin (R-1579, F. Hoffmann-La Roche)

Carmegliptin (IC_{50}=6.8 nM) has a tricyclic base, its selectively inhibits DPP-4 compared to DPP-8, DPP-9 (more than by 100 times) and DPP-2 (more than by 2000 times). Its half-life is 6–8 h, and the bioavailability at 1 mg/kg is 33% in monkeys and 28% in rats [134 Mattei]. The drug is not metabolized, excreted unchanged through the liver and kidneys. Its use (once *p.o.* at the dose of 3 mg/kg) significantly reduces the concentration of glucose in the blood, inhibits the activity of plasma DPP-4 by 40 and 60% after 24 and 48 hours, respectively.

After the administration of 10 mg/kg carmegliptin in ZFR rats, in the course of the oral glucose tolerance test, Mattei R. et al. [129] observed an improvement in the glucose tolerance (30% compared with the control). In the db/db mice, there was a significant (p ≤0.05) decrease in the glucose concentration measured on an empty stomach 2 h after its administration compared

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with the control group. The authors also investigated the carmegliptin efficacy in the ZFR rats at the dose of 20 mg/kg administered for 7 days in the euglycemia model (Euglycemic Hyperinsulinemic Clamp). In this experiment, carmegliptin increased the insulin sensitivity, which was manifested by the maintenance of the normal blood glucose concentration after the administration *p. o.,* compared with the control group.

Kuhlmann O. et al. [130] noted that after the administration of carmegliptin in healthy volunteers, the plasma glucose concentration measured on an empty stomach and after a meal decreased, the secretion of GLP-1 and insulin increased, and the body weight decreased, lipid metabolism and the state of β-cells improved.

CONCLUSION

Despite a fairly large number of registered iDPP-4s, the interest of researchers in this therapeutic target does not fade away. The above DPP-4 ibids have different chemical structures, but they share a moderate hypoglycemic activity, which is expressed in a decrease in the level of HbA1c and AUC of glucose after a meal or an oral glucose tolerance test. For the drugs of this group, there is a high safety use, no effect on the body weight of patients and the possibility of the effective combination with other hypoglycemic drugs. Many of them are already used for the treatment of type 2 diabetes; others are in different phases of clinical trials. The drugs differ in the DPP-4 isoform selectivity, metabolism and pharmacokinetic profile. These factors determine their individual advantages in specific clinical situations.

Serious obstacles, due to which new DPP-4 inhibitors under development can fail in clinical trials, are the pharmacokinetic profile, inhibition of cytochrome P450 enzymes, and selectivity for DPP-4 isoenzymes, which depend on the chemical structure of a particular compound. This class of drugs is promising both in monotherapy for type 2 diabetes and when combined with other hypoglycemic drugs. The interest in the development of such drugs is fueled by the results of the studies that reveal the breadth of their pleiotropic effects due to the spectrum of biological effects of this enzymes group.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Denis V. Kurkin – idea, structure planning, graphic design; Dmitry A. Bakulin, Yuliya V. Gorbunova, Valeria B. Saparova – materials collecting, manuscript draft writing; Evgeniy I. Morkovin, Andrey V. Strygin, Elena V. Volotova – final version of the manuscript editing; Igor E. Makarenko, Roman V. Drai, Vladimir I. Petrov – consultations on highly specialized problems, editing and approval of the manuscript final version.

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RESEARCH ARTICLE

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Protective role of 3-oxypyridine derivatives in rats' steroid-induced osteoporosis associated with reduced oxidative stress and recovery of nitric oxide formation

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From the point of view of the mechanisms for the implementation of pathogenetic links in the development of steroid-induced osteoporosis considered in the paper, the increased risk of the oxidative stress in osteoblasts, as well as the development of the vessels endothelial dysfunction of the microcirculatory bloodstream in the bone tissue, are of particular interest. They lead to the impaired bone tissue trophism and progression of osteoporosis.

The aim of the study was research of the osteoprotective effects of a 3-hydroxypyridine derivatives composition on the model of steroid-induced osteoporosis.

Materials and methods. To model osteoporosis pathology, the animals (male Wistar rats) were injected with methylprednisolone (MP) at the dose of 5 mg/kg (intraperitoneally) every 5th day for 5 weeks. As a non-selective blocker of NO synthase, L-NAME was used at the dose of 25 mg/kg (intraperitoneally). Derivatives of 3-hydroxypyridine (hereinafter referred to as composition No. 1) were administrated at the dose of 50 mg/kg (*per os*) In all experimental groups, the level of microcirculation and the bone mineral density, as well as the analysis of histomorphological and biochemical samples, were assessed.

Results. The study results showed that composition No. 1 (50 mg/kg) has an osteoprotective activity, effectively prevents a decrease in the level of the regional bone tissue microcirculation and in the development of an endothelial dysfunction. That makes it possible to increase the bone mineral density and to slow down the thinning of bone trabeculae. In addition, composition No. 1 (50 mg/kg) reduces the production of reactive oxygen species and increases the NO bioavailability.

Conclusion. The data obtained indicate that the studied composition of 3-hydroxypyridine derivatives is considered a promising compound for the prevention and treatment of steroid-induced osteoporosis.

Keywords: 3-hydroxypyridine derivatives; osteoporosis; reactive oxygen species; oxidative stress; nitric oxide; endothelium

Abbreviations: ROS – reactive oxygen species; MP – methylprednisolone; L-NAME – L-Nitro-arginine methyl ester; NO – nitric oxide; GC – glucocorticoid; NFκB – nuclear factor-κB; RANK – receptor activator for nuclear factor kappa B; RANKL – ligand of receptor activator for nuclear factor kappa B; OPG – osteoprotegerin; NOS – NO-synthase; SOD – superoxide dismutase; MDA – malondialdehyde; EDC – endothelial dysfunction coefficient; LPO – lipid peroxidation; GP – glutathione peroxidase; CSF – colony-stimulating factor; eNOS – endothelial NO-synthase; NOX – nicotinamide adenine dinucleotide phosphate oxidase.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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Протективная роль производных 3-оксипиридина при стероид-индуцированном остеопорозе у крыс, связанная со снижением оксидативного стресса и восстановлением образования оксида азота

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С точки зрения рассматриваемых механизмов реализации патогенетических звеньев развития стероид-индуцированного остеопороза особый интерес представляет повышенный риск окислительного стресса в остеобластах, а также развитие эндотелиальной дисфункции сосудов микроциркуляторного русла костной ткани, приводящее к нарушению трофики костной ткани и прогрессированию остеопороза.

Цель. Изучить остеопротекторные эффекты композиции производных 3-оксипиридина на модели стероид-индуцированного остеопороза.

Материалы и методы. Для моделирования патологии остеопороза животным (самцы крыс линии Wistar) внутрибрюшинно в течение 5 недель вводили метилпреднизолон (МП) в дозе 5 мг/кг каждые 5 дней. В качестве неселективного блокатора NO-синтазы в работе использовали L-NAME в дозе 25 мг/кг, внутрибрюшинно. Производные 3-оксипиридина (в дальнейшем по тексту как композиция № 1), вводились в дозе 50 мг/кг перорально. Во всех экспериментальных группах проводилась оценка уровня микроциркуляции и минеральной плотности костной ткани, анализ гистоморфологических и биохимических проб.

Результаты. Результаты показали, что композиция № 1 (50 мг/кг) оказывала остеопротекторное действие, эффективно предотвращала снижение уровня регионарной микроциркуляции в костной ткани и развитие эндотелиальной дисфункции, что позволило увеличить минеральную плотность костей и замедлить истончение костных трабекул. Кроме того, композиция № 1 (50 мг/кг) снижала выработку активных форм кислорода и увеличивала биодоступность NO.

Заключение. Полученные данные свидетельствуют о том, что изучаемая композиция производных 3-оксипиридина, считается перспективным соединениям для профилактики и лечения стероид-индуцированного остеопороза.

Ключевые слова: производные 3-оксипиридина; остеопороз; активные формы кислорода; оксидативный стресс; оксид азота; эндотелий

Список сокращений: АФК – активные формы кислорода; МП – метилпреднизолон; L-NAME – L-нитро-L-аргининметиловый эфир; NO – оксид азота; ГК – глюкокортикоид; NF-кВ – ядерный фактор-кВ; RANK – активатор рецептора NF-кВ; RANKL – активатор рецептора лиганда NF-кВ; OPG – остеопротегерин; NOS – NO-синтаза; СОД – супероксиддисмутаза; МДА – малоновый диальдегид; КЭД – коэффициент эндотелиальной дисфункции; ПОЛ – перекисное окисление липидов; ГП – глутатионпероксидаза; CSF – колониестимулирующий фактор; eNOS – эндотелиальная NO-синтаза; NOX – никотинамидадениндинуклеотидфосфатоксидаза.

INTRODUCTION

Being widely used in various fields of medicine (rheumatology, pulmonology, hematology, gastroenterology, dermatology, and transplantology), glucocorticoids (GCs) remain one of the effective methods for the treatment of many inflammatory and autoimmune diseases, [1]. However, long-term GC therapy has a number of side effects, one of the most significant among which is steroid-induced osteoporosis. This is the most common form of iatrogenic and secondary osteoporosis [2], which causes a decrease in the bone mineralization and, as a result, fractures

in 30–50% of patients. In terms of prevalence, steroid-induced osteoporosis ranks second among all forms of osteoporosis, second only to postmenopausal and senile ones [3]. The pathophysiology of glucocorticoid-induced osteoporosis is determined by various factors [4–8], including the receptor activator for nuclear factor kappa B (RANK), its ligand (RANKL), and osteoprotegerin (OPG). As a member of the tumor necrosis factor (TNF) superfamily, RANKL regulates osteoclast differentiation, activation, and survival by binding to its cognate RANK receptor, which can interact with several TNF (TRAF) receptor-associated factors to activate signaling molecules [9]. Reactive oxygen species (ROS) are considered the main factors in the RANKL-induced effect on the bone tissue, including in steroid-induced osteoporosis [10–12], which makes it possible to determine one of the most important therapeutic strategies for correcting this pathology.

Another potential target for the treatment of osteoporosis may be nitric oxide (NO). Endogenous NO is formed from L-arginine as a result of a reaction catalyzed by an enzyme of the calmodulin-dependent NO synthases (the NOS family). Endothelial NOS (eNOS), of the three isoforms of NOS, contributes the most to the development of osteoporosis. Strong evidence for a role of NO in the osteoblast function comes from the eNOS knockout animal studies, which report severe defects in the bone formation and an osteoblast activity in both *in vivo* and *in vitro* studies. [13]. In addition, a preventive administration of NO donators (nitroglycyrin and L-arginine) delimits the bone loss, increases bone strength by reducing the development of osteoporosis [14, 15].

Recommendations for the treatment of glucocorticoid-induced osteoporosis include routine calcium and vitamin D supplementations, bisphosphonate therapy, selective estrogen receptor modulators, human monoclonal antibodies to RANKL and its intracellular factor, and a recombinant parathyroid hormone [16-18]. All pharmacological approaches are still controversial and show inconsistent and variable results, which may depend on age, sex, dose and duration of treatment. In addition, a long-term use of certain drugs can lead to serious complications, including kidney damage, venous thrombosis, and an increased risk of developing tumors.

In this regard, the search for new effective approaches for the correction of steroid-induced osteoporosis seems to be a very promising direction in pharmacology.

Derivatives of 3-hydroxypyridine belong to the simplest heterocyclic analogues of aromatic phenols and have a wide spectrum of activity, like antioxidant, antihypoxic, anti-inflammatory, anti-ischemic [19], cardio- and endothelioprotective activities [20]. A huge list of pharmacological effects suggests that a new complex of 3-hydroxypyridine derivatives, consisting of one molecule of 2-ethyl-6-methyl-3-hydroxypyridinium 3-pyridinocarbonoate and three molecules of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate (hereinafter referred to as composition No. 1), obtained by topochemical synthesis (All-Union Scientific Center for the Safety of Biologically Active Substances, Staraya Kupavna, Russia) can become a promising compound for the prevention and treatment of steroid -induced osteoporosis.

THE AIM of the study was research of the osteoprotective effects of a 3-hydroxypyridine derivatives composition on the model of steroid-induced osteoporosis.

MATERIALS AND METHODS

Methods of obtaining and analysis

Chemical reagents necessary to prepare the compound were purchased from commercial suppliers who have a certificate for chemical products (Sigma-Aldrich, USA). The way of composition No. 1 synthesis consisted of the following stages: 26.0 g (0.1 g/mol) of 2-ethyl-6-methyl-3-hydroxypyridinium 3-pyridinocarbonoate was loaded into the homogenizer; while stirring, 93.2 g (0.3 g/mol) 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate was gradually added. The mass was homogenized for 10–15 min at a stirring speed of 300-400 rpm. Next, the particle size of the resulting powder which should be no more than 10 μm, was checked and, if necessary, additionally homogenized. The output was 119.0 g of white fine crystalline powder with a melting point of 139–143°C. The resulting compound is soluble in water with slight opalescence. The following was found out, %: C 62.48; H 7.98; N 9.39 C62 H94 N8O15; m.m. 1 191.46. The result of the calculation, %, was the following: C 62.50; H 7.95; N 9.41; O 20.14. The chemical formula of the compound (composition No. 1) is shown in Fig. 1.

Study design

All experimental studies were carried out in accordance with the Rules of Laboratory Practice approved by the Order of the Ministry of Health of Russia No. 708n, dated Aug 23, 2010, with strict observance of the European Convention for the Protection of Vertebrate Animals used for experiments or other scientific purposes (Directive 2010/63/EU). The experimental studies were approved by the Bioethical Commission of Belgorod State National Research University of the Ministry of Health of Russia (Protocol No. 11/9 dated Feb 12, 2022). The vivisection was carried out in accordance with the ethical principles for the treatment of laboratory animals as set out in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (CETS No. 123).

The experiments were performed on 60 healthy non-morbid male Wistar rats weighing 220–300 grams.

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The animals were obtained from the nursery "Stolbovaya" of the Institution of Science and Technology of the Federal Medical and Biomedical Institute of the Federal Medical and Biological Agency (Moscow Region), were kept under standard conditions that corresponded to the sanitary rules for the arrangement, equipment and maintenance of experimental and biological clinics (vivariums) No. 1045-73.

At the first stage, the laboratory animals were randomly divided into 6 experimental groups: group I – intact animals, intraperitoneally (ip) injected with saline; group II – the animals ip injected with methylprednisolone (MP) at the dose of 5 mg/kg for 5 weeks every 5th day; group III – the animals ip injected with L-NAME at the dose of 25 mg/kg for 35 days; group IV – the animals ip injected with methylprednisolone (MP) at the dose of 5 mg/kg+L-NAME 25 mg/kg ip for 35 days; group V – the animals intragastrically administrated with MP+composition No. 1 at the dose of 50 mg/kg ip with MP+L-NAME+composition No. 1 at the dose of 50 mg/kg BID for 35 days.

On day 36, the animals were withdrawn from the experiment with a further evaluation of densitometry, functional, biochemical and histomorphometric tests. The design of the experiment is shown in Fig. 2.

Bone density test

Densitometry in the animals was performed after a preliminary putting the animals into narcotism with a solution of tiletamine and zolazepam (60 mg/kg) and chloral hydrate (300 mg/kg). The indicator, expressed in g/cm^3, was determined for the proximal metaphysis, diaphysis, and distal metaphysis of the femur. The assessment of the bone density (BD) was carried out using the IN-VIVO MS FX PRO multifunctional laboratory X-ray unit manufactured by Bruker (USA) with a molecular imaging system using licensed Bone Density Software.

Vascular tests

The impact of the bone tissue on the microcirculatory bed is one of the promising approaches in the correction of osteoporosis, and therefore, in all the experimental groups, the microcirculation in the cancellous bone tissue of the proximal metaphysis of the right femur was assessed. To obtain the data on the bone microcirculation, BIOPAC Systems Equipment (USA) was used: an MP100-150 polygraph with an LDF100C laser Doppler flowmetry module and a TSD144 sensor. The results of the laser Doppler flowmetry (LDF) were recorded using Acq Knowledge software (versions 3.8–4.2). Microcirculation parameters were expressed in perfusion units (p.u.). After measuring the intraosseous microcirculation level, a test was performed for an endothelium-dependent vasodilation (acetylcholine 40 μg/kg intravenously (IV)) and an endothelium-independent vasodilation (sodium nitroprusside 30 μg/kg IV), with a further determination of the endothelial dysfunction coefficient (EDC) [21].

Biochemical blood assay

To assess the biochemical parameters in the animals of the experimental groups after conducting vascular tests, the blood was taken using a syringe from the tail vein, followed by the determination of the total calcium content (mmol/l) in the blood plasma by colorimetry with o-cresolphthalein and alkaline phosphatase (U/l) in the blood serum (colorimetric, kinetic methods), on the spectrophotometer SF-46 (LOMO, Russia). The serum levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) (Nanjing Jiancheng Biological Engineering Research Institute, China) were determined according to the manufacturers' instructions [22].

Morphofunctional assessment of bone tissue state

The object of the study for the histological analysis was tibias, which were initially fixed in 10% formalin. The proximal segment was dissected from the fixed bones for 1 cm from the articular surface of the condyles. According to the recommended protocol[1], the material was decalcified in the Surgipath Decalcifier II liquid (Leica, Germany). The decalcified fragments were automatically embedded in paraffin, followed, according to Mallory, by staining the 7 μm thick sections with hematoxylin and eosin. Micropreparations were studied by a scanning method under the microscope "Mikmed" with a video camera "DV1000". Using the McrAView 7.3.1.7 program (LOMO, Russia), the thickness of the bone trabeculae and the cortical bone of the diaphysis was measured.

Statistical analysis

The data were tested for the normal distribution using the Shapiro-Wilk test. Normally distributed data were compared using a conventional one-way analysis of variance (ANOVA) with Tukey's post-hoc test. The non-normally distributed data were compared using the Kruskal-Wallace test and Dunn's nonparametric post-hoc test. The differences were determined at a significance level of 0.05. The statistical analysis was carried out using GraphPad Prism 9.2.0 software (GraphPad Software, USA).

RESULTS AND DISCUSSION

The structure of the supramolecular complex (composition No. 1) was confirmed on the basis of the spectroscopic data: IR spectrum (ν, cm^{-1}): 3412 (OH) 3290 (NH), 2941 (CH), 2673 (N^+), 1781 (C=N–), 1634 (C=C), 1561 (COO–). The mass spectrum of the protonated supramolecular complex in the positive ion scanning mode [M+H] is M/z 1195.46, which corresponds to m.m. 1191.46. The ^{1}H NMR spectra are shown in Fig. 3.

[1] Decalcification. Cardiovascular Pathology (5th Edition), 2022. Available from: https/www.sciencedirect.com/topics/medicine-and-dentistry/decalcification

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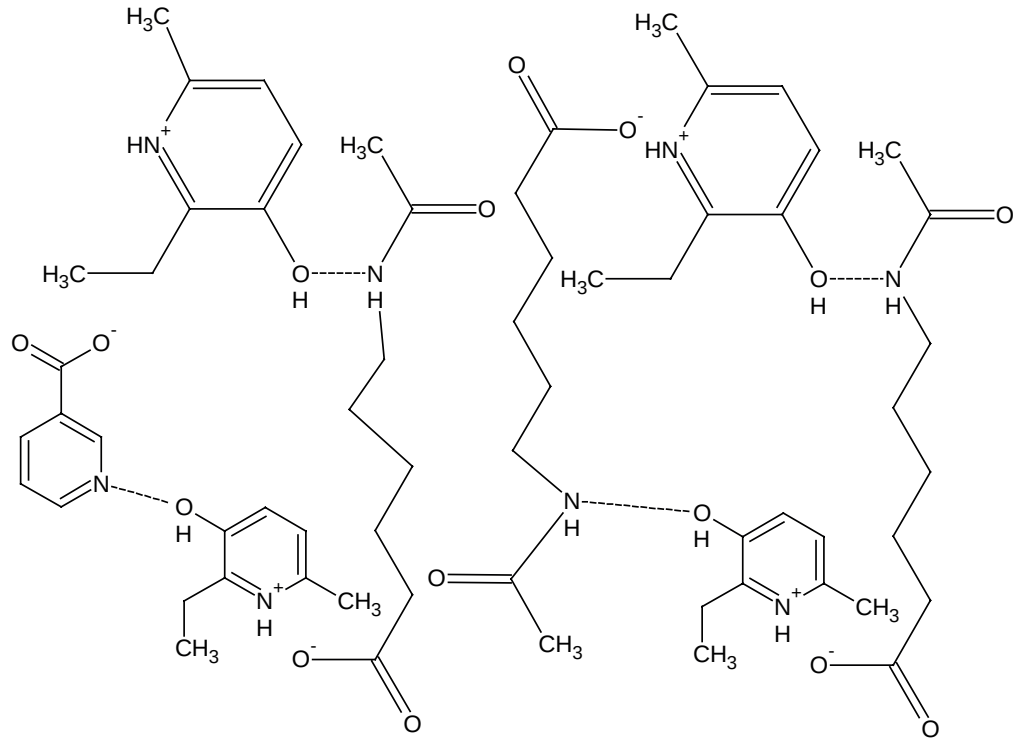

Figure 1 – Structural formula of the compound (composition No. 1)

