Научно-практический журнал
Scientific and Practical Journal

ISSN 2307-9266
e-ISSN 2413-2241

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

PHARMACY & PHARMACOLOGY

2022 Том / Volume X

№ 2

Обзоры, лекции
Reviews, Lectures

Фармакогнозия, ботаника
Pharmacognosy, Botany

Фармацевтическая технология и биотехнология
Pharmaceutical Technology and Biotechnology

Фармацевтическая и токсикологическая химия
Pharmaceutical and Toxicological Chemistry

Фармакология и клиническая фармакология
Pharmacology and Clinical Pharmacology

Информационные технологии в фармации
Information Technologies in Pharmacy

Организация и экономика фармацевтического дела
Organization and Economy of Pharmacy

Экономика и менеджмент медицины
Economy and Management of Medicine

Фармацевтическое образование
Pharmaceutical Education

Дискуссии, рецензии, юбилеи, научные школы, история фармации и фармакологии
Discussions, Referee Reports, Anniversaries, Schools of Thought, History of Pharmacy and Pharmacology

www.pmedpharm.ru www.pharmpharm.ru

ПМФИ
Филиал ВолгГМУ

Соматулин® Аутожель®

ланреотид

Эффективность и благоприятный профиль безопасности

- Соматулин® Аутожель® обеспечивает стабильный контроль ИФР-1 и ГР, а также размера опухоли без отрицательного влияния на гликемию у пациентов с акромегалией и сахарным диабетом[1,8]

Улучшение качества жизни

- Соматулин® Аутожель® приводит к улучшению качества жизни путем уменьшения количества инъекций до 6-8 в год за счет увеличения интервала между инъекциями[7]

Новый, удобный, предварительно заполненный шприц для выполнения подкожных инъекций

- Глубокое подкожное введение препарата Соматулин® Аутожель® позволяет минимизировать боль в месте инъекции и снизить возможность развития нежелательных явлений[3,4]
- Соматулин® Аутожель® подходит для самостоятельного введения благодаря предзаполненному шприцу, готовому к применению[2, 5-7]

1. Caron P.J. et al. Clinical Endocrinol 2015. 2. Инструкция по медицинскому применению препарата Соматулин® Аутожель® 120 мг. 3. Salvatori R. et al. Pituitary 2010;13(2:115–22 4. Haramati N. et al. Arch Fam Med.1994;3(2):146–148 5. 5. Adelman D.T. et al. Medical Devices: Evidence and Research 2012. 6. Salvatori R. et al. Pituitary 2010. 7. Neggers SJCMM, et al. Eur J Endocrinol 2015;173:313–323. 8. Caron P. J. et al. Clinical Endocrinology. – 2017. – Т. 86. – №. 4. – С. 541-551

Краткая информация из инструкции по медицинскому применению препарата Соматулин® Аутожель® ЛСР-003497/09 **ТОРГОВОЕ НАЗВАНИЕ:** Соматулин® Аутожель® **МНН:** ланреотид. **ЛЕКАРСТВЕННАЯ ФОРМА:** гель для подкожного введения пролонгированного действия. **СОСТАВ:** Соматулин® Аутожель® 120 мг. Активное вещество: ланреотида ацетат –149,4 мг/шприц (125,5 мг/шприц). Вспомогательные вещества: вода для инъекций –357,8 мг/шприц, уксусная кислота ледяная – до pH 6,1 ± 0,3. Общая масса – 510,0 мг/шприц, масса, вводимая при инъекции, – 488,0 мг/шприц, вводимая доза ланреотида –120,0 мг/шприц. **ФАРМАКОТЕРАПЕВТИЧЕСКАЯ ГРУППА:** соматостатина аналог синтетический. **КОД АТХ:** H01CB03. **ПОКАЗАНИЯ К ПРИМЕНЕНИЮ** - Терапия пациентов с акромегалией, у которых концентрация ГР и/или инсулиноподобного фактора роста-1 (ИФР-1) остается повышенной после оперативного лечения и/или лучевой терапии; или пациентов, которым показано проведение медикаментозной терапии. Результатом терапии у пациентов с акромегалией является снижение концентраций ГР и ИФР-1 или нормализация их концентрации; - Терапия клинических симптомов акромегалии; **ПРОТИВОПОКАЗАНИЯ:** повышенная чувствительность к ланреотиду или родственным пептидам. Противопоказано у детей и подростков до 18 лет.С осторожностью: холелитиаз, беременность, период грудного вскармливания, сахарный диабет, начало терапии у пациентов с брадикардией. **СПОСОБ ПРИМЕНЕНИЯ И ДОЗЫ** Акромегалия: У пациентов, получающих терапию аналогами соматостатина в первый раз, рекомендуемая начальная доза составляет 60 мг – 120 мг каждые 28 дней. Например, у пациентов, ранее получавших инъекции препарата Соматулин®, лиофилизат для приготовления суспензии для внутримышечного введения пролонгированного действия в дозе 30 мг каждые 14 дней, начальная доза препарата Соматулин® Аутожель®, гель для подкожного введения пролонгированного действия, должна составлять 60 мг каждые 28 дней. У пациентов, получавших инъекции препарата Соматулин®, лиофилизат для приготовления суспензии для внутримышечного введения пролонгированного действия, в дозе 30 мг каждые 10 дней, начальная доза препарата Соматулин® Аутожель®, гель для подкожного введения пролонгированного действия, должна составлять 90 мг каждые 28 дней. У пациентов, получавших инъекции препарата Соматулин®, лиофилизат для приготовления суспензии для внутримышечного введения пролонгированного действия, в дозе 30 мг каждые 7 дней, начальная доза препарата Соматулин® Аутожель®, гель для подкожного введения пролонгированного действия, должна составлять 120 мг каждые 28 дней. В дальнейшем, у всех пациентов доза должна подбираться индивидуально в зависимости от ответной реакции пациента (которая оценивается на основании выраженности клинических симптомов и/или снижения концентрации ГР и/или ИФР-1). Если ожидаемый ответ не был достигнут, доза может быть увеличена. У пациентов, у которых на фоне терапии получены концентрации ГР ниже 1 нг/мл (около 2 мЕД/л), сывороточные концентрации ИФР-1 нормализовались и наиболее обратимые признаки акромегалии исчезли, ежемесячная доза может быть снижена. При необходимости, можно назначать препарат Соматулин® Аутожель® в дозе 120 мг с увеличенным интервалом - каждые 42-56 дней. У пациентов, получающих препарат Соматулин® Аутожель® в дозе 60 мг или 90 мг каждые 28 дней, при достижении хорошего контроля над заболеванием (концентрация ГР менее 2.5 нг/мл (около 5 мЕД/л), но выше 1 нг/мл (около 2 мЕД/л); нормализация концентрации ИФР-1), доза препарата должна поддерживаться на этом же уровне, или возможно применение препарата Соматулин® Аутожель® в дозе 120 мг с увеличенным интервалом введения - 56 или 42 дня, соответственно. У пациентов, у которых клинические симптомы и биохимические параметры заболевания адекватно контролировать не удалось (концентрация ГР выше 2.5 нг/мл (около 5 мЕД/л) или концентрация ИФР-1 выше нормы), доза препарата Соматулин® Аутожель® может быть увеличена до максимальной: 120 мг каждые 28 дней. Всем пациентам показан регулярный долгосрочный контроль клинических симптомов, концентрации ГР и ИФР-1. **ПЕРЕДОЗИРОВКА:** показано проведение симптоматической терапии. **ВЗАИМОДЕЙСТВИЕ С ДРУГИМИ ЛЕКАРСТВЕННЫМИ СРЕДСТВАМИ:** может снижать абсорбцию в кишечнике одновременно принимаемых препаратов, в том числе циклоспорина (может возникнуть необходимость корректировать дозу). При одновременном применении аналогов соматостатина и бромокриптина может повыситься биодоступность бромокриптина. При одновременном применении с лекарственными средствами, снижающими частоту сердечного ритма (такими как бета-андреноблокаторы), может понадобиться коррекция дозы одновременно принимаемого препарата. Может снижать метаболический клиренс препаратов, метаболизируемых с помощью ферментов цитохрома P450. Необходимо соблюдать осторожность при совместном применении с препаратами, метаболизируемыми в основном с помощью CYP3A4 и имеющими низкий терапевтический индекс (хинидин, терфенадин).
ВЛИЯНИЕ НА СПОСОБНОСТЬ УПРАВЛЯТЬ ТРАНСПОРТНЫМИ СРЕДСТВАМИ И РАБОТАТЬ С МЕХАНИЗМАМИ: было сообщено, что на фоне терапии препаратом может развиться головокружение. При появлении данного побочного действия не следует управлять транспортными средствами и работать с механизмами. **ФОРМА ВЫПУСКА:** гель для подкожного введения пролонгированного действия 60 мг,90 мг, 120 мг. Шприц с защитным устройством в комплекте с иглой, упакованный в пакет из полиэтилентерефталата/алюминия/полиэтилена, вместе с инструкцией по применению помещен в пачку картонную. **УСЛОВИЯ ХРАНЕНИЯ:** 2–8 °C (в холодильнике), в недоступном для детей месте. Не замораживать! **СРОК ГОДНОСТИ:** 2 года. Не применять по истечении срока годности. **УСЛОВИЯ ОТПУСКА ИЗ АПТЕК:** по рецепту.