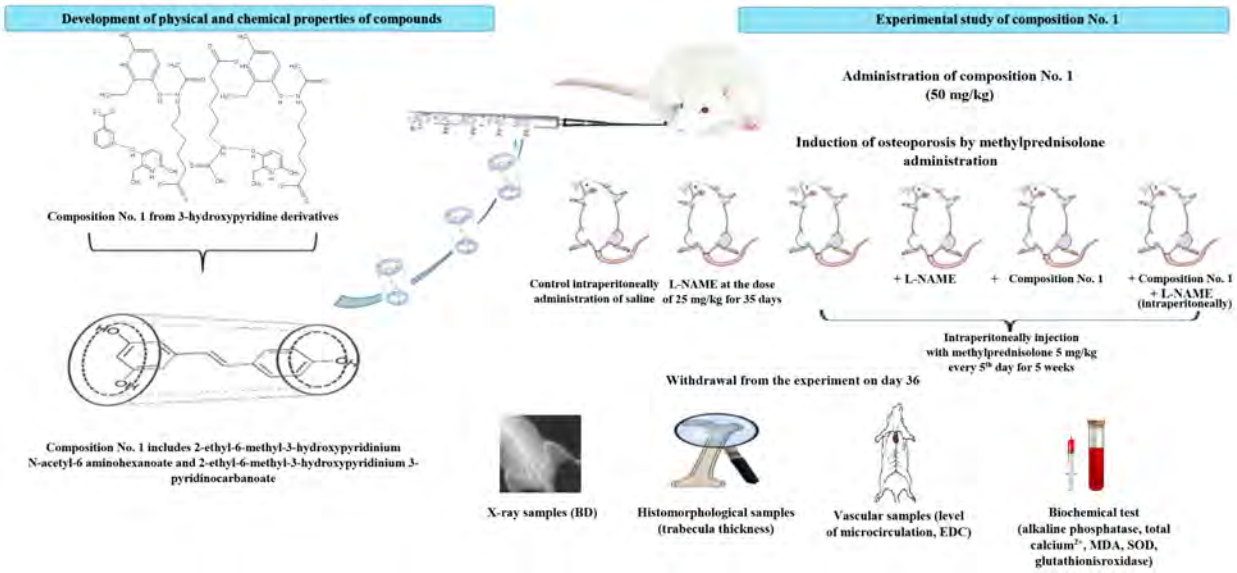

Figure 2 – Experiment design

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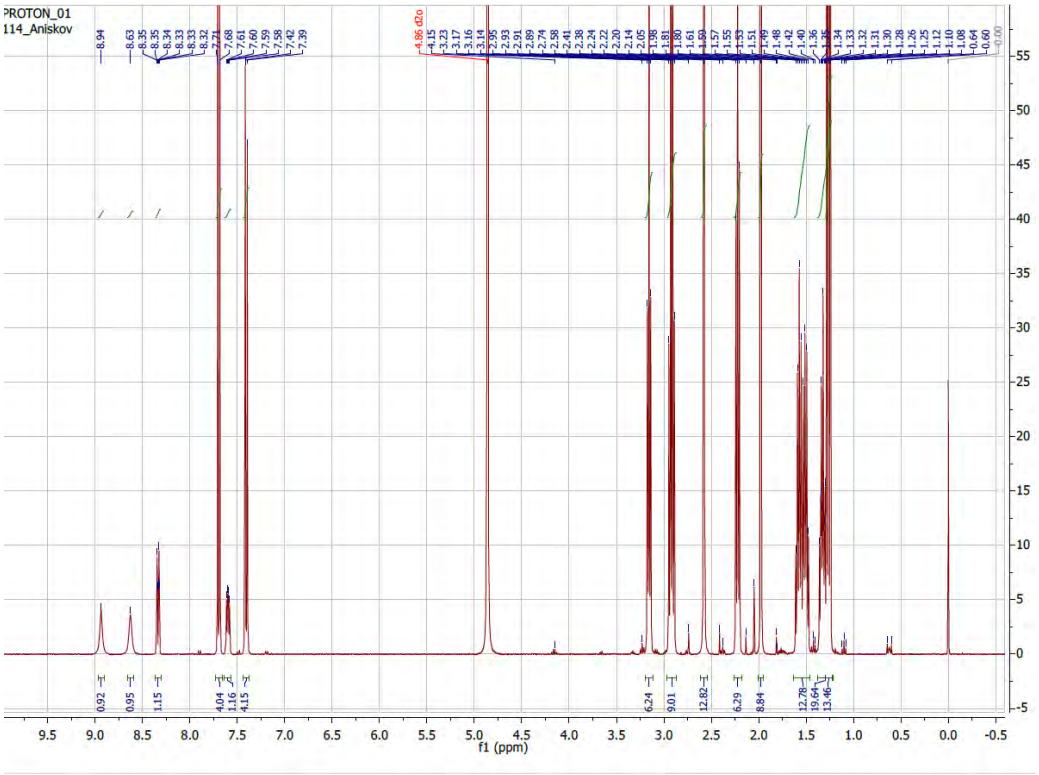
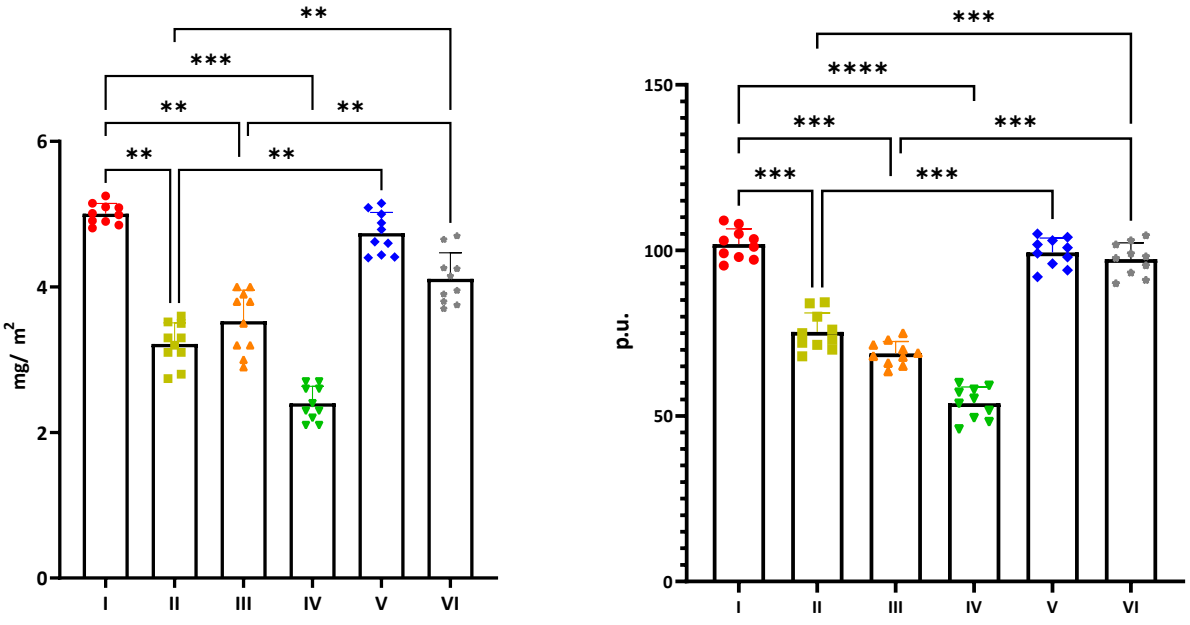


Figure 3 – ^{1}H NMR spectrum of composition No. 1



A **B**

Figure 4 – Effect of composition No. 1 on bone density (A) and the level of microcirculation (B) in steroid-induced osteoporosis

Note: (*here and in Fig. 3-5*): I – intact; II – MP; III – L-NAME; IV – MP+L-NAME; V – MP+composition No. 1; VI – MP+L-NAME+composition No. 1

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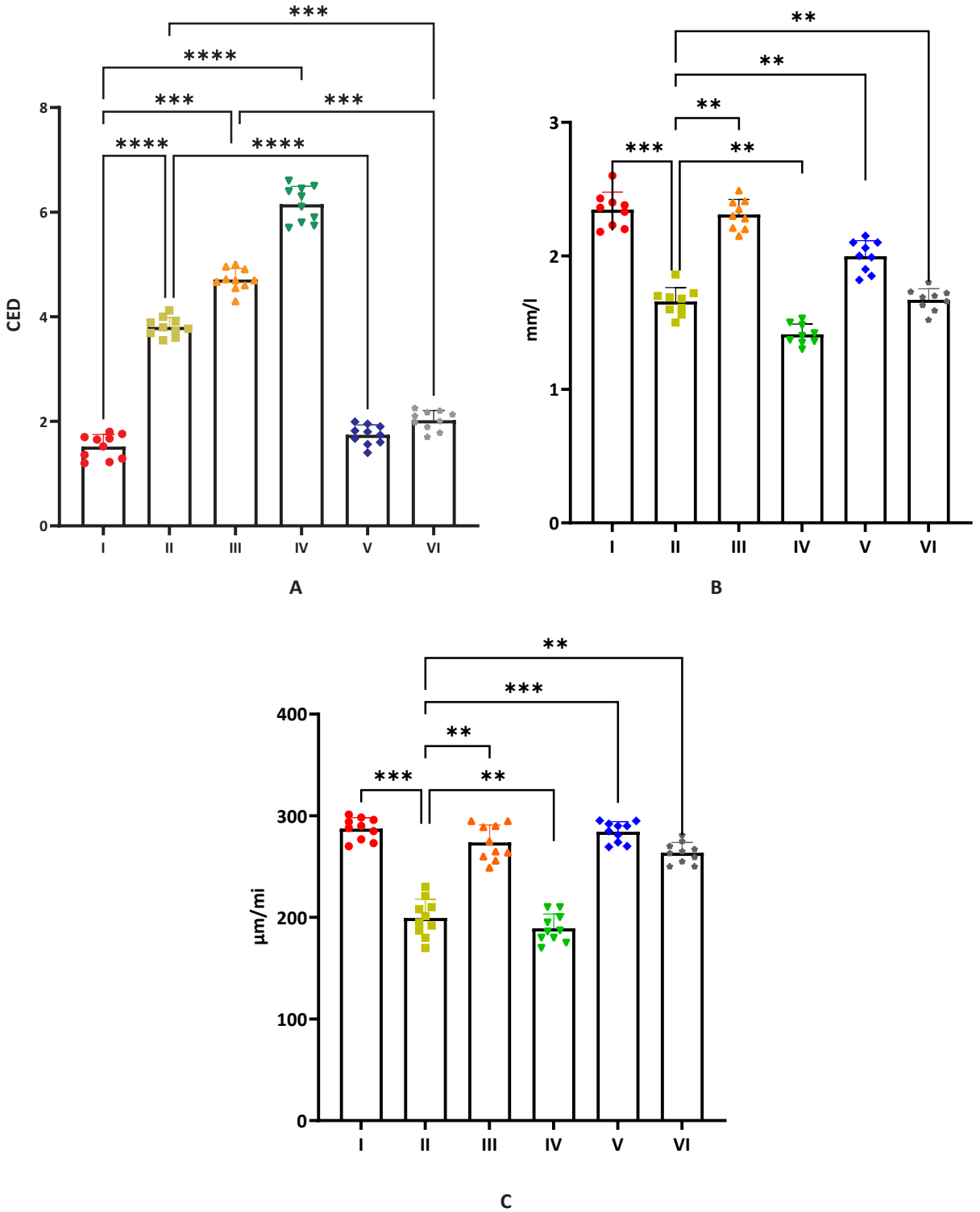

Figure 5 – Effect of composition No. 1 on coefficient of endothelial dysfunction (A), the level of alkaline phosphatase (B) and the calcium content in blood serum (C)

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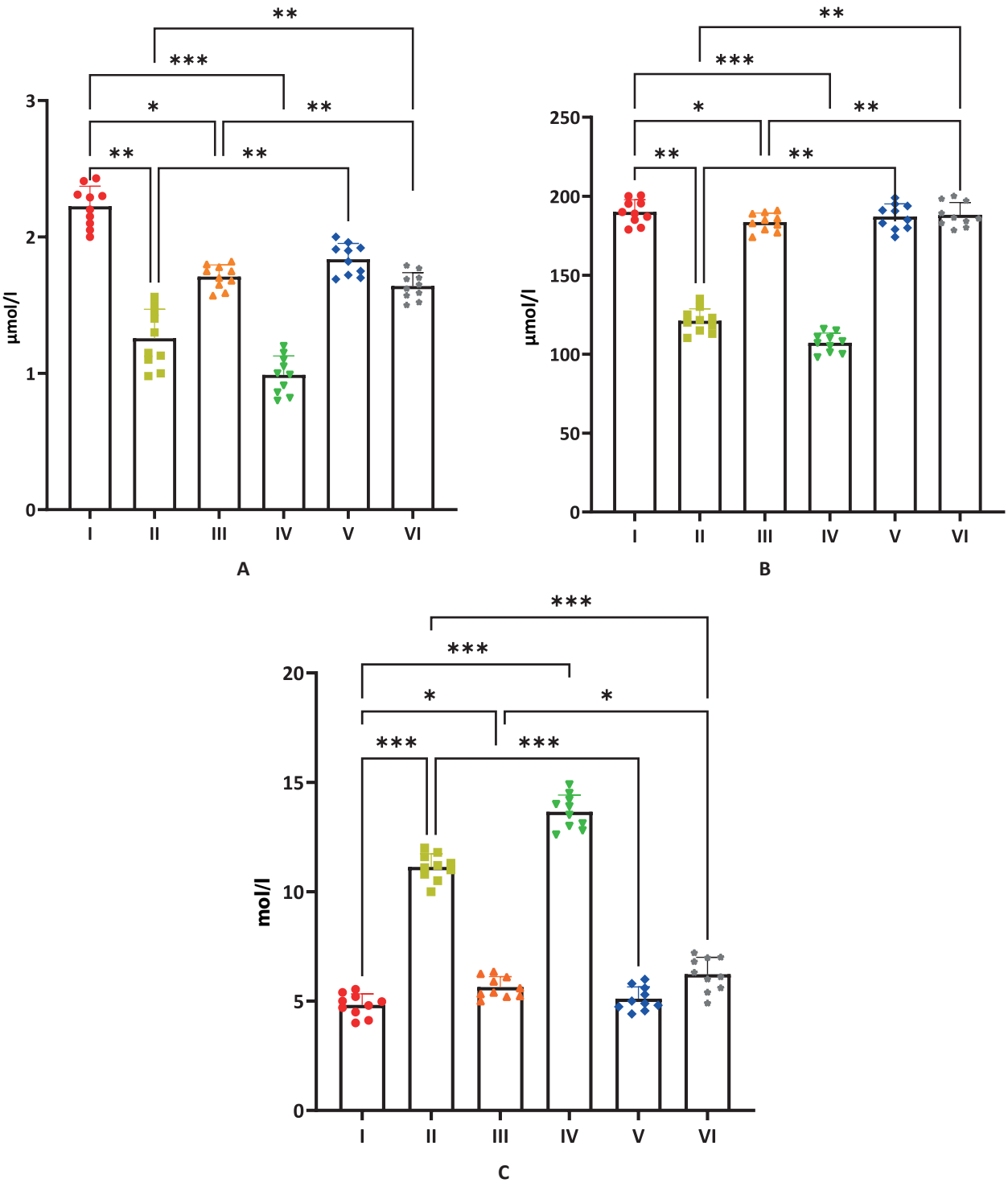

Figure 6 – Effect of composition No. 1 on the content of superoxide dismutase (A), the level of glutathione peroxidase (B) and malondialdehyde (C)

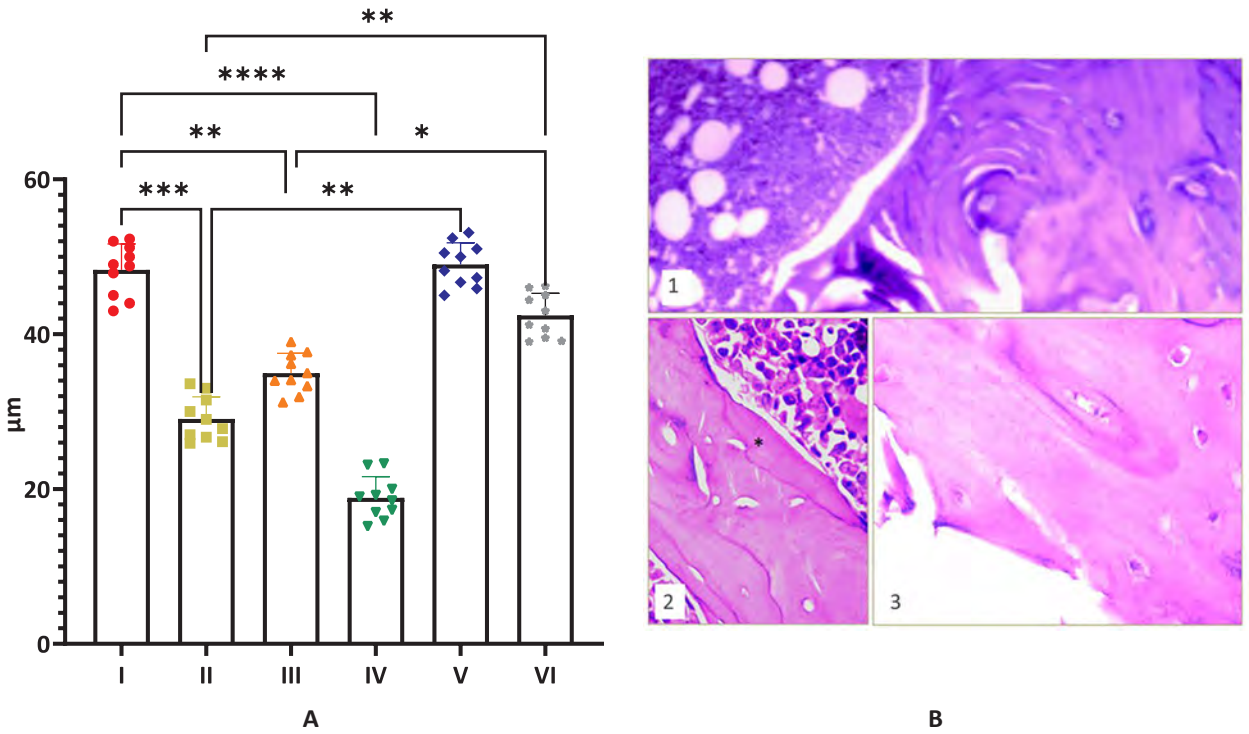

Figure 7 – Effect of composition No. 1 on bone trabeculae thickness (A) and tibia microstructure (B)

Note: tibia microstructure in group VI: MP+composition No. 1+L-NAME; 1 – cortical bone and adjacent area of spongy substance without specific changes; 2 – bone trabecula *of spongy substance with a newly formed layer of lamellar bone tissue; 3 – intact internal structure of bone trabecula. Stained with hematoxylin and eosin, magnification ×100 (A), ×400 (B).

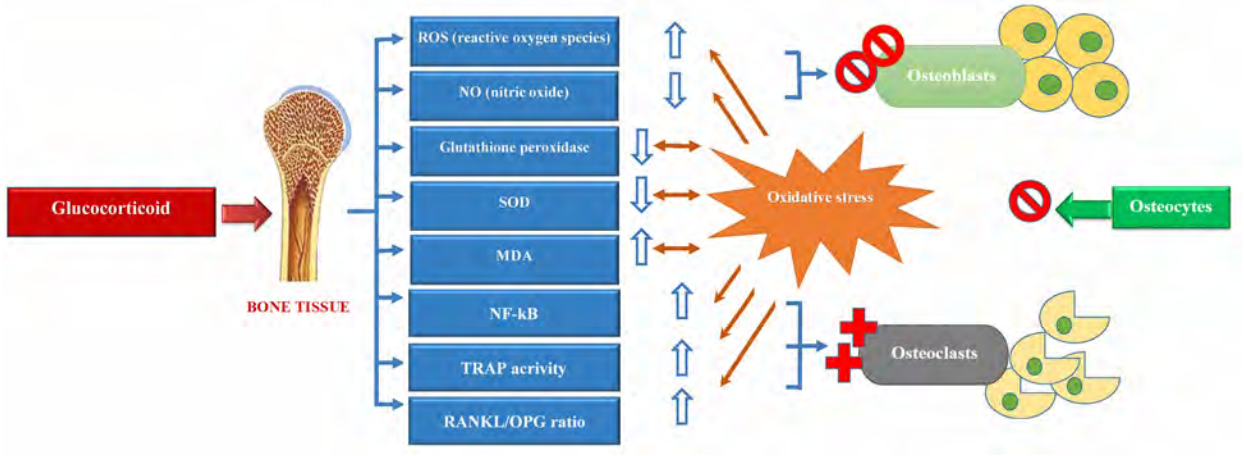

Figure 8 – Possible mechanisms of bone tissue damage in steroid-induced osteoporosis

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The ^{1}H NMR spectrum of the sample composition No. 1 showed the key signals associated with nicotinate anion: 8.94 (br s, 0.25H), 8.63 (br s, 0.25H), 8.34 (dt, J=8.0, 1.8 Hz, 0.25H) 7.60 (dd, J=7.9, 5.1 Hz, 0.25H) and 6-acetihexanoate anion 3.16 (t, J=6.9 Hz, 2H), 2.58 (s, 3H), 2.22 (t, J=7.4 Hz, 2H), 1.98 (s, 9H), 1.63–1.46 (m, 4H), 1.38–1.29 (m, 4H). The ratio of anionic residues is 1:3 (nicotinate/hexanoate). The key signals of 2-ethyl-6-methyl-3-hydroxypyridine were notified: 7.69 (d, J=8.7 Hz, 1H), 7.40 (d, J=8.7 Hz, 1H), 2.92 (q, J=7.6 Hz, 2H, (2-ethyl (CH2)), 1.26 (t, J=7.6 Hz, 4H, 2-ethyl (CH3)).

Composition of 3-hydroxypyridine derivatives prevents the development of steroid-induced osteoporosis

At the beginning of the experiment, significant differences in the baseline bone density were not observed in any of the 6 experimental animals' groups. In all the experimental groups, the mean values of the femur density were 4.91±1.03 mg/cm^2, which confirms the absence of clinical osteoporosis signs in all the experimental animals at the beginning of the experiment (Fig. 2A). However, after 5 weeks of the experiment, a significant and trustworthy decrease in the bone density by 37% (p <0.05) was noted in group II; in group III, there was a decrease by 29% (p <0.05) and in group IV – by 52% (p <0.05). A combined use of MP+L-NAME (group IV) contributed to the maximum reduction in the bone density (Fig. 4A). The administration of composition No. 1 led to the prevention of a decrease in the bone density, bringing the values closer to the group of intact animals (Fig. 4A).

The microcirculation analysis of the proximal metaphysis parameters of the femur in the intact animals showed the indicator of 101.1±4.15 p.u. (Fig. 4B), while in group II, it significantly decreased to the values of 72.09±3.26 p.u. (p <0.05); in group III – up to 67.8±4.12 p.u. (p <0.05); in group IV, there was a maximum decrease in the microcirculation index to the values of 55.3±4.12 p.u. (p <0.05). Against the background of the MP and L-NAME administration, the test compound, composition No. 1, effectively prevented a decrease in the level of the regional blood flow in the femoral bone tissue. The indicator of the microcirculation level in the MP+composition No. 1 group (group V) was 100.8±3.23 p.u.; in the MP+L-NAME+composition No. 1 (group VI) it was 98.1±4.79 p.u., respectively (p <0.05) (Fig. 4B).

A test of vasodilation with an endothelium-dependent (the intravenous acetylcholine administration) and endothelium-independent (the intravenous sodium nitroprusside administration) was conducted. In the calculation of the EDC, the following values were established: 1.22±0.01 (p <0.05) in the group of intact animals; 3.6±0.07 (p <0.05) in the MP group; 4.66±0.09 (p <0.05) in the L-NAME group; 6.31±0.04 (p <0.05) in the MP+L-NAME group (Fig. 5A).

The administration of composition No. 1 contributed to the correction of the endothelial damage. Thus, in the MP+composition No. 1 group, CED decreased to 1.4±0.02, in the MP+L-NAME+composition No. 1 group – to 2.1±0.03, respectively, which confirms an increase in the NO bioavailability with the administration of composition No. 1.

For a biochemical analysis of bone metabolism processes, the concentrations of Ca^{2+} and bone alkaline phosphatase (an osteosynthesis marker) were determined. In the MP and MP+L-NAME groups, there was a statistically significant decrease in serum calcium levels by 33% and 41% (p <0.05), respectively. In the rest of the experimental groups, no statistically significant difference was observed. The administration of composition No. 1 helped to prevent the loss of Ca^{2+} caused by the administration of MP and L-NAME (Fig. 5B).

After two weeks of the experiment, the MP and MP+L-NAME groups showed a significant decrease (p <0.05) in serum alkaline phosphatase levels. After 5 weeks of treatment the animals with composition No. 1, serum alkaline phosphatase levels remained significantly lower than in the MP and MP+L-NAME groups, which confirms the effectiveness of composition No. 1 in the treatment of bone metabolism disorders (Fig. 5C).

When studying the effect of composition No. 1 on the oxidative stress markers in the blood serum, it was found out that initially, the contents of SOD and glutathione peroxidase in the MP and MP+L-NAME groups were significantly lower in comparison with the group of intact animals (p <0.05) by 32 and 41.3%, respectively (Fig. 6A). The administration of composition No. 1 to the animals made it possible to statistically significantly increase the concentration of SOD and glucation peroxidase relative to the groups of animals with MP and MP+L-NAME (Fig. 6A and 6B). There was a change in the level of malondialdehyde (MDA) in the group of animals with MP and MP + L-NAME, in the form of a significant secretion increase to the level of 4.9±0.1 and 5.2±0.2 mol/l (p <0.05), respectively (Fig. 6C). The use of composition No. 1 as a pharmacological support made it possible to statistically significantly decrease the concentration of the lipid peroxidation product – MDA-relative to the group of the animals with pathology modeling (groups II and IV) (Fig. 6C).