Полная информация по препарату содержится в инструкции по медицинскому применению. Материал предназначен для специалистов здравоохранения. Информация о лекарственном средстве предоставляется медицинским работникам в соответствии с пп. 4 п. 1 и п. 2 ст. 74 ФЗ «Об основах охраны здоровья граждан в Российской Федерации» для информирования пациентов об аналогичных лекарственных средствах. Имеются противопоказания. Перед использованием следует ознакомиться с инструкцией или обратиться за консультацией к специалисту. ООО «Ипсен»: 109147, г. Москва, ул. Таганская, д. 17–23, этаж 2, ком. 10–27, 30–39 4/1–14; тел. +7 (495) 258-54-00, факс +7 (495) 258-54-01; www.ipsen.ru. Служба медицинской поддержки по препаратам компании «Ипсен» (в рамках инструкции по применению): тел. 8 (800) 700-40-25 (бесплатный номер телефона по всей Российской Федерации); электронная почта Medical.Information.Russia.CIS@ipsen.com. Контакты для информации о нежелательных явлениях/реакции, для претензий на качество продуктов компании: +7 (916) 999-30-28 (круглосуточно); электронная почта pharmacovigilance.russia@ipsen.com

SOM-RU-000279 -01092020

На правах рекламы.

IPSEN
Innovation for patient care

Scientific and Practical Journal

PHARMACY & PHARMACOLOGY

Scientific and practical journal
Volume X, Issue 2, 2022
The mass media registration certificate:
ПИ №ФС77–67428 от 13.10.2016
ISSN 2307-9266 e-ISSN 2413-2241

Founder: Volgograd State Medical University. 1, Pavshikh Bortsov Sq., Volgograd, Russia, 400131
Editors office address: 11, Kalinin ave., Pyatigorsk, Russia, 357532
Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University
Phone number: +7(8793) 32-44-74. E-mail: pharmjournal@mail.ru
www.pharmpharm.ru
Union catalogue. Russian Press / Newspapers an journals. Code 94183
A4 size, 1000 issues circulation. Price free

Journal "Pharmacy & Pharmacology" is recommended International Comittee Of Medical Journal Editors and included in Higher Attestation Commission, Scopus, Web of Science (ESCI), Russian citation database, eLibrary, ARISTI (All-Russian Institute of Scientific and Technical Information), RSL (Russian State Library), CyberLeninka, Socionet, EMBASE, Chemical Abstracts (CAS), Directory of Open Access Journals (DOAJ), EBSCO Discovery Service, RNMJ, University of CAMBRIDGE, Ulrich'sWeb, Google Scholar, Biefeld Academic Search Engine (BASE), Directory of Open Access Scholarly Resources (ROAD), Research Bible, Open Archives Initiative, Academic Keys, JournalTOCs, WorldCat, OpenAIRE, University of Oxford, The British Library, Universitait Gent, Université de Montréal, University of Saskatchewan.

Printed in the LLC "Amirit" in accord with provided materials, 410004, Saratov, 88, Chernishevsky Str.

Научно-практический журнал

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

Периодичность 6 номеров в год

Том 10, Выпуск 2, 2022

Свидетельство регистрации СМИ:
ПИ №ФС77–67428 от 13.10.2016 г.

ISSN 2307-9266 e-ISSN 2413-2241

Учредитель: Федеральное государственное бюджетное образовательное учреждение высшего образования «Волгоградский государственный медицинский университет» Минздрава России.
400131, Россия, г. Волгоград, площадь Павших Борцов, д. 1
Адрес издательства: 357532, г. Пятигорск, пр-кт Калинина, 11.
Пятигорский медико-фармацевтический институт – филиал ФГБОУ ВО ВолгГМУ Минздрава России
Телефон: +7 (8793) 32-44-74. E-mail: pharmjournal@mail.ru
www.pharmpharm.ru
Объединенный каталог. Пресса России. Газеты и журналы. Индекс 94183
Формат А4, тираж 1000 экз. Цена свободная.

Журнал «Фармация и фармакология» включен в перечень рецензируемых научных изданий, входящих в международные реферативные базы данных и системы цитирования, и в соответствии с пунктом 5 правил формирования перечня рецензируемых научных изданий, в которых должны быть опубликованы основные научные результаты диссертаций на соискание ученой степени кандидата наук, на соискание ученой степени доктора наук (Перечень ВАК), Scopus, Web of Science (ESCI), РИНЦ, eLibrary, ВИНИТИ, РГБ, Киберленинка, Соционет, EMBASE, Chemical Abstracts (CAS), Directory of Open Access Journals (DOAJ), EBSCO Discovery Service, RNMJ, University of CAMBRIDGE, Ulrich'sWeb, Google Scholar, Biefeld Academic Search Engine (BASE), Directory of Open Access Scholarly Resources (ROAD), Research Bible, Open Archives Initiative, Academic Keys, JournalTOCs, WorldCat, OpenAIRE, University of Oxford, The British Library, Universitait Gent, Université de Montréal, University of Saskatchewan.

Отпечатано в соответствии с предоставленными материалами в ООО «Амирит», 410004, г. Саратов, ул. Чернышевского, 88.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

CONTENS / СОДЕРЖАНИЕ

REVIEWS, LECTURES / ОБЗОРЫ, ЛЕКЦИИ

RESEARCH ARTICLE / ОРИГИНАЛЬНЫЕ СТАТЬИ

Pharmacology and Clinical Pharmacology / Фармакология и клиническая фармакология

REVIEWS, LECTURES

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

ACTIVITY MODULATION OF VARIOUS NITRIC OXIDE SYNTASES AS AN APPROACH TO ENDOTHELIAL DYSFUNCTION THERAPY

D.V. Kurkin, E.E. Abrosimova, D.A. Bakulin, N.S. Kovalev, M.A. Dubrovina, A.V. Borisov, A.V. Strygin, E.I. Morkovin, I.N. Tyurenkov

Volgograd State Medical University,
1, Pavshikh Bortsov Sq., Volgograd, Russia, 400131

E-mail: strannik986@mail.ru

Received 16 Nov 2021 After peer review 10 Feb 2022 Accepted 11 March 2022

Nitric oxide as a therapeutic approach to the treatment of cardiovascular diseases attracted the attention of researchers at the end of the 19th century. As a vasodilator, nitric oxide may be a unique therapeutic agent for the treatment of hypertension and, as a result, renal failure and left ventricular hypertrophy.

The aim of the article is to analyze the literature data on possible ways of modulating the activity of various nitric oxide synthases as an approach to the treatment of endothelial dysfunction.

Materials and methods. When searching for materials for writing a review article, such abstract databases as PubMed, Google Scholar, e-Library, etc., were used. The search was carried out on the publications for the period from 1990 to 2021. The following words and phrases were chosen as parameters for the literature selection: nitric oxide; NO synthase; endothelial dysfunction; NO synthase activator; NO synthase inhibitor.

The following words and phrases were chosen as parameters for the literature selection:

Results. The article presents the history of the nitric oxide discovery and its biological role, the process of its biosynthesis, as well as the isoforms of its synthesizing enzymes (NOS): neuronal – nNOS, endothelial – eNOS and inducible iNOS, and their role in normal and pathological physiology. The process of NOS uncoupling (its molecular mechanisms) has been considered as the basis of endothelial dysfunction.

The examples of the pharmacological correction (BH_4, arginase inhibitors, statins, resveratrol) are presented. In addition, NO synthase activators (calcium dobesilate, cavNOxin, and some NOS transcription activators), as well as non-selective (L-NMMA, 1-NNA, L-NAME, ADMA, 546C88, VAS203) and selective (L-NIO, 7-nitroindazole, aminoguanidine, L-NIL, GW273629, GW274150, cavtratin) inhibitors of nitric oxide synthasehave been described.