To confirm the morphofunctional and biochemical samples, histomorphological studies of the animals' proximal femurs were carried out. When studying the intact animals' materials, no features that distinguish the structure of the studied tibia areas from the typical structure, were found out (Fig. 7A).

The bone trabeculae thickness averaged 47.9±1.8 µm. With the administration of MP and MP+L-NAME, the reproduction of the bone changes characteristic of osteoporosis was achieved. The bone trabeculae

thickness decreased and amounted to 31.5±2.2 and 23.1±1.3 μm, respectively (Fig. 7A), which characterizes thinning of the bone trabecula of the spongy substance.

A corrective effect of the studied composition No. 1 is evidenced by both qualitative and morphometric parameters of spongy substance trabeculae. The general architectonics of the cortical bone and spongy substance in the MP+composition No. 1 group approximated the intact animals. The group had both cellular manifestations of the osteoplastic activity and the result of imperfect osteogenesis in the form of lamellar bone structures on the surface of the bone trabeculas (Fig. 7B).

The variety of biological effects of steroid hormones and the complexity of their metabolic pathways make it difficult to fully understand the pathogenetic aspects of the steroid-induced osteoporosis development and its progression.

The oxidative stress can play a central role among a lot of factors contributing to the development of steroid-induced osteoporosis. This has been confirmed in a number of experimental studies [23]. For the pharmacological correction of the oxidative stress in steroid-induced osteoporosis, the influence of antioxidants of various chemical nature, i.e. natural pharmaceutical, is being studied. However, their osteoprotective activity is insufficient and requires a further experimental confirmation [24, 25].

For the creation of new drugs, the choice of compounds of well-studied chemical structures, in particular, pyridine derivatives, remains relevant as a precursor [19]. The validity of this direction lies in the fact that pyridines, while having a low toxicity, exhibit a wide range of pharmacological activity. The supramolecular complex studied in the work (composition No. 1) is represented as one molecule of 2-ethyl-6-methyl-3-hydroxypyridinium 3-pyridinocarbonoate and three molecules of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate. 2-ethyl-6-methyl-3-hydroxypyridinium 3-pyridinocarbonoate has been proven to have antihypoxic, antioxidant, and endothelioprotective activities. The second component – the compound 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate is known as a tool able of accelerating the wound surface cleansing from the necrotic masses, reducing exudative processes, activating the growth of granulation tissue, vascularization and epithelialization of wounds; it also stimulates the formation of bone marrow, accelerating the healing of bone fractures [26].

Taking into account the relationship between the oxidative stress and the development of endothelial dysfunction in the violation of bone remodeling processes, in the framework of this study, the authors tried to assess the influence of the both factors on the bone tissue damage. As it is known, the structure of the bone tissue microvasculature differs significantly from the morphology of the vascular bed of other body tissues. Bone microvessels have only endothelium and do not have muscle or connective tissue layers. Thus, it is the endothelium that mediates the entire humoral regulation of the exchange between osteoblasts, osteoclasts, and blood. To confirm the contribution of NO to the development of osteoporosis, the animals were injected with a selective inhibitor of eNOS, the compound L-NAME. An intraperitoneal administration of L-NAME at the dose of 25 mg/kg daily for 35 days led to a decrease in the bone density, which was accompanied by a statistically significant decrease in the level of microcirculation in the bone, and an increase in the EDC, which indicates the involvement of the endothelial dysfunction in the development of osteoporosis. A combined administration of glucocorticosteroid MP at the dose of 5 mg/kg every 5th day for 5 weeks (intraperitoneally) and L-NAME at the dose of 25 mg/kg for 35 days (intraperitoneally) increased the bone tissue damage, herewith, significantly reducing microcirculation and increasing EDC.

Due to the high reactivity of free radicals, their action in the body is controlled by endogenous and exogenous antioxidants, as well as enzymes of the antioxidant system. The endogenous antioxidant system does not always cope with this process, leading to the development of various pathological conditions. In the course of the study, it was found out that under the conditions of the MP and L-NAME administration, composition No. 1 contributed to the activity preservation of endogenous antioxidant defense enzymes, a decrease in the intensity of lipid peroxidation, which was expressed in an increase in the activity of superoxide dismutase, glutathione peroxidase, and a decrease in the formation of MDA relative to the group of the animals with modeling steroid- induced osteoporosis. However, in the MP group, there was a more pronounced decrease in the amount of antioxidant enzymes SOD and GP, as well as an increase in the level of MDA. The L-NAME administration had no statistically significant effect on the level of antioxidant enzymes.

A colony stimulating factor (M-CSF) and a receptor activator ligand NF-κB (RANKL) are known to influence the osteoclast differentiation and lead to the abnormal bone resorption. The importance of RANKL induction of ROS production in modulating osteoclast differentiation is well known [27]. The stimulation of RANKL causes a significant increase in intracellular ROS due to the activation of the tumor necrosis factor receptor-associated (TNF-α) and nicotinamide adenine dinucleotide phosphate oxidase (NOX) [28]. This is confirmed by a number of works verifying the osteoprotective activity of antioxidants in osteoporosis, in particular, with the use of N-acetyl-L-cysteine and ascorbic acid [29].

Therefore, targeting intracellular ROS can represent a potential therapeutic approach to prevent a bone resorption and treat disorders of the bone metabolism.

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Another important link in the pathogenesis of osteoporosis is a decrease in the blood supply to the bones, accompanied by an endothelial dysfunction and leading to the inhibition of the osteoblast activity, as well to the increased activity of osteoclasts [30]. It is now known that NO has a direct stimulatory effect on osteoblasts, positively influencing the bone tissue. At the same time, many works show a relationship between the oxidative stress and the presence of the endothelial dysfunction [31, 32].

The oxidative stress leads to a decrease in the formation of endothelial NO, which, in turn, disrupts the microcirculation in the damaged bone tissue, *inter alia* steroid-induced osteoporosis. Thus, the oxidative stress generated by the MP administration in combination with the blockade of eNOS by the L-NAME administration, confirms the hypothesis of a detrimental effect of glucocorticosteroids on the bone tissue through an increase in ROS and a decrease in the NO production (Fig. 8).

CONCLUSION

Thus, summing up the study on the osteoprotective effect of a new compound based on 3-hydroxypyridine derivatives, composition No. 1 (50 mg/kg), it can be concluded that the studied compound prevents a decrease in the microcirculation level in the thigh bone tissue, exhibits a pronounced endothelioprotective effect, increasing the bioavailability of NO, and also improves the performance of biochemical and morphometric tests against the background of steroid-induced osteoporosis. The observed improvements can be associated with the compound's effect on reducing the ROS production and inhibiting the RANKL-induced NF-κB activation. However, further in-depth studies are required to elucidate the exact mechanism of action of the compound. The data obtained characterize the prospects of studying composition No. 1 for the correction and prevention of steroid-induced osteoporosis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Anton P. Danilenko – article writing, research design developing; Konstantin S. Trunov – results evaluation and interpretation; Mikhail V. Pokrovsky – study and all stages of the experiment planning; Lyudmila M. Danilenko – article writing, results interpreting; Mikhail V. Korokin – statistical data processing, text editing; Oleg S. Gudyrev – methodology development, research conducting; Aleksey A. Khentov – results interpretation; Natalya P. Masalytina – sampling for histomorphological and biochemical studies, literature sources analysis; Irina A. Tatarenkova – literary sources analysis, text editing; Albina V. Cherednichenko – pathology modeling, experimental work carrying out; Elizaveta V. Boeva – pathology modeling, experimental work; Ivan S. Koklin – literature analysis, graphic materials preparation; Eduard I. Taran – literature analysis, graphic materials preparation.

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RESEARCH ARTICLE

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Bioequivalence study of generic nirmatrelvir in healthy volunteers

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Nirmatrelvir is an antiviral drug that, in combination with ritonavir, is an effective agent for the etiotropic therapy of patients with mild to moderate COVID-19.

The aim of the study was to evaluate bioequivalence of the generic drug nirmatrelvir Arpaxel in combination with ritonavir and the original drug Paxlovid, which is a combination of nirmatrelvir/ritonavir, in a single dose administration to healthy volunteers.

Materials and methods. This research was an open-label, randomized, two-period crossover bioequivalence study. It included 2 periods, in each of which the volunteers received either a test drug (nirmatrelvir at the dose of 300 mg) in combination with ritonavir (100 mg), or a reference drug (a combination of nirmatrelvir 300 mg and ritonavir 100 mg), given as a single dose. A wash-out period between each of the administrations was 7 days. The blood sampling to determine the concentration of nirmatrelvir was carried out in the range from 0 to 36 h in each of the study periods. A nirmatrelvir concentration was determined by a validated HPLC-MS/MS method with a lower quantitation limit of 10 ng/mL. Bioequivalence was assessed by comparing 90% confidence intervals (CIs) for the ratio of geometric means of $AUC_{(0–36)}$ and C_{max} of the test drug and reference drugs with the established equivalence limits of 80.00–125.00%.

Results. In the study were included 68 healthy volunteers, 67 participants of which were included in the bioequivalence population. The pharmacokinetic parameters of the drugs were comparable to each other. The 90% confidence interval for the ratio of the geometric mean of the maximum drug concentration in the blood plasma and the area under the pharmacokinetic curve «concentration-time» from zero to the last blood draw within 36 hours of nirmatrelvir was 87.26–100.83 and 93.27–103.74%, which meets the criteria for assessing bioequivalence. The test drugs were well tolerated by the volunteers. The incidence of adverse events was similar for the test and reference drugs. No serious adverse events were recorded during the entire study.

Conclusion. As a result of this study, bioequivalence of the test and reference drugs has been established.

Keywords: COVID-19; bioequivalence; pharmacokinetics; nirmatrelvir; ritonavir; generic drug

Abbreviations: COVID-19 – a novel coronavirus infection; CI – confidence interval; AUC – area under the concentration-time curve; $AUC_{0–36}$ – area under the pharmacokinetic «concentration-time» curve from zero to the last blood sampling at which the concentration of the drug is equal to or higher than the lower limit of quantification within 36 hours; $AUC_{0–\infty}$ – area under the pharmacokinetic «concentration-time» curve, starting from the zero value of the time, extrapolated to infinity; C_{max} – the maximum concentration of the drug in the blood plasma; T_{max} – time to reach the maximum concentration; HPLC-MS/MS – high performance liquid chromatography with tandem mass spectrometry; AE/SAE – adverse event/serious adverse event; BMI – body mass index; PK – pharmakokinetics; ЭСG – electrocardiography.

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Исследование биоэквивалентности воспроизведенного препарата нирматрелвира у здоровых добровольцев

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Нирматрелвир представляет собой противовирусный препарат, который в сочетании с ритонавиром является эффективным средством для этиотропной терапии пациентов с COVID-19 легкого и среднетяжелого течения.

Цель. Оценить биоэквивалентность воспроизведенного препарата нирматрелвира Арпаксел в сочетании с ритонавиром и оригинального препарата Паксловид, представляющего собой комбинацию нирматрелвир/ритонавир, при однократном применении здоровыми добровольцами.

Материалы и методы. Данное исследование представляло собой открытое рандомизированное простое перекрестное исследование биоэквивалентности. Оно включало 2 периода, в каждом из которых добровольцы получали либо исследуемый препарат (нирматрелвир в дозе 300 мг) в комбинации с ритонавиром (100 мг), либо референтный препарат (комбинация нирматрелвира 300 мг и ритонавира 100 мг) однократно. Отмывочный период между каждым из приемов составил 7 сут. Отбор образцов плазмы крови для определения концентрации нирматрелвира производили в интервале от 0 до 36 ч в каждом из периодов исследования. Концентрацию нирматрелвира определяли валидированным методом ВЭЖХ-МС/МС с нижним пределом количественного определения 10 нг/мл. Для оценки биоэквивалентности проводили сопоставление 90% доверительных интервалов (ДИ) для отношения средних геометрических $AUC_{(0–36)}$ и C_{max} препаратов с установленными пределами эквивалентности 80,00–125,00%.

Результаты. В исследование были включены 68 здоровых добровольцев, из них в популяцию для оценки биоэквивалентности вошли 67 участников. Фармакокинетические параметры препаратов были сопоставимы между собой. Доверительный интервал 90% для отношения средних геометрических показателей максимальной концентрации препарата в плазме крови и площади под фармакокинетической кривой «концентрация-время» от нуля до последнего отбора крови в пределах 36 ч нирматрелвира составили 87,26–100,83 и 93,27–103,74%, что соответствует критериям оценки биоэквивалентности. Препараты исследования хорошо переносились добровольцами. Частота нежелательных явлений была схожей для исследуемого и референтного препаратов. В течение всего исследования не было зарегистрировано ни одного серьёзного нежелательного явления.

Заключение. В результате данного исследования была установлена биоэквивалентность исследуемого и референтного препаратов.

Ключевые слова: COVID-19; биоэквивалентность; фармакокинетика; нирматрелвир; ритонавир; воспроизведенный препарат

Список сокращений: COVID-19 – новая коронавирусная инфекция; ДИ – доверительный интервал; AUC – площадь под кривой «концентрация–время»; $AUC_{0–36}$ – площадь под фармакокинетической кривой «концентрация–время» от нуля до последнего отбора крови при котором концентрация препарата равна или выше нижнего предела количественного определения в пределах 36 ч; $AUC_{0–\infty}$ – площадь под фармакокинетической кривой «концентрация–время», начиная с нулевого значения времени, экстраполированная до бесконечности; C_{max} – максимальная концентрация препарата в плазме крови; T_{max} – время достижения максимальной концентрации; ВЭЖХ–МС/МС – высокоэффективная жидкостная хроматография с тандемной масс–спектрометрией; НЯ/СНЯ – нежелательное/серьезное нежелательное явление; ИМТ – индекс массы тела; ФК – фармакокинетика; ЭКГ – электрокардиография.

INTRODUCTION

Nirmatrelvir is an antiviral drug effective against SARS-CoV-2, the mechanism of action of which is realized by inhibiting the SARS-CoV-2 viral protease 3CLpro and blocking a virus replication [1–3]. To increase the systemic exposure of nirmatrelvir, it is used in combination with ritonavir, which is a strong inhibitor of CYP3A4 and significantly slows down the metabolism of nirmatrelvir [4]. Thus, in this combination, ritonavir acts as a pharmacokinetic enhancer (booster). Ritonavir did not shown own clinical efficacy against COVID-19.

The original nirmatrelvir Paxlovid[1] was developed by Pfizer, USA, and is available as a co-packaged combination of nirmatrelvir 150 mg tablets and ritonavir 100 mg tablets. The pharmacodynamic properties of nirmatrelvir[2] have been confirmed in preclinical and clinical studies [5–7]. *In vitro* data confirm the selectivity of nirmatrelvir against 3CLpro SARS-CoV-2 [8, 9]. During the clinical development, the efficacy of the nirmatrelvir/ritonavir combination in the treatment of mild to moderate COVID-19 was proven [10, 11].

The largest randomized placebo-controlled study (EPIC-HR) of the Paxlovid efficacy and safety was conducted with the participation of 2246 patients [12]. Based on the primary endpoint of this study, in the population of patients who had the symptoms onset no more than 3 days before the randomization, the incidence of COVID-19 – related hospitalization or death from any cause within 28 days was 0.717% (5/697) in the test drug group and 6.452% (44/682) in the placebo group. The difference between the groups was statistically significant (p <0.001). In addition, no deaths were reported in the test drug group, while 9 deaths (1.32%) were observed in the placebo group. It is also worth noting that in clinical studies, nirmatrelvir in combination with ritonavir was well tolerated and demonstrated a favorable safety profile.

In addition, the effectiveness of the nirmatrelvir/ritonavir combination has been confirmed by the data obtained in real clinical practice [13–16].

Despite the fact that the original drug containing the nirmatrelvir/ritonavir combination has been approved for use in many countries including the USA, Australia and Europe, it is currently not approved in Russia. The development and entry into the market of generic drugs of this combination will meet a high demand for the effective etiotropic therapy for COVID-19 in a pandemic [17–19].

The LLC "Drug Formulation" (R-Pharm group) has developed a generic drug of nirmatrelvir called Arpaxel; it contains 150 mg of nirmatrelvir in the dosage form of film-coated tablets. The packaging of the developed drug does not contain a co-packaged ritonavir drug, since its mono-drugs are commercially available. In order to confirm the bioequivalence of the developed generic and original drugs, this bioequivalence study has been conducted.

THE AIM of the study was to evaluate bioequivalence of the generic drug nirmatrelvir Arpaxel in combination with ritonavir and the original drug Paxlovid, which is a combination of nirmatrelvir/ritonavir, in a single dose administration to healthy volunteers.

MATERIALS AND METHODS

Study design

This Clinical Bioequivalence Study No. CJ051032185 was an open-label, randomized, two-period crossover study in healthy volunteers. The study design is shown in Fig. 1.

The study was conducted on the basis of the Clinical site LLC "Eco-Safety Scientific Research Center". The clinical stage of the study was carried out from November 2022 to January 2023.

The study fully complied with the ethical principles set out in the last revision of Helsinki Declaration, the rules of Good Clinical Practice of the Eurasian Economic Union, the rules of good clinical practice of the International Council for Harmonization (ICH E6 GCP R2), as well as other legislative acts applicable to this study. The clinical trial protocol was approved by the Ministry of Health of Russia (Permit No. 640 dated 2022 Nov 07) and the Ethics Council (extract from Protocol No. 317 dated 2022 Sep 06), as well as the local ethics committee at the research center (the extract from Protocol No. 262 dated 2022 Nov 17).

Objects of study

A total of 68 healthy volunteers were included in the study. Inclusion criteria were used: a male gender, the age of 18–45 years, a verified diagnosis of "healthy", a body mass index (BMI) 18.5–30 kg/m^2. The main exclusion criteria were the presence of chronic diseases of various organ systems, mental problems, hypersensitivity to the test drugs, lactose intolerance, the use of prohibited therapy drugs before the start of the study, or the presence of a positive PCR test for SARS-CoV-2. A volunteer was excluded from the study if he or she had withdrawn their formed consent, had received prohibited therapy, had grossly violated the requirements and procedures of the protocol, had experienced adverse events in which the volunteer's further participation in the study might be unsafe, or death. Before the start of the study, all participants familiarized themselves with the procedure for conducting the study and signed an informed consent form. A randomization into groups was performed in the ratio of 1:1 using the method of randomization envelopes.

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Drugs administration

The test drug was Arpaxel (film-coated tablets, 150 mg), in combination with Norvir® (ritonavir), film-coated tablets, 100 mg. The reference drug was the original drug nirmatrelvir/ritonavir – Paxlovid, film-coated tablets (150+100 mg). The volunteers were randomized to one of the groups – TR or RT. In group 1 (TR), the volunteers received the test drug in combination with ritonavir in period 1, and the reference drug in period 2. In group 2 (RT), the drugs were taken in the reverse order. The volunteers were given each of the drugs on an empty stomach as a single dose – 300 mg nirmatrelvir (2 tablets) and 100 mg ritonavir (1 tablet). The wash-out period between the doses of the drugs was 7 days. According to the literature data, the half-life ($T_{1/2}$) of nirmatrelvir administrated as a single dose in combination with ritonavir, is 6–7 h [20][3]. Thus, in order to minimize the risks of the first dose of the drugs affecting the nirmatrelvir pharmacokinetics (PK) in the 2nd period, the wash-out period should be at least 5 half-lives, i.e., at least 36 h.

The drugs were taken under the supervision of medical personnel. During the study, a special regimen of eating and drinking was kept to. The use of the drugs and dietary supplements that could have a pronounced effect on the hemodynamics or a liver function, could be inhibitors or inducers of CYP3A4 or P-glycoprotein, was forbidden. The drugs that increase the pH of the gastric juice, as well as a regular oral or patented use of other medicines and biologically active additives, were forbidden, too.

Sampling and sample preparation

To assess the concentration of nirmatrelvir in the volunteers' blood, the venous blood was sampled at the following time points: before taking the drug, then after 15, 30, 45 min, 1 h, 1 h and 15 min, 1 h and 30 min, 1 h and 45 min, 2 h, 2 h and 15 min, 2 h and 30 min, 2 h and 45 min, 3 h, 3 h and 15 min, 3 h and 30 min, 3 h and 45 min, 4 h, 4 h and 30 min, 5, 6, 8, 10, 12, 24 and 36 h after administration. The biosampling time points for the PK analysis were chosen in such a way as to obtain the most complete information in each of the of concentration-time curve. Given that the median time to reach the maximum concentration (T_{max}) of nirmatrelvir, according to the literature data, is 3 h [24], the chosen approach complies with the recommendations for choosing time points: at least at 3 points of the initial phase of increasing concentration, and at least at 5 points of the phase of decreasing concentration.

In case of the biosampling time deviation from the dew point, it was necessary to register its actual time. The actual time of biosampling was used to calculate pharmacokinetic parameters.

Blood sampling was carried out in test tubes containing K_2EDTA as an anticoagulant. After that, the samples were centrifuged in a Biosan LMC-3000 centrifuge (Biosan, Latvia) with an acceleration of 2000 g for 15 min to separate the plasma. The obtained samples were stored frozen at the temperature not exceeding – 65°C.

Analytical method

Nirmatrelvir plasma concentrations were determined by a validated high performance liquid chromatography with a tandem mass spectrometry (HPLC-MS/MS) method.

The sample preparation was performed by precipitating blood plasma proteins with chilled acetonitrile (LC/MS, Biosolve B.V., Netherlands) containing 0.1% formic acid. The HPLC system of the Sciex 5500 system (SCIEX, USA) and a hybrid triple quadrupole mass spectrometer with an electrospray ionization QTRAP 5500 (SCIEX, USA) were used for the study. Chromatographic separation was performed using a Waters Acquity BEH C18 Column, S-1.7 µm, 50×2.1 mm, chromatographic column in a gradient elution mode with a flow rate of 0.5 mL/min. A solution of ammonium formate (Sigma-Aldrich, USA) and formic acid (PA-ACS, Panreac, Spain) in water was used as mobile phase A, and a solution of formic acid in acetonitrile (HPLC-S, Biosolve B.V., Netherlands) was used as phase B). During the 1st minute of the analysis, 45% of phase A and 55% of phase B were injected. During the 2nd min, only phase B was injected, and at the end of the 2nd min and until the end of the analysis, 45% of phase A and 55% of phase B were injected. Ezetimibe was used as an internal standard. The retention time for nirmatrelvir was 0.5 min, for ezetimibe, it was 0.6 min. The total analysis time was 2.5 min. The lower limit of quantitation for nirmatrelvir was 10 ng/mL.

The data analysis was performed using Analyst 1.7.2 Software (SCIEX, USA). The analyte concentration was determined by the calibration curve of the dependence of the chromatographic peaks' areas of the analyte and the internal standard on the nominal concentration. To construct a calibration curve, a linear regression with the $1/x^2$ normalization was used. The correlation coefficient was not less than 0.99.

Safety assessment

The safety endpoints included the incidence and severity of all adverse events (AEs) and serious adverse events (SAEs). The incidence of grade 3–4 AEs, the incidence of the adverse events that led to an early termination of participation in the study, and the frequency of the AEs associated with study medications, were determined by CTCAE 5.0.