Conclusion. Nitric oxide synthases continue to be promising targets for the development of agents that modulate their activity to correct various pathologies. As a therapeutic approach, modulation of the nitric oxide synthase activity can be implemented to treat endothelial dysfunction, which is the cause for complications of many diseases.

Keywords: nitric oxide (II); NO synthase; endothelial dysfunction; cavNOxin; resveratrol; aminoguanidine

Abbreviations: 7-NI – 7-Nitroindazole; ADDP – 6-Acetyl-7,7-dimethyl-5,6,7,8-tetrahydropterin; ADMA – asymmetric dimethylarginine; AMPK – adenosine monophosphate-activated protein kinase; AP-1 – activating protein-1; AP-2 – activating protein-2; ApoE – apolipoprotein E; BH_2 – dihydrobiopterin; BH_3 – trihydrobiopterin; BH_4 – tetrahydrobiopterin; CaD – Calcium Dobesilate; cNOS – constitutive nitric oxide synthase; CO – carbon monoxide; DDAH – dimethylarginine dimethylaminohydrolase; DHFR – dihydrofolate reductase; eNOS, NOS3 – endothelial nitric oxide synthase; ER – estrogen receptor; FGF – fibroblast growth factor; FPP – farnesyl pyrophosphate; GGPP – geranylgeranyl diphosphate; GSH – glutathione; GSSG – glutathione disulfide; GTPCH-I – guanosine triphosphate cyclohydrolase I; HUVEC – human umbilical vein endothelial cells; IFN-γ – interferon gamma; iNOS, NOS2 – inducible nitric oxide synthase; L-NAME – N^G-nitro-L-arginine; L-NIO – N(5)-(1-Iminoethyl)-L-ornithine; L-NMMA – N^G-monomethyl-L-arginine; L-NNA – N^G-nitro-l-arginine; NF-1 – neurofibromin 1; NF-kB – nuclear factor-kB; nNOS, NOS1 – neuronal nitric oxide synthase; NO – nitric oxide (II); NOHA – N^G-hydroxy-L-arginine; NOCCL – NO-cleavable cross-linker; Nrf2, NFE2L2 – nuclear factor erythroid 2, (NF-E2)-related factor 2; ODQ – 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one; PCSK9 – proprotein convertase subtilisin/kexin type 9; PI3K – phosphoinositide 3-kinase; PKA – protein kinase A; PKC – protein kinase C; ROCK – Rho-associated protein kinase; ROS – reactive oxygen species; SREBP-2 – Sterol regulatory element-binding protein 2; TGF – transforming growth factor; TNFα – Tumor necrosis factor alpha; VEGF-A – vascular endothelial growth factor A; ABP – arterial blood pressure; ROI – reactive oxygen intermediate; GTP – Guanosine-5'-triphosphate; DNA – deoxyribonucleic acid; GIT – gastrointestinal tract; LDL(s) – low-density lipoproteins; LPS – lipopolysaccharide; mRNA – messenger ribonucleic acid; RA – rheumatoid arthritis; sGC – soluble guanylate cyclase; FAD – flavine adenine dinucleotide; FMN – flavin mononucleotide; cGMP – cyclic guanosine monophosphate; CNS – central nervous system.

For citation: D.V. Kurkin, E.E. Abrosimova, D.A. Bakulin, N.S. Kovalev, M.A. Dubrovina, A.V. Borisov, A.V. Strygin, E.I. Morkovin, I.N. Tyurenkov. Activity modulation of various nitric oxide syntases as an approach to endothelial dysfunction therapy. *Pharmacy & Pharmacology.* 2022;10(2): 130-153. **DOI:** 10.19163/2307-9266-2022-10-2-130-153

Для цитирования: Д.В. Куркин, Е.Е. Абросимова, Д.А. Бакулин, Н.С. Ковалев, М.А. Дубровина, А.В. Борисов, А.В. Стрыгин, Е.И. Морковин, И.Н. Тюренков. Модуляция активности различных синтаз оксида азота в качестве подхода к терапии эндотелиальной дисфункции. *Фармация и фармакология.* 2022;10(2):130-153. **DOI:** 10.19163/2307-9266-2022-10-2-130-153

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОБЗОРЫ, ЛЕКЦИИ

DOI: 10.19163/2307-9266-2022-10-2-130-153

МОДУЛЯЦИЯ АКТИВНОСТИ РАЗЛИЧНЫХ СИНТАЗ ОКСИДА АЗОТА В КАЧЕСТВЕ ПОДХОДА К ТЕРАПИИ ЭНДОТЕЛИАЛЬНОЙ ДИСФУНКЦИИ

Д.В. Куркин, Е.Е. Абросимова, Д.А. Бакулин, Н.С. Ковалев, М.А. Дубровина, А.В. Борисов, А.В. Стрыгин, Е.И. Морковин, И.Н. Тюренков

Федеральное государственное бюджетное образовательное учреждение высшего образования
«Волгоградский государственный медицинский университет»
Министерства здравоохранения Российской Федерации
400131, Россия, г. Волгоград, пл. Павших Борцов, д. 1

E-mail: strannik986@mail.ru

Получена 16.11.2021 После рецензирования 10.02.2022 Принята к печати 11.03.2022

Оксид азота как терапевтический подход к лечению сердечно-сосудистых заболеваний привлек внимание исследователей еще в конце XIX века. Являясь вазодилататором, оксид азота может быть уникальным терапевтическим средством для лечения гипертензии и, как следствие, почечной недостаточности и гипертрофии левого желудочка.

Цель. Проанализировать данные литературы о возможных путях модуляции активности различных синтаз оксида азота в качестве подхода к терапии эндотелиальной дисфункции.

Материалы и методы. При поиске материала для написания обзорной статьи использовали такие реферативные базы данных, как PubMed, Google Scholar, e-Library, и др. Поиск осуществлялся по публикациям за период с 1990 по 2021 гг. Параметрами для отбора литературы были выбраны следующие слова и словосочетания: оксид азота; NO-синтаза; эндотелиальная дисфункция; активатор NO-синтазы; ингибитор NO-синтазы.

Результаты. В статье представлена история открытия оксида азота и его биологическая роль, процесс его биосинтеза, а также изоформ ферментов его синтезирующих (NOS): нейрональной – nNOS, эндотелиальной – eNOS и индуцибельной iNOS и их роль в нормальной и патологической физиологии. Рассмотрен процесс разобщения NOS (его молекулярных механизмов) в качестве основы эндотелиальной дисфункции. Представлены примеры фармакологической коррекции (BH_4, ингибиторы аргиназы, статины, ресвератрол). Кроме того, описаны активаторы синтаз NO (кальция добезилат, cavNOxin, и некоторые активаторы транскрипции NOS), а также неселективные (L-NMMA, 1-NNA, L-NAME, ADMA, 546C88, VAS203) и селективные (L-NIO, 7-нитроиндазол, аминогуанидин, L-NIL, GW273629, GW274150, кавтратин) ингибиторы синтаз оксида азота.

Заключение. Синтазы оксида азота продолжают оставаться перспективными мишенями для разработки средств, модулирующих их активность для коррекции различных патологий. В качестве терапевтического подхода модуляция активности синтазы оксида азота может быть реализована для лечения эндотелиальной дисфункции, являющейся причиной осложнений многих заболеваний.

Ключевые слова: оксид азота (II); NO-синтаза; эндотелиальная дисфункция; cavNOxin; ресвератрол; аминогуанидин