[3] WHO/PQT: medicines Guidance Document 28 April 2022 Notes on the design of bioequivalence study: Nirmatrelvir+ritonavir. URL: https://extranet.who.int/pqweb/sites/default/files/documents/BE_Nirmatrelvir_Ritonavir_28April2022.pdf

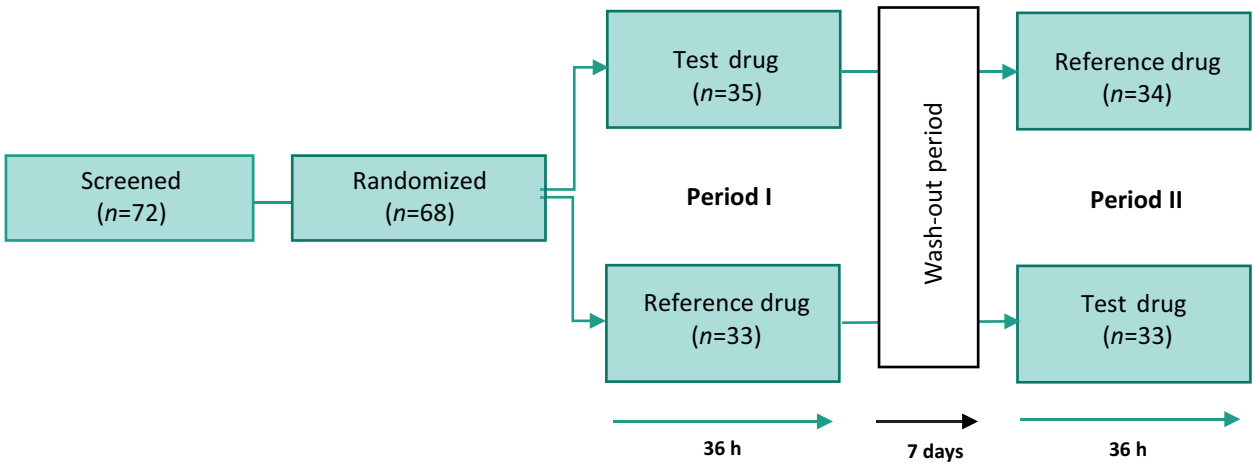

Figure 1 – Study design CJ051032185

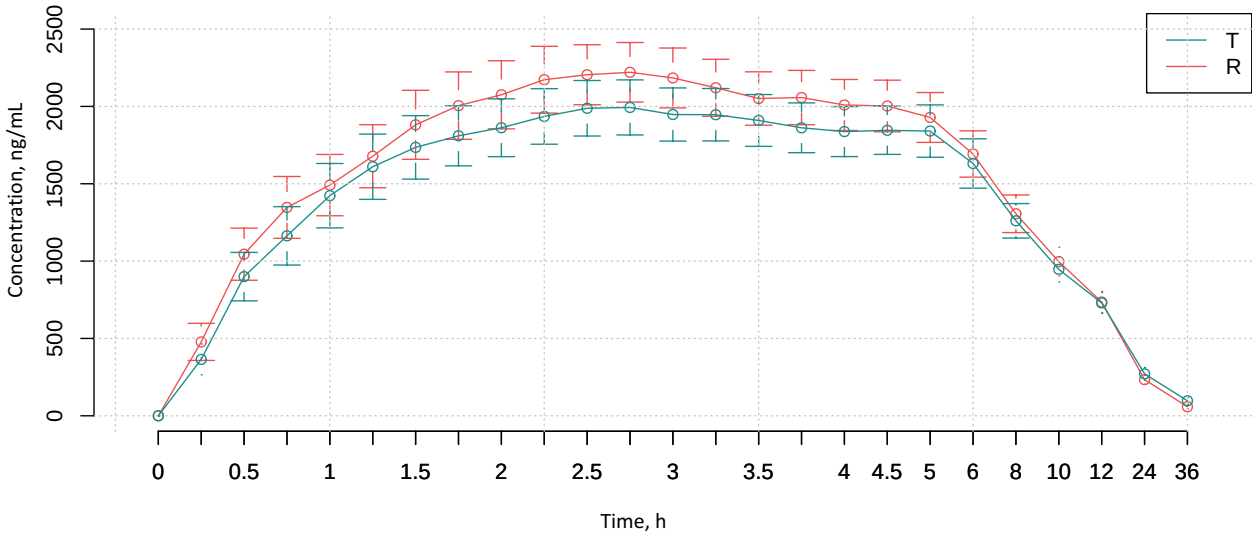

Figure 2 – Dynamics of nirmatrelvir concentration (mean±95% CI) after taking the test drug in combination with ritonavir (T) (*n*=68) and the reference drug Paxlovid (R) (*n*=67)

Note: R – reference drug, T – test drug+ritonavir.

Table 1 – Obtained values of pharmacokinetic parameters after taking test and reference drugs (N=68)

Pharmacokinetic parameters	Test drug (T) (*n*=68)	Reference drug (R) (*n*=67)
$AUC_{(0–36)}$, (ng/mL)*h	24 902.7±7 959.97	25 343.81±7 789.17
C_{max}, ng/mL	2 411.17±808.21	2 580.54±869
$AUC_{(0–\infty)}$, (ng/mL)*h	25 930.98±8 594.38	25 981.73±7 981.04
T_{max}, h	2.5 [1.7; 3.25]	2.25 [1.75; 3]
$T_{1/2}$, h	7.73±2.85	6.46±1.75

Note: *n* – the number of participants; C_{max} – maximum concentration; T_{max} – time to reach C_{max}; $T_{1/2}$ – half-life; $AUC_{(0–36)}$ – total area under the concentration-time curve in the time interval from 0 to 36 h; $AUC_{(0–\infty)}$ – area under the concentration-time curve in the time interval from 0 to infinity. All indicator values are presented as an arithmetic mean (standard deviation), except T_{max}, which presented as a median (minimum – maximum).

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Table 2 – Calculated 90% CI values for ratios of pharmacokinetic parameters of nirmatrelvir after taking test and reference drugs

Parameter	Geometric mean T/R ratio	Calculated values of 90% CI	CV_{intra} [1]
$AUC_{(0–36)}$	98.4	93.27-103.74	18.57
C_{max}	93.8	87.26-100.83	25.43

Note: CV_{intra} [1] – intra-individual coefficient of variability; CIs – confidence intervals; T – test drug + ritonavir; R – reference drug.

Table 3 – Summary of AEs frequency after taking each of the drugs

Adverse effect	Test drug (T) (N=68), *n* (%)	Reference drug (R) (N=67), *n* (%)	P[1] value
Laboratory and instrumental data			
Increase in blood bilirubin level	1 (1.47)	0 (0.00)	0.317
Decrease in platelet count	2 (2.94)	1 (1.47)	0.564
Increase in lymphocyte count	0 (0.00)	1 (1.47)	0.317
Hematopoietic system disorders			
Anemia	0 (0.00)	3 (4,41)	0.083
Nervous System Disorders			
Headache	0 (0.00)	1 (1.47)	0.317
Dysgeusia	7 (10.29)	4 (5.88)	0.317
Vascular disorders			
Hypertension	20 (29.41)	17 (25.00)	0.67
General disorders and reactions at the injection site			
Asthenia	0 (0.00)	1 (1.47)	0.317

Note: 1 is the level of significance when comparing the occurrence frequency of at least one specified adverse event (AE) after taking drugs (McNemar criterion). N is the number of subjects who took the specified drug or any of the drugs at least once. When calculating the frequency (%), the number of volunteers in the general population is taken as 100%. All AEs listed in the table belong to the 1st degree of severity.

For a close monitoring of safety in this study, a periodic evaluation of the vital signs, laboratory parameters (clinical and biochemical blood tests, urinalysis), a physical examination, ECG were performed. All the AEs were recorded from the start of the test drugs administration to the end of the follow-up. The AEs were coded using the Regulatory Dictionary of Medical Terms (MedDRA) [21].

Statistical analysis

The following parameters were used to calculate the sample size: the study power was 0.8 (80%); the level of statistical significance α was 0.05 (5%); according to literature sources, the intra-individual coefficient of the variation for nirmatrelvir C_{max} was 0.36 (36%). Taking into account the potential risk of dropping out from the study up to 25% of volunteers, 68 volunteers were planned to be included in the study.

A statistical analysis was performed using the freely distributed software package for a statistical analysis R-4.2.0 (R Foundation for Statistical Computing, Austria).

The nirmatrelvir pharmacokinetics was assessed using the following parameters: $AUC_{(0–36)}$ – the area under the concentration-time curve in the time interval from 0 to 36 hours; C_{max} – the maximum concentration; T_{max} – the time to reach C_{max}; $T_{1/2}$ – half-life; $AUC_{(0–\infty)}$ is the area under the "concentration-time" curve in the time interval from 0 to infinity.

The comparison of the drugs PK parameters was performed using the Wilcoxon test for linked samples. The frequencies of adverse events were compared using the McNemar test. Bioequivalence was assessed using the analysis of variance (ANOVA), which is a parametric method applied to log-transformed PK values (AUC and C_{max}). The statistical analysis also took into account various sources of variability that could affect the variables under study. The following parameters were used as fixed factors for constructing the ANOVA model: the drug use sequence; a period and drug; the subject of the study, nested in the sequence; cohort; the subject nested in the sequence nested in the cohort; the sequence nested in the cohort.

Based on the residual variation obtained from ANOVA, a 90% CI was calculated for the ratio of the geometric means of the logarithmically transformed baseline PK parameters ($AUC_{(0–36)}$ and C_{max}) calculated for test and reference drugs. To establish bioequivalence, the obtained CIs were compared with the prespecified bioequivalence limits, taken equal to 80.00–125.00%.

RESULTS

Population

A total of 68 male volunteers were included in the study: 35 in the TR group and 33 in the RT group. Since the hospitalization of all the 68 volunteers to the

clinical site for the study procedures was difficult, the volunteers were divided into 2 cohorts, which were included in the study 9 days apart. All the volunteers received at least one dose of test or reference drugs and were therefore included in the safety population. All the 68 volunteers were included in the pharmacokinetic analysis population. The bioequivalence population included 67 volunteers, since 1 volunteer from the TR group had been withdrawn from the study before the start of period 2 due to the withdrawal of an informed consent. All but one volunteer were white people, and that only volunteer belonged to asians. The mean age of the volunteers was 27.10 (±5.75) years, their body weight was 74.74 (±9.57) kg, and the BMI was 23.37 (±2.51) kg/m^2. Demographic and baseline characteristics of the volunteers did not differ between the groups.

Pharmacokinetics and bioequivalence

Pharmacokinetic parameters of nirmatrelvir after the administration of the test or reference drugs, are presented in Table 1. Fig. 2 is a graph of mean nirmatrelvir concentrations after studying the drugs.

After taking the test drug, $AUC_{(0–36)}$ was 24 902.7±7 959.97 ng*h/mL, and after taking the reference drug, it was 25 343.81±7 789.17 ng*h/mL. The maximum concentration of nirmatrelvir C_{max} was 2 411.17±808.21 and 2 580.54±869 ng/mL for the test and reference drugs, respectively. The values of the main pharmacokinetic parameters obtained after the use of the test and reference drugs, in general, were comparable between the drugs. Statistically significant differences between the drugs were observed in terms of $T_{1/2}$ (p=0.01), however, it should be noted that this indicator does not affect the assessment of the drugs bioequivalence, and there was no significant difference between the main pharmacokinetic parameters (C_{max} and AUC).

To assess the drugs bioequivalence, the $AUC_{(0–36)}$ parameter was used, since the obtained values of this indicator were more than 80% of the $AUC_{(0–\infty)}$ values. The calculated 90% CI for the ratio of geometric mean $AUC_{(0–36)}$ of the test and reference drugs was 93.27–103.74%. For the ratio of geometric mean C_{max} of the compared drugs, the 90% CI was 87.26-100.83%. The intervals obtained during the study, fully correspond to the established equivalence limit for $AUC_{(0–36)}$ and C_{max} – 80.00–125.00%, which clearly demonstrates the bioequivalence of the test and reference drugs (Table 2).

ANOVA results showed that the sources of variation such as drug differences, cohort, and period did not significantly affect the variables being evaluated. However, the sequence of the drug administration was found out to have a statistically significant effect on $AUC_{(0–36)}$ and $AUC_{(0–\infty)}$, and the cohort: period – on C_{max}.

When analyzing the results obtained, it was concluded that the statistically significant effect of the drug administration sequence and the period within the cohort identified during the analysis of variance, was due to external random factors and had no clinical significance.

Safety

In the study, a total of 61 AEs were reported in 43 volunteers. The data on AEs are presented in Table 3. The most common AEs (more than 5% in any of the groups) were dysgeusia and hypertension. Dysgeusia was observed in 7 (10.29%) volunteers after taking the test drug and in 4 (5.55%) volunteers after taking the reference drug. Hypertension was observed in 20 (29.41%) and 17 (25.00%) volunteers after taking the test and reference drugs, respectively. There were no significant differences between the compared drugs in the frequency of registration of adverse events (p >0.05). According to CTCAE 5.0, all the AEs registered during the study were classified as grade 1. No grade 2-5 AEs were registered.

According to the investigators, fewer than half of the reported AEs were related to the test drug. For the majority of AEs, the degree of association with the test drug was considered “doubtful”. The association of AEs with the drug use was established in 14 (20.59%) and 13 (19.12%) volunteers after taking the test drug in combination with ritonavir and the reference drug, respectively.

During the study, an association of AEs with the study medications was classified as “possible” for 1 case of anemia, 1 case of asthenia, and 14 cases of hypertension (7 after taking the test drug in combination with ritonavir and 7 after taking the reference drug). The drug association was classified as probable for 1 case of AE, a headache. The drug association was classified as certain for all 11 cases of AE dysgeusia. The frequencies of these events were comparable between the drugs. The reported AEs that the investigators considered drug-related, were consistent with the spectrum of adverse drug reactions of nirmatrelvir/ritonavir.

DISCUSSION

Nirmatrelvir has an antiviral activity against SARS-CoV-2 by inhibiting the main viral protease 3CLpro. The inhibition of 3CLpro by nirmatrelvir leads to the disruption of the polyprotein precursors processing, resulting in the termination of the viral replication [22, 23]. Due to the fact that nirmatrelvir is a substrate for the CYP3A4 enzyme, it is recommended to take it simultaneously with the pharmacokinetic enhancer ritonavir. In its turn,

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ritonavir is an inhibitor of CYP3A4, i.e., it reduces the rate of nirmatrelvir metabolism and thereby increases its systemic exposure [24, 25]. In Russia, ritonavir is registered as a part of combined drugs, as well as in the form of an independent drug. Due to the fact that ritonavir mono-preparations are commercially available, LLC "Drug Formulation" has developed a generic drug of nirmatrelvir, which does not include tablets with nirmatrelvir. In order to implement the state registration of the drug, a clinical bioequivalence study has been conducted.

The results of the clinical study showed that the PK parameters of the generic drug nirmatrelvir, when used together with ritonavir, are comparable to the PK parameters of the original drug nirmatrelvir/ritonavir. When conducting a statistical analysis of bioequivalence, the obtained 90% CI for the ratios of the geometric mean AUC and C_{max} nirmatrelvir values do not go beyond the specified intervals of 80.00–125.00% for these indicators, which confirms the bioequivalence of the test and reference drugs.

The data obtained as results of the study, are also consistent with the information available in the literature on the pharmacokinetics of the original drug nirmatrelvir/ritonavir. According to the data in the FDA fact sheet for healthcare providers on the original drug Paxlovid, as well as in the official instructions for the medical use of nirmatrelvir preparations placed in the State Register of Medicines, the geometric mean for nirmatrelvir C_{max} value after a single use on an empty stomach in combination with ritonavir is 2.21 mcg/mL, i.e., about 2 210 ng/mL. In the present study, the geometric mean C_{max} of the generic nirmatrelvir preparation practically did not differ from the literature data and was equal to 2274 ng/mL. The geometric mean $AUC_{(0-\infty)}$ of the original drug, according to the literature data, and the generic drug according to our study, are 23.01 (23 010 ng*h/mL) and 24 596 ng*h/mL, respectively. According to the literature data, the median T_{max} and mean $T_{1/2}$ of the original drug are 3.00 and 6.05 h, respectively. In this study, the generic drug showed similar values – 2.5 and 7.7 h for the median T_{max} and mean $T_{1/2}$.

The test and reference drugs were well tolerated by the volunteers. After taking the test and reference drugs, the incidence of AEs had no statistically significant differences. The AEs that were characterized by the investigators as drug-related were consistent with the published safety data for nirmatrelvir/ritonavir preparations. The most common adverse events, which occurred in more than 5% of participants, were dysgeusia and hypertension. According to the information specified in the instructions for the medical use of nirmatrelvir medicinal products, dysgeusia is a common (1–10%) adverse reaction when using these drugs. The hypertension AE is a common adverse reaction (1–10%) for ritonavir preparations in combination with nirmatrelvir/ritonavir used as a pharmacokinetic enhancer for nirmatrelvir.

CONCLUSION

In accordance with the protocol and requirements for confirming the bioequivalence of two drugs in agreement with international guidelines and legal requirements of the EAEU, the 90% CI obtained in this study for the ratio of the geometric mean $AUC_{(0-36)}$ and C_{max}, is fully within the range of 80.00–125.00%. Thus, it can be concluded that the test and reference drug nirmatrelvir are bioequivalent. It is also worth noting that the compared drugs showed a good tolerability and comparable safety profiles.

Based on the results of the bioequivalence study, Arpaxel was registered in the Russian Federation under the procedure for registering the drugs intended for use under the threat of occurrence and liquidation of emergencies.

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CONFLICT OF INTEREST

The clinical trial was organized by the sponsor JSC R-Pharm. The authors of the article Valentina G. Mozgovaya, Olga V. Filon, Anna V. Zinkovskaya, Antonina N. Dolgorukova, Elizaveta K. Khanonina, Vasily G. Ignatiev, Mikhail Yu. Samsonov are employees of JSC “R-Pharm”.

AUTHORS’ CONTRIBUTION

Rodion A. Oseshnyuk – research conducting; Anna Yu. Boroduleva, Pavel D. Sobolev, Ajyyna G. Nikiforova – analytical part development and validation; biosamples analysis; Svetlana A. Lesnichuk, Bair B. Garyaev, Anna A. Abramova – results analysis, text writing and editing; Valentina G. Mozgovaya – research design development, text writing and editing; Olga V. Filon – research design development, text writing and editing; Anna V. Zinkovskaya, Antonina N. Dolgorukova – statistical processing of research results; Elizaveta K. Khanonina – literary sources analysis, text writing and editing; Vasily G. Ignatiev, Mikhail Yu. Samsonov – aim setting, research design development.

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RESEARCH ARTICLE

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Post-exposure prophylaxis of COVID-19: results of double-blind, placebo-controlled, multicenter clinical study evaluation of efficacy and safety of double-stranded sodium salt RNA drug

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The aim of the study was to evaluate the efficacy and safety of an RNA double-stranded sodium salt drug, a lyophilisate for a solution preparation for an intramuscular and subcutaneous administration, as a means of post-exposure COVID-19 prophylaxis in comparison with placebo.

Material and methods. A double-blind, placebo-controlled, multicenter, randomized phase III clinical trial was conducted to evaluate the efficacy and safety of a double-stranded sodium salt RNA drug (RADAMIN®VIRO), a lyophilisate for preparing a solution for intramuscular and subcutaneous administration as a means of post-exposure prophylaxis of COVID-19. The study was conducted in 10 research centers in the Russian Federation from May 31, 2022 to January 17, 2023. The study included

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ОРИГИНАЛЬНАЯ СТАТЬЯ
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men and women aged ≥18 years who cohabitate with a person with a documented COVID-19 diagnosis and do not have symptoms characteristic of COVID-19. At the randomization stage, the subjects were assigned to one of two groups: group 1 (*n*=400) received a study drug RADAMIN®VIRO 5 mg (1 vial) intramuscularly once a day; group 2 (*n*=400) received placebo 1 vial intramuscularly once a day. The total duration of the study for each subject was no more than 30 days.

Results. By day 10–11, in the double-stranded sodium salt RNA drug group, the proportion of the subjects with confirmed COVID-19 and at least 1 symptom characteristic of COVID-19 was 5.76% (23/399), and in the placebo group – 11.03% (44/399). The difference in proportions between the study drug and placebo groups was 0.0526 (5.26%), the 95% confidence interval (CI) for the difference in proportions between the groups was [0.0123;0.0937]). More than 94% of single-dose subjects did not become infected with COVID-19 with any symptoms during the 11 days of the follow-up. As a result of a comparative analysis, it was shown that the infection frequency in the study drug group was statistically significantly (almost twice) less than in the comparison group, which indicates a high efficiency and expediency of using the double-stranded sodium salt RNA drug as a means of the post-exposure COVID-19 prophylaxis.

Conclusion. Thus, regardless of the vaccination availability, the effectiveness and feasibility of using the study double-stranded sodium salt RNA drug as a means of the post-exposure COVID-19 prophylaxis was demonstrated not only in medical institutions (outpatient clinics and hospitals), but also in caregivers and/or the persons in contact with COVID-19 patients. The situation was the same in the organizations and enterprises in case of evolution of a mass infection threat and the availability of appropriate medical personnels.

Keywords: coronavirus; COVID-19; RNA double-stranded sodium salt; RADAMIN©VIRO; prophylaxis; interferon inducer

Abbreviations: WHO – World Health Organization; AE – adverse events; SAE – serious adverse events; IG – Interim guidelines "Prevention, diagnosis and treatment of a new coronavirus infection"; CCs – comorbid conditions; ARI – acute respiratory infection; NAAT – nucleic acid amplification test; NSAIDs – non–steroidal anti–inflammatory drugs; MedDRA – Medical Dictionary for Regulatory Activities; CI – confidence interval; COVID–19 – coronavirus disease; SARS-CoV-2 – coronavirus, the causative agent of COVID–19; CTs – clinical trials, IWRS – Interactive Web Randomization System; eIRC – electronic individual registration card; GFR – glomerular filtration rate.

Постконтактная профилактика COVID-19: результаты двойного слепого плацебо-контролируемого многоцентрового клинического исследования по оценке эффективности и безопасности применения препарата РНК двуспиральной натриевой соли

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Цель. Оценка эффективности и безопасности применения препарата РНК двуспиральной натриевой соли, лиофилизат для приготовления раствора для внутримышечного и подкожного введения, в качестве средства постконтактной профилактики COVID-19 в сравнении с плацебо.

Материал и методы. Проведено двойное слепое плацебо-контролируемое многоцентровое рандомизированное клиническое исследование III фазы по оценке эффективности и безопасности применения препарата РНК двуспиральной натриевой соли (РАДАМИН®ВИРО), лиофилизат для приготовления раствора для внутримышечного и подкожного введения в качестве средства постконтактной профилактики COVID-19. Исследование проведено в 10 исследовательских центрах на территории РФ в период с 31.05.2022 г. по 17.01.2023 г. В исследование включались мужчины и женщины в возрасте ≥18 лет, совместно проживающие с лицом с документально подтвержденным диагнозом COVID-19 и не имеющие симптомов, характерных для COVID-19. На этапе рандомизации субъекты распределялись в одну из двух групп: 1 группа (*n*=400) получала исследуемый препарат РАДАМИН®ВИРО по 5 мг (1 флакон) внутримышечно однократно; 2 группа (*n*=400) получала плацебо по 1 флакону внутримышечно однократно. Общая продолжительность исследования для каждого субъекта составляла не более 30 дней.