Список сокращений: 7-NI – 7-нитроиндазол; ADDP – 6-ацетил-7,7-диметил-5,6,7,8-тетрагидроптерин; ADMA – асимметричный диметиларгинин; AMPK – аденозинмонофосфат-активируемая протеинкиназа; AP-1 – activating protein-1 (активирующий белок-1; AP-2 – активирующий белок-2; ApoE – аполипопротеину E; BH_2 – дигидробиоптерин; BH_3 – тригидробиоптерин; BH_4 – тетрагидробиоптерин; CaD – кальция добезилат; cNOS – конститутивная изоформа NO синтазы; CO – монооксид углерода; DDAH – диметиларгинин диметиламиногидролаза; DHFR –дигидрофолатредуктаза; eNOS, NOS3 – эндотелиальная синтаза оксида азота; ER – рецепторы эстрогена; FGF – фактор роста фибробластов; FPP – фарнезилпирофосфат; GGPP – геранилгеранилпирофосфат; GSH – глутатион; GSSG – дисульфид глутатиона; GTPCH-I – гуанозин трифосфат циклогидролаза I; HUVEC – эндотелиальные клетки пупочной вены человека; IFN-γ – интерферон-γ; iNOS, NOS2 – индуцибельная синтаза оксида азота; L-NAME – N^G-нитро-L-аргинин; L-NIO – N(5)-(1-иминоэтил)-L-орнитин; L-NMMA – N^G-монометил-L-аргинин; L-NNA – N^G-нитро-L-аргинин; NF-1 – нейрофибромин 1; NF-kB – ядерный фактор-kB; nNOS, NOS1 – нейрональная синтаза оксида азота; NO – оксид азота (II); NOHA – N^G-гидрокси-L-аргинин; NOCCL – NO-расщепляемый сшивающий агент; Nrf2, NFE2L2 – ядерный фактор-2, подобный фактору эритроидного происхождения 2; ODQ –1H-[1,2,4]оксадиазоло-[4,3-a]хиноксалин-1-он; PCSK9 – пропротеин конвертаза субтилизин кексин 9; PI3K – фосфатидилинозитол-3-киназа; PKA – протеинкиназа A; PKC – протеинкиназа C; ROCK – Rho-ассоциированная протеинкиназа; ROS – активные формы кислорода; SREBP-2 – белок, связывающий регуляторный элемент стерола 2; TGF – трансформирующий фактор роста; TNFα – фактор некроза опухоли альфа; VEGF-A – фактор роста эндотелия сосудов A; АД – артериальное давление; АФК – активные формы кислорода; ГТФ – гуанозинтрифосфат; ДНК – дезоксирибонуклеиновая кислота; ЖКТ – желудочно-кишечный тракт; ЛПНП – липопротеины низкой плотности; ЛПС – липополисахарид; мРНК – матричная рибонуклеиновая кислота; РА – ревматоидный артрит; рГЦ – растворимая гуанилатциклаза; ФАД – флавинадениндинуклеотид; ФМН – флавинмононуклеотид; цГМФ – гуанозинмонофосфат; ЦНС – центральная нервная система.

INTRODUCTION

Nitric oxide (II) (NO) is characterized by a wide range of properties: from a toxic air pollutant to a pro-inflammatory mediator and regulator of the most important body functions (vasomotor, neurotransmitter).

NO, a colorless toxic gas, was first studied by a British chemist Joseph Priestley in 1772. A student of Theophile-Jules Peluza (who had developed nitrocellulose and other nitrosulfates), the Italian chemist Ascanio Sobrero, discovered nitroglycerin in 1847, for which he notified its

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

ability to reproducibly cause severe headaches. Another student of Peluz, Alfred Nobel, combined this highly unstable compound with kieselgur and patented it in 1867 as a more stable commercial explosive dynamite.

In the workers involved in the production of nitroglycerin, the disappearance of angina pectoris symptoms was notified. In 1879, the British physician William Murrell proposed nitroglycerin for the angina pectoris treatment. In 1977, Ferid Murad discovered that the positive effect of nitroglycerin on vascular smooth muscles is due to the release of NO. The 1998 Nobel Prize in Physiology and Medicine was shared by Ferid Murad and Robert F. Furchgott, who, together with John Zawadzki in 1980, established the importance of the endothelial relaxing factor caused by the vasodilating action of acetylcholine.

In the late 1970s, Robert Furchgott began to investigate vasoactive effects of acetylcholine, leading to the discovery of NO.

In the early 1980s, several studies were carried out, as a result of which the chemical properties and the action mechanisms of the endothelium-dependent relaxation factor were established. The similarity between the properties of NO and the properties of the endothelium-dependent relaxation factor had been determined by 1986.

In 1987, two separate laboratories – R. Furchgott and J. Zawadzki's, as well as Louis J. Ignarro and Salvador Moncada's [1] – published the definitive evidence that NO is an endothelium-dependent relaxation factor [2].

The discovery of NO as an endogenously generated signaling agent of a particular importance for the cardiovascular system set off a new era in the study of biological signaling in the late 1980s and early 1990s. This discovery changed the paradigm and demonstrated that a small, freely diffusible molecule can be synthesized endogenously to act as a specific signaling factor through interactions with a receptor protein [3].