Результаты. В группе препарата РНК двуспиральной натриевой соли доля субъектов с подтвержденным COVID-19 и наличием как минимум 1 симптома, характерного для COVID-19, к 10–11 сут составила 5,76% (23/399), а в группе плацебо – 11,03% (44/399). Разница в долях между группами исследуемого препарата и плацебо составила 0,0526 (5,26%), 95% доверительный интервал (ДИ) [0,0123; 0,0937]. Более чем у 94% субъектов, которым однократно вводили лекарственный препарат, не наблюдалось заражение COVID-19 с проявлением каких-либо симптомов в течение 11 дней наблюдения. В результате сравнительного анализа было показано, что частота заражения в группе исследуемого препарата была статистически значимо (практически в 2 раза) меньше, чем в группе сравнения, что говорит о высокой эффективности и целесообразности применения препарата РНК двуспиральной натриевой соли в качестве средства постконтактной профилактики COVID-19.

Заключение. Таким образом, была продемонстрирована эффективность и целесообразность применения исследуемого препарата РНК двуспиральной натриевой соли в качестве средства постконтактной профилактики COVID-19 вне зависимости от наличия вакцинации не только в медицинских учреждениях (амбулаториях и стационарах), но и у субъектов, осуществляющих уход и/или контактировавших с больными COVID-19, а также в организациях и на предприятиях при возникновении угрозы массового заражения и наличия соответствующего медицинского персонала.

Ключевые слова: коронавирус; COVID-19; РНК двуспиральной натриевая соль; РАДАМИН®ВИРО; профилактика; индуктор интерферонов

Список сокращений: ВОЗ – Всемирная организация здравоохранения; НЯ – нежелательное явление; СНЯ – серьёзные нежелательные явления; АПФ2 – ангиотензинпревращающий фермент 2; ВМР – Временные методические рекомендации «Профилактика, диагностика и лечение новой коронавирусной инфекции»; ОРВИ – острая респираторная вирусная инфекция; СЗ – сопутствующие заболевания; МАНК – метод амплификации нуклеиновых кислот; НПВП – нестероидные противовоспалительные препараты; MedDRA – медицинский словарь терминов международной медицинской терминологии; ДИ – доверительный интервал; COVID-19 – коронавирусная инфекция; SARS-CoV-2 – коронавирус, возбудитель COVID-19; КИ – клинические исследования; IWRS – модуль рандомизации пациентов; э-ИРК – электронная индивидуальная регистрационная карта; СКФ – скорость клубочковой фильтрации.

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INTRODUCTION

The COVID-19 pandemic has damaged many aspects of society, but at the same time has had a powerful driver on the development of the pharmaceutical industry, in particular, the development and implementation of effective drugs for the treatment and prevention of coronavirus infection. During the pandemic, clinical trials were accelerated and the procedure for registering new drugs was simplified, careful monitoring, but at the same time, permanent monitoring of the drugs safety and efficacy was observed [1, 2].

The new coronavirus infection (COVID-19) turned into a pandemic in March 2020 and was characterized by high contagiousness, damage not only to the lungs, but also to other organs and systems (digestive tract, cardiovascular system, kidneys) and a lethality of about 2%[1]. SARS-CoV-2 is RNA single-stranded virus, which, binds with ACE2, entering at the human body through the cell membrane, penetrates the cell, multiplies with the release of new virions from the infected cell, the development of a local and systemic inflammatory response, with damage to target organs [3].

Despite the fact that at present there are the signs of the COVID-19 pandemic subsiding and the epidemiological situation has switched on to a "managed" mode, due to virus mutations (decrease in virulence), increases of collective immunity and number of COVID-19 vaccinated and recovering, the risk of infection, especially with new sublines of SARS-CoV-2 persists, and the search for new means and methods of treatment and prevention of a new coronavirus infection is relevant now [4–6]. Social distancing (self-isolation, breakup, public places and enterprises; the cancellation of mass events), quarantine measures limited to the recording of disease cases, isolation of severely ill patients in a hospital, mild cases at home and contact tracing[2], have historically been the first approaches to prevention of COVID-19, but had a limited effect [7] and, unfortunately, have not made the spread of the infection possible to take complete control of it[3].

The presence of niches where the SARS-CoV-2 virus circulates both during the peaks of the pandemic and outside the pandemic waves, which include asymptomatic carriers and/or mild cases with symptoms of seasonal acute respiratory infection (ARI) [8, 9] explained the low effectiveness of social prevention measures and the need for immunization and other ways to prevent infection [10, 11]. However, the problems of vaccination against a new coronavirus infection remain unresolved [12, 13]. One of the reasons for this is a high contagiousness of SARS-CoV-2 "Omicron" and its subvariants[4]. Moreover, the virus has a reduced "recognition" of post-infection and post-vaccination antibodies [14].

Some patients (including vaccinated) carry a new coronavirus infection asymptomatically or in a mild form, but the spread of the virus by humans continues [15]. No "superinfectors" should be disregarded: to the high replication of the virus in the oral cavity, nasopharynx and oropharynx, they can release large concentrations of the virus during close contacts in sneezing and coughing [16, 17].

Despite the unprecedented lockdown measures, families always have the members involved in the process flow production (doctors, pharmacists, employees of law enforcement agencies, emergency services, transport), forced to be at the workplace all the time. In this regard, it is possible that the disease of one family member poses a threat of infection not only to all their family members, but also to those around them [18]. The search for methods of drug prevention that affect the signaling pathways of interferon, which have an immunomodulatory and early antiviral effect after contact with patients with COVID-19, is extremely relevant and especially significant for controlling morbidity in contact persons in the family, in labor collectives, crowded places and among medical workers [19].

Nonspecific prophylactic measures aimed at the infection source, include an early diagnosis and an active detection of the infected, including those who are asymptomatic, the isolation of patients and persons with a suspected disease, and the prescription of the etiotropic therapy [20, 21]. Cytokines are regulatory peptides produced by body cells that play one of the main roles in individual reactivity associated with clinical manifestations.

The antiviral response mediated by interferons has a direct relationship with the viral load, which depends on the infecting dose and the degree of the immune replication control [22].

In the early stages of infection, the use of the drugs based on the RNA double stranded sodium salt can act as a factor determining the virus replication control and, at the same time, maintain the endogenous control mechanism of the interferons content in the body no higher than protective concentrations [23].

In the Russian Federation, a medicinal product based on the RNA double-stranded sodium salt (LS-000381 dated Dec 27. 2021) RADAMIN©VIRO is registered for the treatment and prevention of influenza and other ARIs, as well as in the prevention and treatment of other infectious and inflammatory diseases, including caused by herpes zoster, genital, simplex, and chlamydia viruses.

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[4] Weekly epidemiological update on COVID-19 – 26 October 2022. Edition 115. Available from: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---26-october-2022.

THE AIM of the study was to evaluate the efficacy and safety of an RNA double-stranded sodium salt drug as a means of a post-exposure COVID-19 prophylaxis in comparison with placebo.

MATERIALS AND METHODS

The efficacy and safety of using the RNA double-stranded sodium salt (lyophilizate for the preparation of a solution for intramuscular and subcutaneous administrations) as a means of a post-exposure COVID-19 prophylaxis, was studied in a double-blind, placebo-controlled, randomized, multicenter, comparative phase III clinical trial (RCT No. 263 dated 12 Apr 2022).

The research was conducted from May 31, 2022 to January 17, 2023 on the basis of 10 research centers in the Russian Federation:

1. LLC "Eco-Safety" Research & Development center, St. Petersburg;
2. LLC OrKli Hospital Company, St. Petersburg;
3. Kirov State Medical University;
4. LLC "Professor's Clinic", Perm;
5. LLC Aurora MedFort, St. Petersburg;
6. National Research Ogarev Mordovia State University, Saransk;
7. Smolensk State Medical University, Smolensk;
8. City Clinical Hospital named after S.I. Spasokukotsky, Moscow City Health Department;
9. Ivanovo Clinical Hospital;
10. Smolensk Clinical Hospital No. 1.

This study was conducted in accordance with the rules of Good Clinical Practice of the International Conference on Harmonization (ICH GCP), the ethical principles set forth in the Declaration of Helsinki of the World Medical Association (Fortaleza, 2013) and the requirements of the Russian legislation.

The study protocol, the Investigator's Brochure, the Subject Information Sheet with the Informed Consent Form for Participation in the Study, were approved by the Ethics Council prior to the inclusion of subjects in the study (Protocol No. 307 dated 04.05.2022). The subjects consented to participate in this study by signing the Informed Consent Form.

Randomization of study subjects into groups

The study screened 804 subjects, 800 subjects of which were randomized (4 subjects were not randomized due to the non-inclusion criteria; Fig. 1).

The subjects were randomized using an interactive on-line randomization system (Interactive web randomization system, IWRS) embedded in an electronic individual registration card (eIRC). Before the start of the study, each investigator-physician who had been delegated the responsibility of transferring the data to the eIRC was provided with an access code (a combination of a username and password) to the eIRC, as well as detailed written instructions for working with the eIRC, including detailed instructions for the randomization procedure. The randomization was carried out according to the following algorithm. Each subject that had met all of the inclusion criteria and had not met any of the exclusion criteria was assigned a three-digit randomization number by the IWRS system. The subject randomization number and other relevant data were entered by the investigator into the Subject Screening/Randomization Journal. If a subject had terminated participation in the study prematurely, their randomization number was not reused and the subject was subsequently unable to participate in the study. Neither the investigator nor the subject knew what therapy the subject was receiving.

Study design

The study included men and women (*n*=800) aged 18 to 80 years who cohabited with a person with a documented COVID-19 diagnosis and had symptoms characteristic of COVID-19, meeting the inclusion criteria and not meeting the non-inclusion criteria. Subjects were screened and randomized into 2 groups in the ratio of 1:1. The choice of drug for patients was carried out in accordance with the randomization number assigned to patients at the time of randomization.

Depending on the randomization, the study subjects received either the study RNA sodium double-stranded drug or placebo. During the randomization phase, the subjects were assigned to 1 of the 2 groups:

Group 1 (*n*=400) received the study RNA double-stranded sodium salt drug (RADAMIN®VIRO, JSC "Biochemist", series 010122) 5 mg (1 vial) intramuscularly once;

Group 2 (*n*=400) received a placebo (sodium chloride, JSC "Biochemist", Russia, series 010122) 1 vial intramuscularly once.

The study drug/placebo was administered by the study center medical staff in the upper outer quadrant of the buttock. The study provides for a single intramuscular RADAMIN® VIRO injection at the dose of 5 mg[5]. This is due to its dosage form and dosing regimen approved by the current instructions for the prevention and treatment of influenza and SARS. A placebo was used as a reference drug, which made it possible to obtain objective results of this study. The study participants who were diagnosed with COVID-19 during the course of the study could receive standard therapy in accordance with the IGs in force at the time of the research. Due to the lack of the approved COVID-19 post-exposure prophylaxis, the drugs that could be used as comparators, and to avoid the data collection/evaluation bias during the study, placebo was used as the study comparator.

[5] Russian State Register of Medicines. Instructions for the medical use of RADAMIN®VIRO. Available from: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=2e3ad776-6616-4e43-99c1-3133cd95b280

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The clinical study included the following steps (Fig. 2):

- screening – no more than 24 h;
- randomization – no more than 1 day;
- study drug/placebo therapy – 1 day;
- post-observation – 28 days.

The duration of the study for the subjects was no more than 30 days, herewith, the study drug/placebo treatment period was 1 day. If a subject had been diagnosed with COVID-19 during the course of the study based on the results of SARS-CoV-2 RNA analyses using the NAAT, the standard therapy presented in the current version of the IG, could be prescribed to the subject at the discretion of the investigator.

Depending on the COVID-19 severity, its treatment could be carried out both at home and in a hospital setting. At Visits 2 and 3, regardless of the presence/absence of COVID-19 symptoms, nasopharyngeal and/or oropharyngeal swabs were collected from the subjects for the SARS-CoV-2 RNA determination by the NAAT to detect COVID-19.

In addition, if a study subject had experienced the symptoms consistent with COVID-19 prior to day 29 after the administration of the study drug/placebo, he was given a COVID-19 Confirmation Visit. However, if the subject developed the symptoms consistent with COVID-19 after being diagnosed with COVID-19 based on the NAAT SARS-CoV-2 RNA results, the COVID-19 Confirmation Visit was not conducted.

If a subject did not need to be hospitalized due to the COVID-19 development, he was not withdrawn from the study, and continued to be monitored. If a subject needed to be hospitalized during the course of the study, he was excluded from the study.

If a subject had been diagnosed with COVID-19, additional procedures outside the Protocol could be performed at the discretion of the investigator physician in accordance with the clinical practice of the research center.

Selection of subjects for analysis

Primary and secondary efficacy outcomes were analyzed using a dataset of the study participants selected according to the protocol compliance, i.e., all the patients who had completed the study in accordance with the Study Protocol. A participant was excluded from the data set if he had met the exclusion criteria. The safety data set included all randomized patients who had been exposed to the study drug, regardless of the degree of adherence to the Protocol during the study. The study screened 804 subjects, 800 of which were randomized. 4 subjects were not randomized due to the non-inclusion criteria. During the course of the study, 1 subject was excluded from the RNA double-stranded sodium salt drug group due to the meeting the exclusion criterion No. 4 “Invalid inclusion of a subject not meeting the inclusion criteria and/or meeting the non-inclusion criteria”, i.e., living with 2 or more persons with documented COVID- 19 at the time of screening. One subject in the placebo group was tested positive for SARS-CoV-2 RNA by the NAAT at screening.

Inclusion Criteria

The subjects meeting the following inclusion criteria were included in the study:

1. Men and women aged 18 to 80 inclusive (subjects) at the time of signing the Informed Consent Form.

2. The subject is living with a person with documented COVID-19 who meets both of the following criteria:

– the first positive result of a laboratory test for the presence of SARS-CoV-2 RNA using nucleic acid amplification tests (NAATs) or SARS-CoV-2 antigen using an immunochromatographic analysis within 72 hours before the randomization of the subject participating in this study;

– the presence of at least 1 of the symptoms characteristic of COVID-19, with the onset of symptoms no more than 5 days before the randomization of the subject participating in this study.

3. A negative result for the presence of SARS-CoV-2 antigen using an immunochromatographic analysis.

4. The absence of the symptoms characteristic of COVID-19.

5. The subject is expected to continue to live with a person with documented COVID-19 for the duration of the participation in the clinical study, no need for hospitalization of a person;

6. A subject’s consent to use reliable methods of contraception throughout the study and for 3 weeks after the end of the study. Reliable means of contraception are: sexual abstinence, the use of a condom in combination with spermicide. The study may also include the women who are unable to bear children (history: hysterectomy, tubal ligation, infertility, menopause for more than 2 years), as well as the men with infertility or a history of vasectomy.

7. An availability of an Informed Consent Form signed and dated by the subject.

8. An availability of a signed and dated by a person documented COVID-19 Informed Consent Form for the collection of information on COVID-19.

Exclusion Criteria

The subjects meeting at least one of the following non-inclusion criteria were not included in the study:

1. Hypersensitivity to the components of the study drug, procaine.

2. The presence of contraindications for intramuscular injections.

3. Contact with 2 or more individuals with documented COVID-19 within 1 month prior to screening, or living with 2 or more individuals with documented COVID-19 at the time of screening.

4. Shared accommodation with > 10 people.

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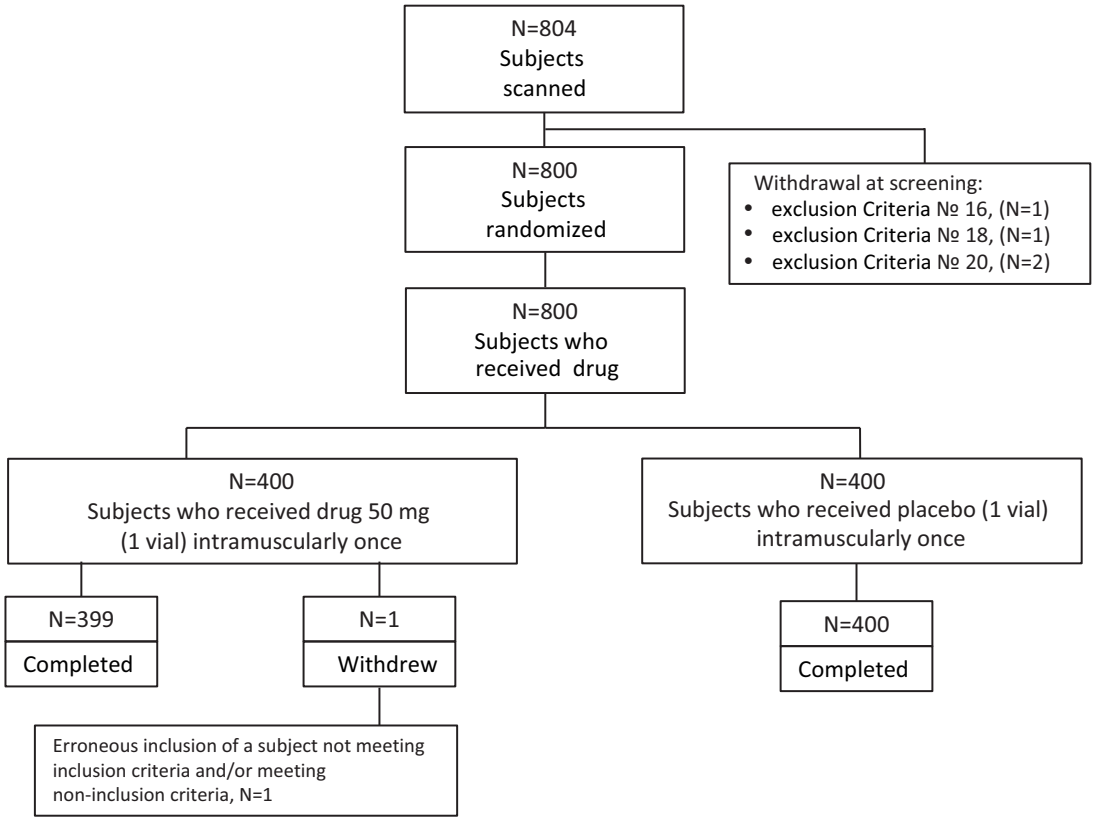

Figure 1 – Randomization of study subjects into groups

Table 1 – Criteria for effectiveness evaluation

No.	Effectiveness criterion	End point
	Primary effectiveness criterion	
1.	Frequency of subjects with confirmed COVID-19 and at least 1 symptom consistent with COVID-19*	Visit 3
	Secondary effectiveness criteria	
2.	Frequency of subjects with confirmed COVID-19 and at least 1 symptom consistent with COVID-19**	Visits 2, 4, 5
3.	Frequency of subjects with confirmed COVID-19 and no symptoms consistent with COVID-19**	Visits 2, 3.
4.	Frequency of Subjects with Confirmed COVID-19 with and without Symptoms of COVID-19**	Visits 2–5
5.	Time till exposure to COVID-19* Infection was understood as the moment of the onset of a symptom characteristic of COVID-19, or the moment of SARS-CoV-2 RNA by NAAT detection, depending on what had been previously detected.	–
6.	Assessment of the severity of symptoms characteristic of COVID-19* Assessment was performed only for the subjects who had developed symptoms of COVID-19 during the study up to and including Visit 3.	Visits 2–5
7.	Duration of symptoms characteristic of COVID-19** Assessment was performed only for subjects who had developed symptoms of COVID-19 during the study up to and including Visit 3. The score was presented for symptoms that had ended before the end of the subject's participation in the study.	–
8.	Estimated severity of COVID-19**. Assessment was performed only for subjects who had tested positive for COVID-19.	To visits 2–5
9.	Frequency of subjects requiring hospitalization due to development of COVID-19.	–

Note: * – Analysis includes subjects with a negative SARS-CoV-2 RNA assay by NAAT selected at screening; ** – Assessment was performed with and without subjects who were positive for SARS-CoV-2 RNA by NAAT at the time of screening. The assessment included subjects who had a COVID-19 confirmation visit between visits and found positive SARS-CoV-2 RNA by NAAT.

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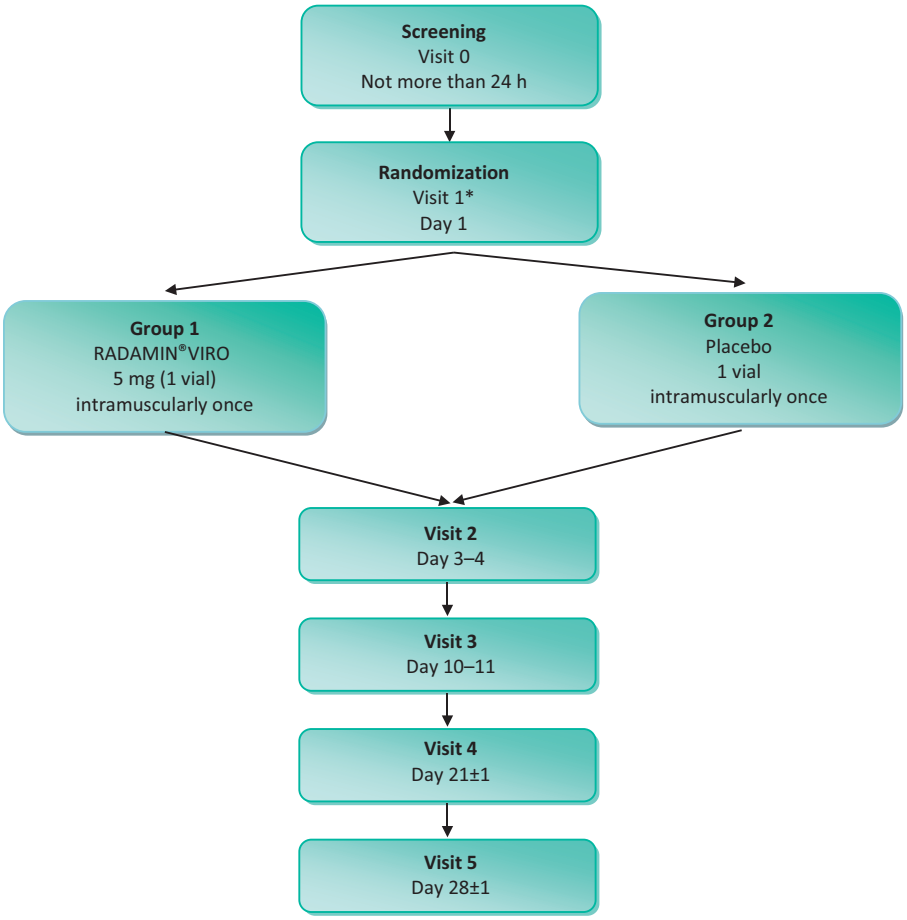

Figure 2 – Study design

Note: * – Visit 1 could be the same as Visit 0. If Visit 1 and Visit 0 were the same, then a physical examination, a pulse oximetry with SpO_2 measurement, a vital signs assessment, concomitant therapy registration was not re-assessed, the inclusion and non-inclusion criteria had been evaluated immediately before randomization, and the exclusion criteria were assessed after the drug use. The visits could take place at the study center or at home. If a subject had experienced the symptoms consistent with COVID-19 before day 29 after the study drug/placebo administration, that subject was eligible for a COVID-19 Confirmation Visit. The visit was carried out as soon as possible, but not later than 3 days after the development of the first COVID-19 symptom.

Table 2 – Baseline demographic, anthropometric and clinical characteristics of patients

Characteristics		RNA double-stranded sodium salt, *n*=400	Placebo, *n*=400
Age, years (M±SD)		44.68±15.60	45.96±14.86
Gender:	males, *n* (%)	169 (42.25%)	174 (43.50%)
	females, *n* (%)	231 (57.75%)	226 (56.50%)
Weight, kg (M±SD)		74.80±13.39	75.84±13.71
Height, cm (M±SD)		171.41±8.00	171.44±8.18
BMI, kg/m^2 (M±SD)		25.41±4.00	25.74±3.99
Comorbid conditions, including:		121 (30.33%)	128 (32.00%)
	obesity	45 (11.28%)	52 (13.00%)
	arterial hypertension	81 (20.30%)	90 (22.50%)
Vaccination against COVID-19		284 (71%)	291 (72.75%)

Note: BMI – body mass index.

Table 3 – Generalized data of comparative evaluation of RNA double-stranded sodium salt efficacy

Checkpoint	Groups	
	Placebo	RNA double stranded sodium salt
Primary criterion		
Frequency of subjects with confirmed COVID-19 and at least 1 symptom consistent with COVID-19*		
Visit 3	11.03% (44/399)	5.76% (23/399)
Secondary efficacy criteria		
Frequency of subjects with confirmed COVID-19 and at least 1 symptom consistent with COVID-19**		
Visit 2	7.00% (28/400)	3.51% (14/399)
Visit 4	11.50% (46/400)	6.02% (24/399)
Visit 5	11.50% (46/400)	6.27% (25/399)
Frequency of subjects with confirmed COVID-19 and no symptoms consistent with COVID-19**		
Visit 2	0.25% (1/400)	0.75% (3/399)
Visit 3	0.00% (0/400)	0.50% (2/399)
Frequency of subjects with confirmed COVID-19 with and without symptoms consistent with COVID-19**		
Visit 2	7.25% (29/400)	4.26% (17/399)
Visit 3	11.25% (45/400)	6.27% (25/399)
Visit 4	11.50% (46/400)	6.52% (26/399)
Visit 5	11.50% (46/400)	6.77% (27/399)
Frequency of COVID-19 infection by day 11 of the study**		
Visit 3	11.25% (45/400)	6.27% (25/399)
Frequency of subjects with COVID-19 symptoms prior to Visit 3*		
Visit 2-5	9.02% (36/399)	17.04% (68/399)
Duration of symptoms characteristic of COVID-19**		
Sore throat, days	4.68±2.53	3.06±1.25
Smell Change	2.25±1.26	11.13±6.45
Severity assessment of COVID-19**		

Checkpoint	Mild illness	Asymptomatic/ completed case	Mild illness	Asymptomatic/ completed case
Visit 2	100% (28/28)	0.00% (0/28)	94.12% (16/17)	5.88% (1/17)
Visit 3	47.73% (21/44)	52.27% (23/44)	64.00% (16/25)	36.00% (9/25)
Visit 4	17.78% (8/45)	82.22% (37/45)	23.08% (6/26)	76.92% (20/26)
Visit 5	15.56% (7/45)	84.44% (38/45)	22.22% (6/27)	77.78% (21/27)
Frequency of subjects requiring hospitalization due to COVID-19 development.				
Visits 1-5	0.00% (0/45)		0.00% (0/27)	

Note: * – The analysis includes subjects with a negative SARS-CoV-2 RNA assay by NAAT selected at screening; ** – The evaluation was carried out with and without the subjects who were positive for SARS-CoV-2 RNA by NAAT at the time of screening. The evaluation included the subjects who had a COVID-19 confirmation visit between the visits and a positive SARS-CoV-2 RNA was found out by NAAT.

Table 4 – Severity of symptoms characteristic of COVID-19

Checkpoint	Placebo			RNA sodium double-stranded		
	Severity of symptoms			Severity of symptoms		
	Absence	Moderate	Severe	Absence	Moderate	Severe
			Sore throat			
Visit 2	94.99% (379/399)	4.76% (19/399)	0.25% (1/399)	97.49% (389/399)	2.51% (10/399)	0.00% (0/399)
Visit 3*	97.24% (388/399)	2.76% (11/399)	0.00% (0/399)	99.25% (396/399)	0.75% (3/399)	0.00% (0/399)
Visit 4	99.75% (398/399)	0.25% (1/399)	0.00% (0/399)	100% (399/399)	0.00% (0/399)	0.00% (0/399)
Visit 5	100% (399/399)	0.00% (0/399)	0.00% (0/399)	100% (399/399)	0.00% (0/399)	0.00% (0/399)
			Fatigue			
Visit 2	94.24% (376/399)	4.76% (19/399)	1.00% (4/399)	96.49% (385/399)	2.76% (11/399)	0.75% (3/399)
Visit 3**	96.24% (384/399)	3.76% (15/399)	0.00% (0/399)	98.50% (393/399)	1.50% (6/399).	0.00% (0/399)
Visit 4	99.75% (398/399)	0.25% (1/399)	0.00% (0/399)	100% (399/399)	0.00% (0/399)	0.00% (0/399)
Visit 5	100% (399/399)	0.00% (0/399)	0.00% (0/399)	100% (399/399)	0.00% (0/399)	0.00% (0/399)
			Chills			
Visit 2***	98.25% (392/399)	1.75% (7/399)	0.00% (0/399)	99.75% (398/399)	0.25% (1/399)	0.00% (0/399)
Visit 3	99.25% (396/399)	0.75% (3/399)	0.00% (0/399)	99.50% (397/399)	0.50% (2/399)	0.00% (0/399)
Visit 4	100% (399/399)	0.00% (0/399)	0.00% (0/399)	100% (399/399)	0.00% (0/399)	0.00% (0/399)
Visit 5	100% (399/399)	0.00% (0/399)	0.00% (0/399)	100% (399/399)	0.00% (0/399)	0.00% (0/399)

Note: * – statistically significant difference between groups; p=0.0314; ** – statistically significant difference between groups, p=0.0472; *** – statistically significant difference between groups; p=0.0191.

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Table 5 – Description of total number of reported AEs in subjects in study groups

AEs (RT* according to MedDRA)	Number of AEs, absolute value (% of AEs total number)		
	Total (*n*=400)	Placebo group (*n*=200)	RADAMIN®VIRO group (*n*=200)
Asthenia	3 (4.92%)	3 (7.14%)	0 (0.00%)
Pain	1 (1.64%)	1 (2.38%)	0 (0.00%)
Pain at injection site	2 (3.28%)	2 (4.76%)	0 (0.00%)
Pain in oropharynx (oropharyngeal)	1 (1.64%)	1 (2.38%)	0 (0.00%)
Viral infection of respiratory tract	6 (9.84%)	2 (4.76%)	4 (21.05%)
Headache	14 (22.95%)	10 (23.81%)	4 (21.05%)
Nasal congestion	6 (9.84%)	3 (7.14%)	3 (15.79%)
Cough	3 (4.92%)	2 (4.76%)	1 (5.26%)
Oropharyngeal discomfort	1 (1.64%)	1 (2.38%)	0 (0.00%)
Sore throat	2 (3.28%)	2 (4.76%)	0 (0.00%)
Pyrexia	4 (6.56%)	3 (7.14%)	1 (5.26%)
Rhinitis	3 (4.92%)	3 (7.14%)	0 (0.00%)
Rhinorrhea	2 (3.28%)	1 (2.38%)	1 (5.26%)
Tension headache	1 (1.64%)	0 (0.00%)	1 (5.26%)
Nausea	1 (1.64%)	1 (2.38%)	0 (0.00%)
Induration at infection site	2 (3.28%)	2 (4.76%)	0 (0.00%)
Fatigue	7 (11.48%)	3 (7.14%)	4 (21.05%)
Erythema at infection site	2 (3.28%)	2 (4.76%)	0 (0.00%)
TOTAL	61 (100%)	42 (100%)	19 (100%)

Table 6 – Frequency of adverse effects according to WHO classification

System organ class and preferred MedDRA term	Number of events (absolute value, %)		*p* value (Pearson's chi-squared test)
	RADAMIN®VIRO (*n*=400)	Placebo (*n*=400)	
Infections and invasions			
Viral infection of respiratory tract	0 (0)	1 (0.3) infrequent	0.3170
Nervous system disorders			
Headache	0 (0)	1 (0.3) infrequent	0.3170
General disorders and reactions at injection site			
Erythema	0 (0)	2 (0.5) infrequent	0.1568
Pain	0 (0)	2 (0.5) infrequent	0.1568
Induration	0 (0)	2 (0.5) infrequent	0.1568

5. Within 6 months prior to the randomization, presence of a laboratory-confirmed COVID-19 case.

6. The subjects vaccinated against COVID-19 less than 4 weeks prior to screening.

7. The use or need to use drugs from the prohibited list at the time of screening.

8. The use of immunostimulating, immunomodulatory or immunosuppressive drugs within 3 months prior to screening.

9. The subjects on the renal replacement therapy or with a history of a severe renal failure (the estimated glomerular filtration rate (GFR) <30 mL/min/1.73 m^2 calculated using the CKD-EPI formula within 6 months prior to screening).

10. Child-Pugh class C primary biliary cirrhosis or history of chronic or active hepatitis B or C.

11. A positive test result for the presence of HIV, syphilis, hepatitis B and/or C at screening.

12. A chronic heart failure (III–IV FC) according to the functional classification of the New York Heart Association (NYHA).

13. A history of malignancy, excluding the subjects with no history of disease in the past 5 years, the subjects with completely healed basal cell skin cancer or completely healed carcinoma *in situ.*

14. A history of alcohol, pharmacological and/or drug dependence and/or at the time of screening.

15. A history of or suspected schizophrenia, schizoaffective disorder, bipolar disorder, or other psychiatric disorder at screening.

16. Any history data that, in the opinion of the investigator, may complicate the interpretation of the results of the study or create additional risk for the subject as a result of his participation in the study.

17. Unwillingness or inability of the subject to comply with Protocol procedures (in the opinion of the investigator).

18. Pregnant or lactating women or women planning a pregnancy.

19. Participation in another clinical study within 3 months prior to the enrollment in the study.

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20. Other conditions that prevent the subject from being included in the study.

Exclusion Criteria

A decision to exclude a subject from the study was made by the investigator. The subject was withdrawn from the study immediately if any of the following situations occurred:

1. The occurrence during the course of the study of any diseases or conditions that worsen the prognosis of the subject, and make it impossible for the subject to continue participation in the clinical study. If a subject was diagnosed with COVID-19 based on the results of SARS-CoV-2 RNA analyses using the NAAT, selected both at the screening stage and after it, and there was no need for hospitalization of the subject, he was not excluded from the study, he continued to be monitored. If a subject needed to be hospitalized during the course of the study, the subject was excluded from the study.
2. Taking drugs of prohibited therapy or the need to prescribe them.
3. Pregnancy (for study participants).
4. Invalid inclusion of a subject that does not meet the inclusion criteria and/or meets the non-inclusion criteria.
5. Other violations of the Protocol which, in the opinion of the investigator, are significant.
6. Refusal of the subject to participate in the study.
7. Other administrative reasons.

Criteria for evaluating effectiveness

The primary endpoint for this study was selected based on the FDA's Guidelines for Drug Development[6] for the Treatment and Prevention of COVID-19. The primary efficacy endpoint analysis included COVID-19 cases prior to Visit 3 (days 10–11). A case of COVID-19 in this study was defined as the absence of a positive SARS-CoV-2 RNA test by the NAAT at the screening stage, the appearance of at least one symptom characteristic of COVID-19 during the study, and the detection of a positive result for RNA during the SARS-CoV-2 study by the NAAT. The primary outcome was assessed prior to Visit 3 (Days 10–11), which was sufficient time to reliably evaluate the efficacy of a single RADAMIN®VIRO dose, taking into account the SARS-CoV-2 incubation period. At the same time, the study subjects were monitored for 28 days after the use of the drug. As a part of the secondary efficacy endpoints, the following indicators were assessed: the incidence of COVID-19 cases with and without symptoms during the entire period of a subject's participation in the study, the severity of the developed COVID-19 disease, the frequency of hospitalizations due to COVID-19, the assessment of the severity and duration of symptoms. The selection of these criteria was also based on the FDA Drug Development Guidelines for the Treatment and Prevention of COVID-19.

The endpoints for evaluating the effectiveness of therapy are presented in Table 1.

Criteria for safety assessment

- Total number of AEs stratified by severity and frequency;
- Frequency of adverse reactions;
- Frequency of SAEs, including those associated with the study drug/standard therapy;
- Proportion of patients with at least one AE.

Statistical analysis

For a statistical analysis, software with validated algorithms for performing statistical analyzes and a proper documentation was used (StatSoft Statistica 13.3).

Descriptive statistics is presented for all efficacy and safety measures collected during the study. Continuous (quantitative) data are presented using the number of the observations, arithmetic mean, a 95% confidence interval (CI) for the mean, a standard deviation, median, an interquartile range (25^{th} and 75^{th} percentiles), minimum and maximum.

Ordinal and qualitative data are presented as absolute frequencies (numbers of observations), relative frequencies (percentage), and a 95% CI (unless otherwise noted).

Checking for the normality of the distribution was carried out by one of the generally accepted methods (Shapiro-Wilk test). In case of a non-Gaussian distribution, non-parametric estimation methods were used to compare indicators.

Significance levels and confidence intervals were calculated as two-tailed, and the statistical significance of differences by default and referred to a significance level of 0.05 (unless otherwise stated).

Demographic data (age, sex), baseline data are presented for the safety population as absolute frequencies (number of observations), relative frequencies (percentage) or using the arithmetic mean, a 95%CI for the mean, standard (root mean square) deviation, median, an interquartile range (25^{th} and 75^{th} percentiles), minimum and maximum depending on the type of the variable. To test the hypothesis about the homogeneity of the study groups in the initial period, null hypotheses were tested (about the absence of differences between the groups) using the Mann-Whitney test (for ordinal indicators or for interval indicators with a distribution that differs from normal) or the χ^2 test (for qualitative signs). In case of finding statistically significant differences between the groups, the magnitude of the differences between the study groups was assessed using confidence intervals.

[6] COVID-19: Developing Drugs and Biological Products for Treatment or Prevention,Guidance for Industry / U.S. Department of Health and Human Services Food and Drug Administration, 2021. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/covid-19-developing-drugs-and-biological-products-treatment-or-prevention

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For the analysis of secondary efficacy parameters, represented by qualitative values, an intergroup comparison of shares was used using a two-tailed version of Fisher's exact test (or χ^2 ("chi-square") test, if all the expected values in the cells of the contingency table for this analysis were 5 or more). To assess the parameters represented by ordinal values, non-parametric methods of the analysis were used: to compare indicators between the groups, the Mann-Whitney test was used; the Wilcoxon test was used for two dependent variables. The Fisher's exact test or the χ^2 ("chi-square") test could be also used for the analysis, if all the expected values in the cells of the contingency table for this analysis are 5 or more.

For comparison between the groups of continuous scores, the Student's *t*-test or the Mann-Whitney test (depending on the conclusion about the nature of the distribution) will be used.

To estimate the time to the event (time-to-event), taking into account censored observations, the Kaplan-Meier method and the construction of survival tables were used as descriptive methods of analysis, and the Cox-Mantel criterion was used to compare the time between the study groups. The differences were considered statistically significant at $p < 0.05$.

For all safety indicators collected during the study, descriptive statistics is presented (means, scatter measures, frequency, 95% confidence intervals, median, quartiles, minimum and maximum values or absolute frequencies (number of observations), relative frequencies (percentage)). The comparison of groups in terms of frequency indicators was carried out using the Fisher's exact test or the chi-square test, depending on the expected value in the cells of the contingency table. For quantitative laboratory results, comparisons between the groups at the respective visits were made using the Mann-Whitney test. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Baseline Patient Characteristics

The study involved 800 male and female subjects. The average age of the subjects in the population was 45.32±15.24 years (from 18 to 80 years), the average body weight was 75.32±13.55 kg (from 44.70 to 130 kg), the average height was 171.42± 8.08 cm (from 150 to 192 cm), the average body mass index (BMI) was 25.58±3.99 kg/m^2 (from 16.16 to 46.87 kg/m^2).

As a result of a comparative analysis of the demographic and anthropometric data of the subjects, no intergroup statistical differences were found out (Table 2).

It is worth noting that the average BMI of the population indicates that the majority of patients were overweight, which means they had an increased risk of contracting a new coronavirus infection and a complicated course of the disease.

A total of 575 subjects vaccinated against the novel coronavirus infection participated in the study: 284 vaccinated subjects in the study drug group and 291 subjects in the placebo group.

344 subjects (43.00%) had comorbidities. In the study drug group, the frequency of subjects with comorbidities was 174 (43.50%), in the placebo group – 170 (42.5%).

A total of 249 subjects with comorbid conditions such as obesity, type 2 diabetes mellitus, hypertension, chronic pyelonephritis, chronic bronchitis, cystitis, impaired glucose tolerance, prostatitis, chronic tonsillitis, rheumatoid arthritis, psoriasis, chronic sinusitis, angina pectoris, a chronic heart failure, asthma, a chronic obstructive pulmonary disease, diabetic neuropathy, dyslipidemia, a non-alcoholic fatty liver disease, herpes virus often recurrent lesions of the genital mucosa (no relapses were recorded during the observation period). In the study drug group, the frequency of the subjects with these diseases was 121 (30.33%), in the placebo group – 128 (32.00%).

Among the identified pathologies, the conditions such as hypertension and obesity were most frequently observed. In the RNA double-stranded sodium salt drug group, the frequency of the subjects with hypertension was 81 (20.30%), in the placebo group – 90 (22.50%). In the study drug group, the incidence of obese subjects was 45 (11.28%), in the placebo group – 52 (13.00%).

Primary efficacy criterion

The analysis included subjects (*n*=798) with a negative SARS-CoV 2 RNA assay by NAAT selected at screening: the RNA double-stranded sodium salt drug group (*n*=399) and the placebo group (*n*=399).

In the double-stranded sodium salt RNA drug group, the proportion of subjects with confirmed COVID-19 and at least 1 symptom characteristic of COVID-19 at Visit 3 was 5.76% (23/399), and in the placebo group it was 11.03% (44/399). The difference in proportions between the RNA double-stranded sodium salt drug and placebo groups was 0.0526 (5.26%), the 95%CI [0.0123; 0.0937]). The comparison of the frequency of subjects with confirmed COVID-19 and at least 1 symptom of COVID-19 at Visit 3 (Day 10-11) between the study drug and placebo groups showed statistically significant differences between study groups (*p*=0.0074).

Despite a close contact with a patient with a confirmed novel coronavirus infection, a single dose of the RNA sodium double-stranded study drug prevented the infection and symptoms in more than 94% of the subjects during 11 days of follow-up. As a result of a comparative analysis, it was shown that the infection frequency in the group of the study drug was statistically significantly (almost twice) less than in the comparison

group, which indicates a high efficiency and expediency of using the RNA double-stranded sodium salt drug as a means of the post-exposure COVID-19 prophylaxis.

Additionally, a primary endpoint analysis was performed taking into account the factor of vaccination.

A total of 575 subjects vaccinated against the novel coronavirus infection participated in the study: 284 vaccinated subjects in the study drug group, 291 subjects in the placebo group. In the RNA double-stranded sodium salt drug group, the proportion of vaccinated subjects with confirmed COVID-19 and at least 1 symptom characteristic of COVID-19 at Visit 3 was 5.28% (15/284), and in the placebo group it was 10.65% (31/291). The analysis revealed statistically significant differences between the studied groups (p=0.017).

Thus, the effectiveness and expediency of using the RNA studied double-stranded sodium salt drug as a means of the post-exposure COVID-19 prophylaxis, regardless of the presence of vaccination, was demonstrated.

Secondary efficacy criteria

The frequency of subjects with confirmed COVID-19 and at least 1 symptom consistent with COVID-19. As a result of a comparative analysis of the frequency of the subjects with confirmed COVID-19 and the presence of at least 1 symptom characteristic of COVID-19, statistically significant differences had been revealed between the study groups by Visit 2 (p=0.0270), by Visit 4 (p=0.0061), by Visit 5 (p=0.0093). A statistically significant decrease in the incidence of COVID-19 infection in the study drug group compared to the placebo group, both in the short term (3-4 days of follow-up) and in the long term (28 days of the follow-up), allow us to make a conclusion about the effectiveness and validity of the studied method of preventing infection with a new coronavirus infection.

Frequency of subjects with confirmed COVID-19 and no symptoms consistent with COVID-19. In the RADAMIN®VIRO group, the frequency of subjects with symptoms of COVID-19 that had appeared before Visit 3 inclusive, was 9.02% (36/399), in the placebo group – 17.04% (68/399). No statistically significant differences between the study groups were found between the frequency of subjects with "confirmed COVID-19" and no symptoms "consistent with COVID-19" at Visits 2 and 3 in the RNA sodium double-stranded drug and placebo groups.

The frequency of subjects with confirmed COVID-19 with and without symptoms consistent with COVID-19. A comparative analysis of the frequency of confirmed COVID-19 subjects with and without COVID-19 symptoms in the RNA sodium double-stranded drug and placebo groups had shown statistically significant differences between the study groups by Visit 3 (p=0.0127), by Visit 4 (p=0.0139), by Visit 5 (p=0.0203). Thus, it was shown that the subjects who had received the study RNA sodium double-stranded drug as a means of the post-exposure COVID-19 prophylaxis, were not only significantly less likely to test positive for COVID-19, but also showed fewer symptoms of the disease, which indicates a decrease in its severity course. This may be associated with the development of an adequate immune response against the background of the use of the studied drug.

Time till COVID-19 infection. As a result of the analysis, it was shown that by day 11 (Visit 3) in the RNA sodium double-stranded drug group, the infection occurred in 6.27% (25/399) of the subjects, and in the placebo group – in 11.25% (45/400). In addition, among all infected subjects in the study drug group, 75% were infected before day 7, while in the placebo group it was before day 5. Thus, the subjects treated with placebo were shown to become infected earlier than the subjects treated with the RNA sodium double-stranded drug. The median time till the exposure to COVID-19 in the study drug group and placebo group was 3 days. A comparative analysis revealed statistically significant differences in time to the COVID-19 infection between the RNA sodium double-stranded drug and placebo groups (p=0.0249). As a result of the analysis, it was shown that when using the study drug, there is a delay in the infection of subjects undergoing the COVID-19 prophylaxis, which may be important in terms of reducing the level of the viral load at the time of infection and reducing the risk of developing a complicated course of the disease.

Assessment of symptoms severity characteristic of COVID-19. The assessment was performed only for the subjects who had developed symptoms of COVID-19 during the study up to and including Visit 3. In the RNA sodium double-stranded drug group, the frequency of subjects with symptoms of COVID-19 that had appeared up to and including Visit 3, was 9.02% (36/399), in the placebo group it was 17.04% (68/399). A comparative analysis of the frequency of subjects with COVID-19 symptoms prior to and including Visit 3, regardless of the presence of laboratory-confirmed COVID-19, showed statistically significant differences between the RNA sodium double-stranded drug group and the placebo group (p=0.0008), which indicates a high efficiency of the study drug in preventing the infection with a novel coronavirus infection and reducing the severity of the disease. In the population, there were statistically significant differences between the study drug group and the placebo group in the frequency of the subjects with the COVID-19 symptoms onset up to and including Visit 3 (p=0.0006); by the frequency of the subjects with varying degrees of severity of the Sore Throat symptom at Visit 3 (p=0.0314); by the frequency of the subjects with different severity of the “Fatigue” symptom by Visit 3 (p=0.0472) by the frequency of the subjects with different severity of the “Chills” symptom by Visit 2 (p=0.0191) (Table 4). In terms of symptoms (a nasal congestion and a runny nose,

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shortness of breath or shortness of breath during the exertion, cough, pains in the muscles and throughout the body, a headache, a fever (body temperature >38°C), a sense of smell in the last 24 h), there were no statistically significant differences between the groups. At the same time, none of the groups showed symptoms such as: vomiting, diarrhea, changes in the taste sensitivity.

Duration of symptoms characteristic of COVID-19. As a result of the comparative analysis, statistically significant differences between the groups were revealed in the duration of symptoms characteristic of COVID-19, namely "Sore throat" (p=0.0173) and the symptom "Smell in the last 24 h» (p=0.0214). There were no statistically significant differences between the groups in the duration of the following symptoms: nasal congestion or runny nose, shortness of breath or shortness of breath with an exertion, cough, fatigue, muscle or whole-body pains, a headache, chills, a fever (body temperature >38°C).

Assessing COVID-19 severity. The assessment was performed only for the subjects who had tested positive for COVID-19 during the course of the study. There were no significant differences in the severity of COVID-19 between the study groups (p ≥0.05).

Frequency of subjects requiring hospitalization due to COVID-19 development. There were no cases of hospitalization of the subjects due to the development of COVID-19 during the study.

Safety Assessment Results

The frequency of subjects with reported cases of AEs was 5.13% (41/800). A total of 61 AEs were noted in 41 subjects. The frequency of the subjects in the RNA sodium double-stranded drug group with reported AEs was 4.0% (16/400). A total of 19 AEs were observed in 16 subjects of the RNA sodium double-stranded drug study group. The incidence of the subjects in the placebo group with reported AEs was 6.25% (25/400). A total of 42 AEs were observed in 25 subjects in the placebo group. All reported AEs in the subjects in the study drug and placebo groups were of a mild severity (Table 5).

According to the investigators, the causal relationship with the study drug therapy was assessed as "not related" in 26.32% (5/19) of cases, as "doubtful" in 73.68% (14/19) of cases; a causal relationship with placebo was assessed as "not related" in 52.38% (22/42) of cases, as "doubtful" in 28.57% (12/42) of cases, as "probable" in 7.14% (3/42) of cases, as "possible" – in 11.90% (5/42) of cases.

An analysis of the frequency of AE outcomes in the subjects treated with the study RNA sodium double-stranded drug showed that "a recovery without consequences" was noted in 94.74% (18/19) of cases and "an improvement" in 5.26% (1/19) cases; the subjects treated with placebo in all cases had "a recovery without consequences".

An analysis of the interventions for AEs frequency in the subjects who received the study drug of the RNA sodium double-stranded drug showed that "no treatment was carried out" in 52.63% (10/19) of cases, "local therapy" was required in 15.79% (3/19) cases and "systemic therapy" was required in 31.58% (6/19) of cases; in the subjects receiving placebo, "not treated" – in 59.52% (25/42) of cases, "topical therapy" was required in 21.43% (9/42) of cases, and "systemic therapy" was required in 19, 05% (8/42) of cases.

There were no statistically significant differences between the study groups in terms of the presence of AEs (p ≥0.05). As a result of the analysis, statistically significant differences were found between the treatment groups in terms of the association of AEs with the drug (p=0.0078), with a predominance of drug-related AEs in the placebo group.

It should be noted that against the background of pharmacotherapy with the study drug, there were no relapses of chronic, as well as previously transferred diseases. In some articles, the COVID-19 infection has been associated with the coinfection or reactivation of human herpesviruses [24, 25]. Thus, it is known that the COVID-19 infection can cause reactivation of the latent human herpes simplex viruses, including urogenital, by enhancing the expression of lytic genes and supporting the antegrade movement of the activated viruses to the epithelial tissues [24]. At the same time, in the course of this study, there were no cases of relapse in the patients with a history of urogenital herpes.

In the course of the study, no adverse events associated with the use of the RNA sodium double-stranded study drug were registered.

In the course of the study, there was not a single confirmed case of pyrogenicity (increased body temperature) occurring with the use of this group of drugs. This effect is associated with an innovative technology for obtaining an active active substance, in which special attention is paid to the purification of the resulting substance from protein components and impurities formed during the microbiological synthesis of double-stranded RNA [26–28].

No cases of SAEs were reported during the course of the study.

In the course of the study, there were no cases of pregnancy of the subject/sexual partner of the study participant.

Additionally, an analysis was made of possible adverse effects associated with the use of study drugs (Table 6).

The analysis of both groups included AEs with a definite, probable and possible drug association. Thus, no adverse reactions associated with the use of the study drug were identified. The study drug based on the RNA double-stranded sodium salt has a high favorable and predictable safety profile.

CONCLUSION

Features of the immune response during viral infection, in particular, the penetration of SARS-CoV-2, determine both the risk of initiating a disease with clinical manifestations and the severity of the infection, including the complications risk. The results of the placebo-controlled study convincingly prove the effectiveness of RADAMIN®VIRO in preventing diseases with a novel coronavirus infection, regardless of the fact of vaccination, gender, age, and concomitant diseases, including such as an overweight and obesity. At the same time, in case of the COVID-19 infection, the symptoms of the disease developed less frequently than in the placebo group patients. A decrease in the frequency of development, duration and severity of the symptom complex characteristic of COVID-19, indicates a high efficiency of the preventive effect, a decrease in the risk of a complicated course of the disease, an acceleration of recovery, and a positive effect of RADAMIN®VIRO on the quality of patients' lives. Thus, it is appropriate to include the studied drug in the schemes for the prevention of a novel coronavirus infection used in medical institutions or at enterprises when cases of the disease are detected and there is a high risk of its mass prevalence.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Larisa A. Balykova – research design development and implementation, text writing and editing; Olga A. Radaeva – study design development, results analysis, text editing; Kira Ya. Zaslavskaya – research design development, text editing, literary sources analysis; Alexey V. Taganov – study design implementation, data processing; Petr A. Bely – study design development, results analysis, text editing; Konstantin A. Zakharov – research design implementation, data processing; Varvara V. Popova – research design implementation, data processing; Tatyana I. Chudinovskikh – research design implementation, data processing; Svetlana V. Teplykh – research design implementation, data processing; Igor V. Balaban – research design implementation, data processing;, Roman S. Kozlov – research design implementation, data processing; Natalya V. Kirichenko – research design implementation, data processing; Elena N. Simakina – study design implementation, data processing; Ksenia N. Koryanova – collection of sources, data processing, article writing; Dmitry Yu. Pushkar – research design development, research design implementation, data processing.

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Synthesis of 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone with analgesic activity

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Quinazolin-4(3H)-one derivatives are characterized by a wide range of pharmacological properties, among which the most significant one is a pronounced effect on the central nervous system. In this regard, a molecular design of biologically active compounds that have an analgesic activity due to the formation of ligand-receptor complexes with nociceptive and dopamine receptors, has been performed.

The aim of the study was a molecular design and a subsequent targeted synthesis of 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone with an analgesic activity, as well as the creation of a mathematical model in order to identify significant molecular descriptors.

Materials and methods. A molecular design was carried out by a logical-structural approach using the PASS program with the identification of the biological activity of the predicted structures, as well as the energy calculation of the ligand-receptor interaction. The synthesis of 2-phenyl derivatives of 4(3H)-quinazolinone was carried out by the reaction of 2-aminobenzamide with aromatic aldehydes in polyphosphoric acid when heated, while the 2-benzyl derivatives were synthesized by fusing amides of anthranilic and homoveratric acids followed by sulfonation with sulfuric acid. The analgesic activity of the synthesized compounds was studied in the models of nociceptive reactions induced by chemical stimuli (a formalin test and “acetic acid writhings”).

Results. A molecular design made it possible to identify promising structures in the series of 4(3H)-quinazolinone derivatives that affect nociceptive and dopamine receptors and have an analgesic activity. A modification was made to the synthesis of 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone in order to increase the yield of the target products by a simpler and more cost-effective method. The predicted compounds were synthesized by cyclocondensation of anthranilic acid amide with aromatic aldehydes or with homoveraic acid amide. It follows from the primary pharmacological studies results that the synthesized substances are promising from the point of view of creating painkillers based on them. A structure-activity relationship between the molecular descriptors, which are largely responsible for the analgesic activity, and the results of biological tests, has been revealed.

Conclusion. The use of computer modelling made it possible to identify the amino acid residues involved in the formation of the ligand-receptor complex with the nociceptive receptor, and to construct a mathematical model to explain the analgesic activity of 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone. Modified procedures for the synthesis of target compounds have been proposed. The obtained coefficients of the approximation between the theoretical values and the data of the pharmacological experiment make it possible to state a sufficient reliability of the carried out studies.

Keywords: molecular design; quinazolin-4(3H)-one derivatives; dopaminergic compounds; nociceptive receptors; analgesic activity; cyclocondensation; anthranilamide; aromatic aldehydes; homoveratric acid amide; molecular descriptors

Abbreviations: CNS – central nervous system; BACs – biologically active compounds; PPA – polyphosphoric acid; LUMO – lowest unoccupied molecular orbital; DMSO – dimethyl sulfoxide.

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Синтез 2-фенил- и 2-бензилпроизводных хиназолин-4(3*H*)-она, обладающих анальгезирующей активностью

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Известно, что производные хиназолин-4(3*H*)-она обладают широким спектром фармакологических свойств, среди которых наиболее значимым является выраженное влияние на центральную нервную систему. В связи с этим нами выполнено молекулярное конструирование биологически активных соединений, обладающих анальгезирующей активностью за счет образования лиганд-рецепторных комплексов с ноцицептивными и дофаминовыми рецепторами.

Цель. Молекулярное конструирование и последующий целенаправленный синтез 2-фенил- и 2-бензилпроизводных хиназолин-4(3*H*)-она, обладающих анальгезирующей активностью, а также создание математической модели с целью выявления значимых молекулярных дескрипторов.

Материалы и методы. Молекулярное конструирование проводилось с помощью логико-структурного подхода посредством программы PASS с выявлением биологической активности прогнозируемых структур, а также расчетом энергии лиганд-рецепторного взаимодействия. Синтез 2-фенилпроизводных хиназолин-4(3*H*)-она осуществляли взаимодействием 2-аминобензамида с ароматическими альдегидами в полифосфорной кислоте при нагревании, а 2-бензилпроизводных – сплавлением амидов антраниловой и гомовератровой кислот с последующим сульфированием серной кислотой. Анальгезирующую активность синтезированных соединений изучали на моделях ноцицептивных реакций, вызванных химическими стимулами (формалиновый тест и «уксусные корчи»).

Результаты. Молекулярное конструирование позволило выявить перспективные структуры в ряду производных хиназолин-4(3*H*)-она, влияющие на ноцицептивные и дофаминовые рецепторы и обладающие анальгезирующей активностью. Осуществлена модификация синтеза 2-фенил- и 2-бензилпроизводных хиназолин-4(3*H*)-она с целью повышения выхода целевых продуктов посредством более простого и экономически выгодного способа. Прогнозируемые соединения синтезированы циклоконденсацией амида антраниловой кислоты с ароматическими альдегидами или с амидом гомовератровой кислоты. Из результатов первичных фармакологических исследований следует, что синтезированные вещества перспективны с точки зрения создания на их основе обезболивающих средств. Выявлена взаимосвязь структура-активность между молекулярными дескрипторами, в значительной степени отвечающими за анальгезирующую активность, и результатами биологических тестов.

Заключение. Использование компьютерного моделирования позволило выявить аминокислотные остатки, участвующие в образовании лиганд-рецепторного комплекса с ноцицептивным рецептором и построить математическую модель, позволяющую объяснить обезболивающую активность 2-фенил- и 2-бензилпроизводных хиназолин-4(3*H*)-она. Предлагаются модифицированные методики синтеза целевых соединений. Полученные коэффициенты аппроксимации между теоретическими значениями и данными фармакологического эксперимента позволяют констатировать достаточную достоверность проведенных исследований.

Ключевые слова: молекулярное конструирование; производные хиназолин-4(3*H*)-она; дофаминергические соединения; ноцицептивные рецепторы; анальгезирующая активность; реакция циклоконденсации; антраниламид; ароматические альдегиды; амид гомовератровой кислоты; молекулярные дескрипторы

Список сокращений: ЦНС – центральная нервная система; БАС – биологически активные соединения; ПФК – полифосфорная кислота; НСМО – низшая свободная молекулярная орбиталь; ДМСО – диметилсульфоксид

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INTRODUCTION

The targeted search for new highly effective and safe drug substances that affect the CNS is an urgent task of pharmacy [1-3]. The empirical approach used in the synthetic preparation of new compounds with a biological activity is insufficiently productive and efficient [4–7]. It is possible to significantly increase the effectiveness of the search for biologically active compounds (BACs) with the molecular modeling aimed at finding structures with the targeted pharmacological action [8–11]. When designing substances that affect the CNS, special attention is paid to the prediction of ligand-receptor interactions, which makes possible not only to purposefully synthesize new pharmacologically active compounds, but also to effectively plan a pharmacological experiment.

The neurotropic effect of a number of 4(3H)-quinazolinone derivatives has been shown, and the search for BACs substances that exhibit analgesic an activity in this group, is of considerable interest [12, 13]. The modified synthesis procedure given in the article, makes possible to expand the boundaries of the preparative possibilities for obtaining 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone, which leads to the synthesis of the target products with desired pharmacological properties. The introduction of substituents in position 2 of the pyrimidine ring containing hydroxy and methoxy groups, leads to the modeling and completion of additional cycles in the creation of a significant series of polyheterocyclic and coordination compounds. The revealed structure-activity relationships and molecular descriptors responsible for the effect on the central nervous system will further make possible a more efficient search for BACs with an analgesic action containing classical pharmacophore fragments inherent in anesthetics.

The choice of hydroxy and methoxy substituents in the 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone synthesized in the phenyl and benzyl fragments is due to their similarity in electronic effects, which made it possible to reveal a correlation between the values obtained in the course of the molecular design and the results of the pharmacological experiment. In addition, since the hydroxy and methoxy substituents are electron-donating, this will make it possible to vary the structure of the resulting quinazolinones over a fairly wide range due to the electrophilic substitution in the phenyl and benzyl fragments in position 2, including, with a subsequent heterocyclization, to one of the nitrogen atoms [14–17]. It is noteworthy that in one of the latest works, in which the prospects of using a number of nitrogen-containing heterocycles were evaluated *in silico*, 2-(4-methoxyphenyl)-3-amino(3,4,5-trihydroxybenzylidene)-4(3H)-quinazolinone was identified as the leader having just such substituents [18].

The unrelenting interest in quinazolinone derivatives is caused by a wide range of their biological activity, which began with a pharmacognostic study of the plant extracts containing alkaloids, the structural basis of which is the core of this heterocycle. One of the first alkaloids of this kind, which was isolated in a pure form from the extract of *Glycosmis arborea* (Roxb.) DC. and other plants of this genus [19], is arborine, which is 2-benzylquinazolinon.

The possibility of using quinaline and quinazoline alkaloids in the fight against COVID-19 is being intensively studied [20], including the study on the basis of a theoretical structure-activity study.

In recent years, interest in quinazolinone derivatives has been significantly stimulated by the search for and study of their luminescent properties [21]. A new trend in this area is the synthesis of ligand systems and the preparation of multifunctional coordination compounds based on them, which have a number of practically significant properties [22, 23].

THE AIM of the study was a molecular design and a subsequent targeted synthesis of 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone with an analgesic activity, as well as the creation of a mathematical model in order to identify significant molecular descriptors.

MATERIALS AND METHODS

Prognostic part

A preliminary prediction of a biological activity was carried out using the web service of the PASS program (Prediction of Activity Spectra for Substances, Russia; Protected Online PASS Application) [24].

Geometry optimization, calculation of the enthalpy and the lowest unoccupied molecular orbital (LUMO) of the structures under study were carried out by semi-empirical AM1 and Monte Carlo methods and using the HyperChem 6.0.9 program (in free access). To model the ligand-receptor interaction with dopamine and nociceptive receptors using the molecular docking method, the Molegro Virtual Docker 6.0.1 program (a demo version) was used, the calculations of which are based on the molecular calculation algorithm – MolDockScore. This program was used to simulate the 50 most stable conformations of the studied substances in the active center of the dopamine and nociceptive receptors. The results obtained were optimized in accordance with the published experimental data of the

X-ray diffraction analysis of the protein-ligand complex [25]. To study the energy components of the spatial-conformational interactions of 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone with the dopamine receptor binding site, the 3D structure of the protein-ligand complex with the identification code 5WIU and the nociceptive receptor 4EA3 was used. These protein-ligand complexes are presented in the RCSB Protein Data Bank.

Synthetic part

General procedure for compounds III–VII synthesis. A mixture solution consisting of 0.01 mol of 2-aminobenzamide (**I**) and 0.01 mol of the corresponding aromatic aldehyde (**II**) in 50 g of polyphosphoric acid was heated for 30 minat the temperature of 80–90°C. The precipitation formed after the reaction mixture hydrolysis, was filtered off and recrystallized from glacial acetic acid. The yields of the reaction products were 75–82%.

Synthesis of 2-(3,4-dimethoxybenzyl)-4(3H)quinazolinone VIII. The mixture melt – 19.5 g (0.1 mol) of homoveratric (3,4-dimethoxyphenylacetic) acid amide and 15.2 g (0.11 mol) of 2-aminobenzamide (**I**) – was heated in an open vessel at 110–120°C until the release of the water vapor (~40–60 min). The melt was cooled to 90–70°C and diluted with 100 ml of acetic acid heated to the same temperature. The crystalline precipitate formed after cooling was filtered off, washed twice with cold isopropyl alcohol, and dried at room temperature. The yield was 79-82%, melting point (m.p.) was 225–226°C (colorless crystals).

^{1}H NMR spectrum (300 MHz), δ, ppm, DMSO-d6: 3.70 (s, 3H, OCH3), 3.74 (s, 3H, OCH3), 3.00 (s, 2H, CH2), 6.91 (d, 1H, Ar), 6.93 (d, 1H, Ar), 7.07 (s, 1H, Ar), 7.58 (t, 1H, Ar), 7.67 (d, 1H, Ar), 7.88 (t, 1H, Ar), 8.11 (d, 1H, Ar).

Synthesis of 6-sulfo-2-(3,4-dimethoxybenzyl)-4(3H)-quinazolinone IX. At room temperature and stirring, 3.0 g (0.01 mol) of 2-(3,4-dimethoxybenzyl)quinazolin-4(3H)-one (**VIII**) was dissolved in 15 ml of concentrated sulfuric acid. The homogeneous reaction mixture was kept for 10-12 h at room temperature and introduced into 150 g of an ice water mixture. The colorless precipitate formed was filtered off and washed thoroughly with water and isopropyl alcohol. The yield was 80%, m.p. >300°C.

^{1}H NMR (600 MHz), δ, ppm, DMSO-d6: 3.55 (s, 6H, 2OCH3), 4.70 (s, 2H, CH2), 6.94 (s, 1H, Ar) , 7.38 (s, 1H, Ar), 7.20–7.55 (d+t, 2H, Ar), 7.83 (t, 1H, Ar), 8.25 (d, 2H, Ar).

The air-dried product was used further without any further purification.

In vivo studies

Study animals

A primary pharmacological screening was performed on adult white Wistar female rats weighing 200–220 g (178 animals, 9–10 animals in a randomly formed group). The animals were kept with a free access to water and food under standard vivarium conditions. The requirement for the care of animals was accomplished in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

The experiments were performed in accordance with the standards of the Russian Federation legislation (GOST R 33044-2014, GOST R 33647-2015), the technical standards of Good Laboratory Practice of the Eurasian Economic Union (ICH GCP). The studies were carried out in compliance with the bioethical standards, approved by the local ethics committee of Stavropol State Medical University (Protocol No. 89 dated March 18, 2020).

Study of specific activity

The assessment of the peripheral level of the pain sensitivity and, consequently, the magnitude of the analgesic activity of the synthesized compounds was determined using models of nociceptive reactions[1] induced by chemical stimuli (a formalin test and "acetic acid writhings") [26].

A formalin test was used to assess the somatic pain. In this test, hyperalgesia was modeled by a subplantar injection – 50 µl of a 2% formalin aqueous solution (CJSC Base No. 1 Himreaktivov, Russia) with an insulin syringe (Elets MPK, Russia) into the dorsal surface of the right hind paw. The phases of the nociceptive response were recorded by a number of pain reactions ("flinches": raising the paw, licking, biting the injection site) from the moment of the formalin administration and throughout the entire observation time – 60 min. The magnitude of the analgesic activity of the studied substances was assessed in total, as well as separately for phases I (the first 10 min) and II (10–60 min) of the nociceptive response to reduce the number of pain reactions in comparison with the indicators in control animals taken as 100%.

The "acetic acid writhings" test was used to evaluate the peritovisceral pain caused by algogens. This test is a model of visceral nociception and is used to study the peripheral analgesic activity of new substances through the chemical stimulation method of peritoneal nociceptors and the corresponding motor responses[2].

[1] Voronina TA, Guzevatykh LS. Metodicheskiye rekomendatsii po izucheniyu anal'geticheskoy aktivnosti lekarstvennykh sredstv [Methodical recommendations for the study of the analgesic activity of drugs]. Guidelines for conducting preclinical studies of drugs. Mironov A.N. editor; Moscow: Grif and K, 2012. – P. 197–218. Russian

[2] Ibid.

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This pain reaction was induced by an intraperitoneal injection of a 1% acetic acid (Laverna, Russia). An analgesic activity was assessed by a decrease in the number of "writhings" ("writhings" are contractions of the abdominal muscles, alternating with their relaxation, stretching of the hind limbs, arching of the back, resembling pain in peritonitis) for a 15-minute observation period after the administration of an acetic acid solution in the % relative to the indicators of the control group of animals, taken as 100%.

The compounds under study (lab codes: III, IV, V, VI, VII, VIII, IX) investigated at the dose of 2/10 of the molecular weight in mg/kg (III – 47.6 mg/kg; IV – 53.6 mg/kg; V – 53.6 mg/kg; VI – 53.6 mg/kg; VII – 56.4 mg/kg; VIII – 59.2 mg/kg; IX – 75.2 mg/kg).

Lidocaine (the injection solution of 20 mg/ml, 2 ml, Dalchimpharm, Russia) at the dose of 1 mg/kg was used as a reference drug. All compounds of the studied series, the reference drug, the saline solution (OJSC "Borisov Plant of Medical Preparations", Belarus) were administered to the control animals (0.4 ml) intraperitoneally once 40 min before the administration of chemical stimuli. The doses of the studied substances and the reference drug were selected taking into account the literature data [27, 28] and the dose titration method.

Statistical processing of results

A statistical analysis of the results was carried out using the following software packages: "Microsoft Excel 2010" (Microsoft Office, USA), "Statistica 10" (Statsoft, USA), "BIOSTAT" (Glantz, McGraw Hill, USA). The normality of the obtained data distribution was determined using the Shapiro-Wilk test. When comparing independent normally distributed data, a one-way analysis of variance with the Dunnett's test (a multiple comparison with the control group) was used. When the distribution of study data was different from the normal, the Kruskal-Wallis test the post hoc Dunn's test was used. The differences were considered statistically significant at $p < 0.05$.

Predictive experiment *in silico*

Molecular descriptors were calculated using the T.E.S.T. (Toxicity Estimation Software Tool. EPA, USA – An official website of the United States government), which makes it possible to obtain information about 794 descriptors associated with the 2D compounds structure. A correlation analysis was carried out between dependent variables representing biological activity data and independent variables, including the energy of interaction with amino acid residues, energy values (Total Energy), as well as molecular descriptors. In constructing the mathematical model, methods of a linear regression and the least squares regression, as well as a sliding control over individual objects (leave-one-outcross-validation), were used.

RESULTS

Based on the logical-structural approach, a group of virtual quinazolin-4(3H)-one derivatives was formed. At the first stage of a computer modeling of the structures, the biological activity was predicted using the PASS program, the results of which for the most promising compounds are shown in Table 1.

Table 1 shows that almost all hydroxyphenyl derivatives isolated from the total array can be characterized by an effect on the central nervous system, have broncholytic, antiulcer and anti-ischemic effects. In addition to the structure of 2-dimethoxyphenyl substituted VII, the structures containing hydroxymethoxyphenyl residues (IV, V, VI) and a dimethoxybenzyl fragment, can affect the release of dopamine. All the predicted compounds, except substance VII, can have an antiviral activity and cardioprotective effects. The introduction of the 2-dimethoxybenzyl fragment of the sulfo group into the aromatic nucleus can lead to an increase in the antiulcer activity and the loss of a stimulating effect on the release of neurotransmitters – dopamine and serotonin.

The results of the molecular docking in a series of 4(3H)-quinazolinone derivatives with nociceptive and dopamine receptors are presented in Table 2.

The values of the minimum and average energies of interaction with nociceptive and dopamine receptors for 2-phenyl and 2-benzyl derivatives of 4(3H)-quinazolinone suggest that compound IX should exhibit the greatest dopaminergic activity, since its ligand-receptor complex with the dopamine receptor is characterized by the greatest sustainability. This compound appears to be superior to other predicted 2-phenyl derivatives, and compound VIII forms less stable ligand-receptor complexes than compound IX.

For the synthesis of 2-phenyl derivatives III–VII, a modified procedure was developed that does not require the use of an oxidizing agent.

Syntheses of 2-phenyl-substituted derivatives of 4(3H)-quinazolinone , realized by the cyclocondensation of anthranilic acid azomethines, which are easily formed by the interaction of aldehydes and its N-acylated amides, are described. However, the cyclization process itself proceeds rather difficultly in the presence of strong oxidizing agents (diacetoxyiodo)benzene or potassium permanganate [29, 30].

The proposed optimized procedure for the synthesis of hydroxy- and methoxyphenyl derivatives of 4(3H)-quinazolinone is based on the interaction of equimolar amounts of anthranilic acid amide and the

corresponding aromatic aldehydes in a polyphosphoric acid (PPA) medium. The desired effect is achieved due to the fact that, unlike aromatic carboxylic acids, their corresponding aldehydes are highly soluble in PPA.

The scheme for obtaining target compounds (III–VII) is shown below (Fig. 1).

In order to increase the yield of target 2-phenyl derivatives of 4(3*H*)-quinazolinone, a synthesis method using polyphosphoric acid is used. This method is simpler and makes it possible to obtain target products with the yields varying from 79 to 82%.

The synthesis of 2-benzyl derivatives of 4(3H)-quinazolinone was carried out by fusing amides of anthranilic and homoveraic acids and led to the formation of compound VIII, followed by sulfonation with sulfuric acid and the formation of compound IX (Fig. 2).

To confirm the molecular design reliability, a study of the pharmacological properties of the synthesized substances was carried out. Previously, the anticataleptic effect of the predicted 2-phenyl and 2-benzyl derivatives of 4(3H)-quinazolinone, as well as the effect on the blood coagulation, was experimentally shown [12, 31].

With reference to these studies, to research their analgesic activity was considered appropriate. Under the influence of the studied compounds in the female animals, a statistically significant decrease in the number of pain responses was observed when substance VIII was used throughout all stages of testing with formalin (Table 3).

In the "acetic acid writhings" test, 4(3H)-quinazolinone derivatives III, VIII, and IX significantly limited the frequency of pain responses in the female rats. At the same time, the effect of substance III was most pronounced, and compounds VIII, IX outperformed the reference drug lidocaine.

Based on the data of the pharmacological studies, it follows that compounds III, VIII, IX reduce the frequency of nociceptive responses in the female rats, showing an analgesic effect.

Next, a study of quantitative structure-activity relationships in the series of 2-phenyl and 2-benzyl derivatives of 4(3H)-quinazolinone, which made it possible to identify molecular descriptors characterizing an analgesic activity, was carried out.

The assessment of the results reliability of the molecular design should be confirmed by a correlation analysis. This analysis was carried out between the theoretically calculated formation energies of the ligand-receptor complex and molecular descriptors with the experimentally obtained results of the analgesic activity. The values of pain responses in female rats had been used as experimental values for these calculations.

The approximation coefficient (R^2) between the minimum binding energies of the studied molecules with the nociceptive receptor and the results of the formalin test is 93.9%, and the average values of the formation energies of ligand-receptor complex – 82.3%. The approximation coefficient between the minimum binding energy of the predicted structures with the dopamine receptor and the results of the formalin test are a great deal less, so they are not given. This fact suggests a great interaction contribution of the studied substances with the nociceptive receptor, which manifests itself in the analgesic activity of 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone. The most stable ligand-receptor complexes of the leader compound (VIII) in the binding site with dopamine and nociceptive receptors are shown in Fig. 3.

The approximation coefficient between the minimum binding energy of the studied molecules with the nociceptive receptor and the values of the "acetic acid writhings" test is 75.48%. At the same time, the coefficient of approximation between the binding energies of the investigated molecules with the dopamine receptor and the results of the "acetic acid writhings" test is insignificant, i.e., there is no correlation.

Subsequently, to identify the correlation between quantum-chemical and topological descriptors with the results of the analgesic activity, the HyperChem (energy calculation) and T.E.S.T. (Toxicity Estimation Software Tool) programs were used [32]. (Table 4).

Table 5 shows the energies of the most stable conformations with isolated amino acid residues in the binding site of BACs and the nociceptive receptor.

To select the most significant amino acid residues when constructing a mathematical model in the Molegro Data Modeler program, a correlation matrix was calculated for the results of the tests. The matrix is shown in Table 6 below.

Based on the number of substances in the sample and the data of the formalin test correlation matrix, the following amino acid residues were selected to build a mathematical model: **Gln 280, Ile 219, Met 134, Phe 215,** and for the "acetic acid writhing" test – **Asp 130, Phe 215, Met 134 , Val 202.** In addition to the indicated interaction energies, two independent variables – Total Energy and SdO – turned out to be the most relevant in constructing the predictive model (Table 7).

The proposed predictive model can be used for the molecular design of highly effective and safe BACs, since it is characterized by sufficiently high approximation coefficients and makes it possible to judge the significant reliability of the studies carried out.

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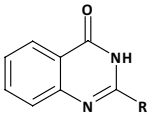
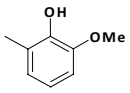
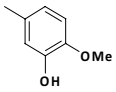
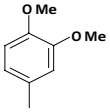
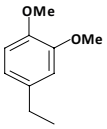
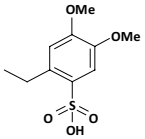
Table 1 – Predicted types of biological activity of hydroxy- and methoxyphenyl, as well as dimethoxybenzyl derivatives of 4(3H)-quinazolinone



R substituent / Biological activities	III 	IV 	V 	VI 	VII 	VIII 	IX 
	Pa, %						
Broncholytic	86.3	81.2	75.1	75.1	80.7	55.7	40.5
Psychotropic (dopamine release stimulant)	39.8	57.4	64.2	64.2	–	60.5	35.9
Psychotropic (stimulator of serotonin release)	54.6	50.3	56.5	56.5	61.2	44.7	–
Antiviral	89.4	84.2	73.4	73.4	–	64.8	52.4
Anti-ischemic	61.2	65.4	70.3	70.3	67.4	59.7	64.8
Histamine inhibitor	54.1	50.3	51.2	51.2	–	50.1	46.5
Neuroprotective	51.6	52.7	56.8	56.8	63.5	57.3	32.7
Antiulcer	47.3	61.4	62.6	62.6	62.1	–	85.7
Cardioprotective	54.6	58.9	65.7	65.7	–	57.6	51.9

Note: Pa (%) characterizes the probability of an pharmacological activity manifestation.

Table 2 – Minimum and average mean energies of interaction with nociceptive and dopamine receptors of 2-phenyl and 2-benzyl derivatives of 4(3H)-quinazolinone

Substances	Minimum energy (nociceptive receptor), kcal/mol	Average energy (nociceptive receptor), kcal/mol	Minimum energy (dopamine receptor), kcal/mol	Average energy (dopamine receptor), kcal/mol
III	-98.443	-75.808	-94.905	-83.205
IV	-99.098	-74.455	-110.399	-93.380
V	-106.250	-78.909	-108.822	-93.935
VI	-99.595	-73.997	-108.96	-92.002
VII	-105.502	-81.574	-106.676	-94.181
VIII	-111.212	-87.347	-110.732	-99.709
IX	-115.508	-91.058	-126.920	-111.839
Lidocaine	-88.152	-68.476	-87.250	-70.356

Table 3 – Influence of 2-phenyl and 2-benzyl derivatives of 4(3H)quinazolinone on parameters of the formalin test and the number of "acetic acid writhings" in female rats (in the % data of the control group)

Substances	Formalin test			Acetic acid writhings
	Whole period	Phase 1 (10 min)	Phase 2 (50 min)	
III	68.0	65.9	69.3	11.7*
IV	71.3	64.5	75.2	45.6
V	111.0	42.9	150.7	63.6
VI	96.7	71.9	111.1	75.6
VII	96.2	71.0	110.8	82.0
VIII	17.0*	29.1*	10.0*	19.5*
IX	43.5	49.2	40.2	32.0*
Lidocaine	50.3	61.4	43.8	39.4*

Note: significant relative to the control group of female rats: * – p <0.05 (Kruskal-Wallis test with Dunn's test).

Table 4 – Total energy of formation calculated by the Monte Carlo method and molecular electrotopological indices for 2-phenyl and 2-benzyl derivatives of 4(3H)-quinazolinone

Substances	Energy, kcal/mol	SdO	SHssNH	Formalin writhings	Acetic writhings
III	-3216.51	11.907	2.0492	68.0	11.7
IV	-3582.71	11.9981	2.0621	71.3	45.6
V	-3582.87	12.0181	2.0631	111.0	63.6
VI	-3836.77	12.0989	2.0662	96.7	75.6
VII	-3574.60	12.0302	2.0866	96.2	82.0
VIII	-4115.67	12.073	2.0158	17.0	19.5
IX	-4471.47	35.7029	2.1112	43.5	32.0
Lidocaine	-3232.35	11.8923	1.8784	50.3	39.4

Table 5 – Interaction energies of 2-phenyl and 2-benzyl 4(3H)-quinazolinone derivatives with amino acid residues of the nociceptive receptor

Substances	Amino acid residues								
	Ala216	**Gln280**	**Ile219**	Leu284	**Met134**	**Phe215**	Phe220	Ser223	**Val202**
III	-0.655	-7.449	-20.062	-9.307	-6.349	-3.098	-9.962	-4.889	0
IV	-13.857	-9.791	-17.865	-1.903	-1.694	-19.934	-3.943	-0.903	-0.3986
V	-11.549	-10.136	-19.453	-8.007	-0.311	-22.050	-9.408	-0.661	-1.7388
VI	-11.974	-5.023	-13.882	-10.253	0	-23.701	-6.637	-0.811	-3.7199
VII	-8.713	-4.146	-19.999	-8.621	0	-22.635	-9.043	-0.581	-3.9376
VIII	-7.605	-22.105	-8.656	-9.932	-12.806	-4.417	-8.089	-2.506	0
IX	-8.843	-13.104	-13.042	-5.753	-6.222	-10.783	-5.317	-1.357	0
Lidocaine	-9.059	-19.582	-14.256	-9.166	-2.763	-3.876	-8.039	-1.298	0

Table 6 – Results of the mathematical model for significant amino acid residues of 2-phenyl and 2-benzyl derivatives of 4(3H)-quinazolinone

Biological activity	Amino acid residues								
	Ala216	**Asp130**	**Gln280**	**Ile219**	**Met134**	Phe135	**Phe215**	Ser223	**Val202**
Formalin writhings	0.099	0.421	0.670	0.570	0.780	0.039	0.648	0.162	0.553
Acetic acid writhings	0.390	0.583	0.356	0.115	0.682	0.453	0.801	0.633	0.834

Table 7 – Results of building a predictive model of analgesic activity for 2-phenyl and 2-benzyl derivatives of 4(3H)-quinazolinone

Biological activity	R^2	R^2 for sliding control	Model
Formalin test	0.915	0.877	Activity=0.00398328×Total Energy–0.271986×SdO+1.24195×Gln280–1.58577×Ile219+2.06023×Met134–1.04422×Phe215+70.3678
"Acetic acid writhings" test	0.954	0.886	Activity=-0.00667588×Total Energy–0.158964×SdO+1.72064×Asp130+1.14011×Met134–0.852054×Phe215–5.75385×Val202+12.5588

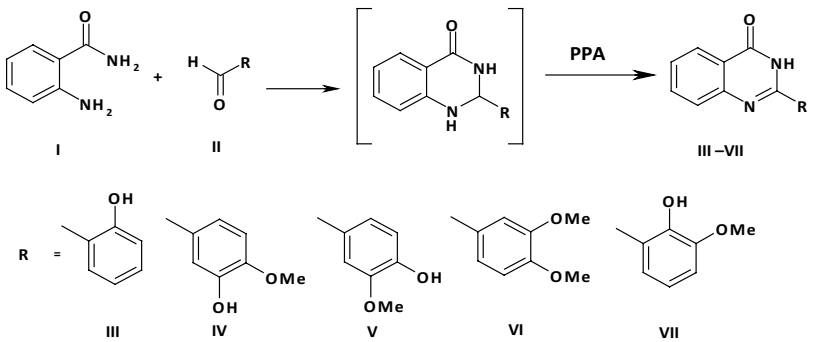


Figure 1 – Scheme for synthesis of 2-phenyl derivatives of 4(3H)-quinazolinone III–VII
Note: PPA – polyphosphoric acid.

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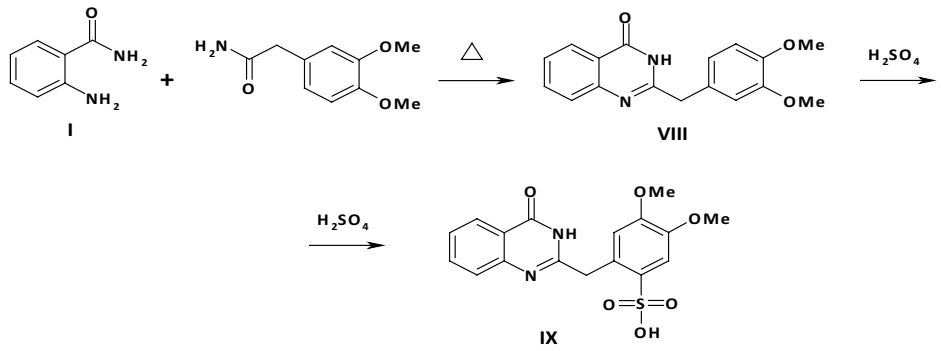
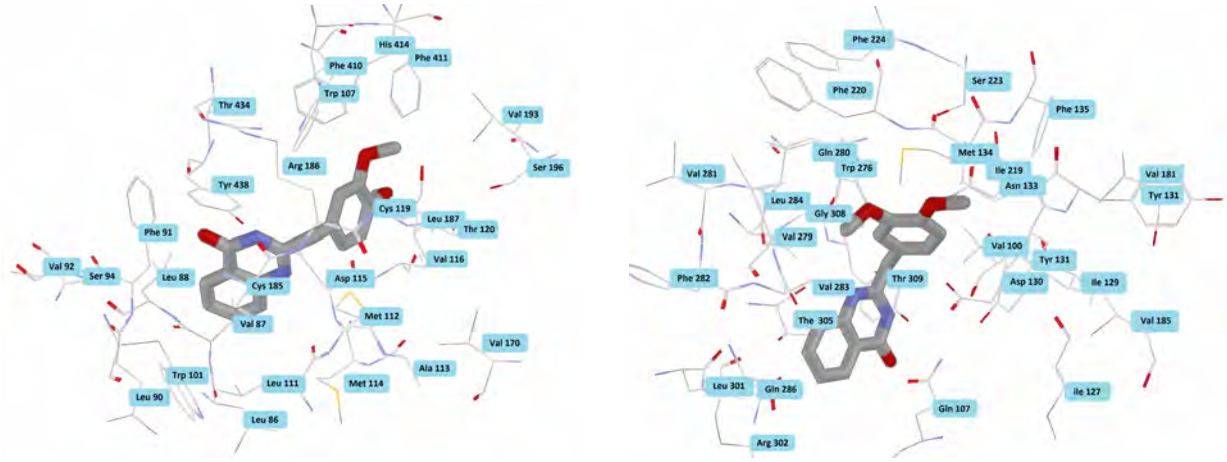


Figure 2 – Scheme for the synthesis of 2-benzyl derivatives of 4(3H)-quinazolinone VIII and IX



A B

Figure 3 – Ligand-receptor complexes of the leader compound (VIII), which have the most stable, with dopamine (A) and nociceptive (B) receptors

DISCUSSION

A neuroprotective activity can manifest itself in a stimulating effect on neurotransmitter systems, which became the basis for a molecular docking of the predicted substances with nociceptive and dopamine receptors.

Primarily, formalin exerts its pharmacological action through the activation of channels of the variable receptor potential of ankyrin 1, TRPA1, which normally respond to cold and stimulate the development of inflammation [33–35]. According to the experimental studies, two phases can be distinguished in the formalin test mechanism. The drugs related to local anesthetics, affect the first phase of the nociceptive response, while non-steroidal anti-inflammatory drugs suppress mainly the second phase of the formalin test [26, 36–38].

The studied compounds presumably have a dopaminergic activity, which is expressed in the anticataleptic effect revealed for 2-phenyl and 2-benzyl derivatives of 4(3H)-quinazolinone in previous studies [12]. Two groups of dopamine receptors are known: the D_1-type group (D_1 and D_5) and the D_2-type group (D_2, D_3 and D_4), which are opposite in their mechanism of action and affect cellular processes in different ways. The dopamine receptors are associated with G proteins. A group of D_1-type receptors has an activating effect on the adenylate cyclase, while D_2-type receptors inhibit its activity, which leads to a decrease in the concentration of cAMP in the cells and the activation of potassium channels [39, 40].

In relation to the nociceptive receptor, all the predicted structures are characterized by a higher binding energy with the receptor than the reference drug, lidocaine. Comparing the results of the molecular docking of 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone, an assumption about the pronounced analgesic activity of the latter can be made. The values of the minimum and average energies of the ligand-receptor complex with the nociceptive receptor formation, are comparable for structures VIII and IX.

The modification of preparative studies consists in carrying out the reaction in a polyphosphoric acid medium, which allows the resulting intermediate dihydroquinazolinone to be converted into the target 4(3H)-quinazolinone heterocycle without an additional oxidation step. We have shown that

When the reaction is carried out in the PPA medium, an additional oxidation process is eliminated. The fact

that the reaction yields exceed 50% at an equimolar ratio of reactants makes it possible to exclude the possibility of the reaction proceeding through a disproportionation. Perhaps the role of the oxidizing agent is performed by atmospheric oxygen, or pentavalent phosphorus PPA.

An important requirement for drug candidates is their water solubility, the increase of which consists, among other things, in the introduction of highly polar groups into the molecule. On this basis, to increase the solubility of the substance in water, the structures that form internal salts, were obtained. The simplest way to achieve this goal is to sulfonate the activated aromatic ring of the nitrogen-containing heterocycle, the heterocyclic fragment of which plays the role of a proton acceptor. Quinazolinones containing two nitrogen atoms seem to be a good model in this respect.

From the data in Table 3 show that the analgesic activity is observed in 2-phenyl and 2-benzyl derivatives of 4(3H)-quinazolinone in the formalin test and in the "acetic acid writhing" test. It should be noted that compared with the activity of other studied compounds and the reference drug lidocaine, the analgesic effect of substance VIII was stable and expressed in the both test methods used. In the "acetic acid writhing" test, the greatest analgesic effect is noted for substance III, and here, the effect is more pronounced than that of lidocaine.

Since compound VIII is active in the first phase of the formalin test, it can be assumed that it exhibits an antineoceptive action, exerting a local anesthetic effect. Compound VIII also blocks the second phase of the test in animals, which suggests a combination of a local anesthetic and anti-inflammatory activity in the mechanism of the analgesic action of this substance. It is possible that the analgesic activity of the studied compound is realized by acting on C-polymodal nociceptors that are sensitive to chemical stimuli, in particular, to formalin.

A correlation analysis of the quantum-chemical parameters of the structures and the results of the pharmacological tests showed that the highest approximation coefficient is observed for 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone and the reference drug lidocaine when used in the calculations of the total energy, and the results of the formalin test (96.08%). However, for the "acetic acid writhing" test, the coefficient is much lower (69.58%). Accordingly, these data make it possible to reveal the differences between the formalin and acetic acid writhing tests for assessing the analgesic activity and the prospects for their use in predicting the analgesic activity for 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone.

CONCLUSION

By means of the molecular design, a targeted synthesis of 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone with an analgesic activity was carried out. The results of correlation studies made it possible to identify molecular descriptors and create a predictive model for the search for new analgesic compounds in the series of 4(3H)-quinazolinone derivatives. The reliability of the molecular design of virtual molecules with the given pharmacological properties has been experimentally proven to a certain extent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Ivan P. Kodonidi –research idea and molecular design methodology of biologically active substances and QSAR calculations of molecular descriptors; Alexander V. Bicherov – synthesis methods development of 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone; Eleonora A. Manvelyan – working out a pharmacological research strategy, results interpretation; editing and approval of manuscript pharmacological part; Aleksandra A. Kolodina – NMR spectra interpretation, 4(3H)-quinazolinone derivatives purification; Aleksandr A. Bicherov – 2-phenyl- and 2-benzyl derivatives of 4(3H)-quinazolinone synthesis; Mikael M. Manvelyan – pharmacological studies conducting; statistical analysis results and interpretation, pharmacological part of the manuscript writing; Aleksandr V. Ivchenko – analysis of the relationship structure-analgesic activity; Natalia N. Vdovenko-Martynova – results interpretation of molecular descriptors calculation; Aida T. Navalieva – predictive models analysis; Maria M. Manvelyan – pharmacological studies conducting.

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Индуктор эндогенных интерферонов I и II типов (α,β,γ)

Быстрое контролируемое действие	Продукция интерферонов через 2-6 часов после введения и возврат к фоновым значениям в течение 2-х суток[1]
Возможность комплексного лечения и профилактики	Применение в комплексной терапии с антибиотиками и противовирусными средствами позволяет повысить эффективность лечения[1]
Гибкие схемы терапии	Грипп, ОРВИ – 1 инъекция, генитальный и опоясывающий герпес – 3 инъекции, инфекционные урогенитальные заболевания (в том числе хламидоз –4 инъекции)[1]

Механизм[1] действия Радамина Виро:

1. Оптимизация воспалительной реакции
2. Активация синтеза белков, тормозящих процесс производства вирусных копий в пораженных клетках
3. Стимуляция репаративных и регенеративных процессов
4. Активация неспецифической резистентности организма

[1] Инструкция по медицинскому применению

РУ: ЛС-000381 от 27.12.2021
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PROMOMED

ИНФОРМАЦИЯ ДЛЯ СПЕЦИАЛИСТОВ СИСТЕМЫ ЗДРАВООХРАНЕНИЯ. ПЕРЕД ПРИМЕНЕНИЕМ РЕКОМЕНДУЕТСЯ ОЗНАКОМИТЬСЯ С ИНСТРУКЦИЕЙ

ПМФИ

ВолгГМУ

MENTE ET ANIMO