Main effects of NO

NO plays an important role in the regulation of various physiological processes that are critical for functioning of many body tissues. When NO signaling is impaired, these central tissue processes can become pathophysiological, causing acute or chronic diseases in the CNS [4–7], blood vessels [8–10], gastrointestinal tract [11, 12], skeletal muscles [13, 14] and the immune system. [15, 16].

NO biosynthesis and signal transduction in the NO-ergic system

Endogenous NO is mainly generated by enzymatic pathways, but there is also a non-enzymatic pathway (Fig. 1A). A non-enzymatic NO production involves the production of NO from nitrite in several ways, especially under conditions of acidosis (e. g, after ischemia), and occurs primarily in tissues predominantly via a nitrite reduction:

$$e^- + 2H^+ + NO_2^- \rightarrow NO + H_2O$$

THE AIM under conditions of ischemia with acidosis, anitrite-mediated NO production approaches a maximum constitutive nitric oxide synthase (NOS) production, making this pathway an alternative in ischemia, i.e. under the conditions when the NO production from NOS is violated [17].

MATERIALS AND METHODS

When searching for materials for writing a review article, such abstract databases as PubMed, Google Scholar, e-Library, etc. were used. The search was carried out on the publications for the period from 1990 to 2021. The following words and phrases were chosen as parameters for the literature selection: nitric oxide; NO synthase; endothelial dysfunction; NO synthase activator; NO synthase inhibitor.

RESULTS AND DISCUSSION

NO synthases and their role

The enzymatic production of NO is catalyzed by various NOS isoforms through a series of redox reactions in the presence of oxygen to cleave L-arginine to L-citrulline and NO via the intermediate metabolite N-hydroxy-1-arginine (NOHA), using NADPH as an electron donor.

Isolated NOS was first isolated by Bredt and Snyder in 1990 [18]. The three major mammalian NOS enzymes had already been cloned and successfully expressed in E. coli or insect cells and had been characterized by 1998.

Three major NOS isoforms have been identified based on their different primary amino acid sequences (only 50–60% identity), tissue and cellular distribution, and a regulation mechanism. Two NOS isoforms are calcium/calmodulin-dependent and constitutively expressed (cNOS, constitutive nitric oxide synthase): NOS of the endothelial origin (NOS3, eNOS, endothelial nitric oxide synthase) and NOS of the neuronal origin (NOS1, nNOS, neuronal nitric oxide synthase), respectively. The third NOS isoform is induced by cytokines, and its activity is independent on calcium/calmodulin (NOS2, iNOS, inducible nitric oxide synthase). The individual properties of each NOS isoform are of great importance, since they are: the magnitude, duration, and cellular sites of the NO production that determine the overall physiological or pathophysiological effect [19].

While iNOS normally produces large amounts of NO and is involved in the immune defense, inflammatory responses, and the NO production by airway epithelial cells, the constitutively expressed nNOS and eNOS isoforms typically generate nanomolar NO concentrations that are important for physiological processes such as neuronal signaling, inhibition of the hemostatic system, vasodilation and control of blood pressure (BP). NO synthesized in such quantities is not rapidly oxidized either,

DOI: 10.19163/2307-9266-2022-10-2-130-153

and can directly interact with metal ions in proteins, for example, with the iron atom in the heme group of hemoglobin, myoglobin, soluble guanylate cyclase (sGC), cytochrome P450, and NOS itself [20].

All NOS isoforms use tetrahydrobiopterin (BH_4, tetrahydrobiopterin), which is synthesized from guanosine triphosphate (GTP) by the enzyme GTP-cyclohydrolase-I (GTPCH-I). Other cofactors are flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and heme. All NOS proteins are homodimers.

Functional NOS transfers electrons from NADPH from the carboxy-terminal reductase domain through the flavins FAD and FMN to heme in the N-terminal oxygenase domain. The oxygenase domain also binds the important cofactor BH_4, molecular oxygen, and the substrate L-arginine. At the heme site, electrons are used to reduce and activate O_2, as well as to oxidize L-arginine to L-citrulline, followed by the release of NO.

To synthesize NO, the NOS enzyme goes through two stages. At the first stage, NOS hydroxylates L-arginine to Nω-hydroxy-L-arginine, which remains bound to the enzyme. At the second stage, NOS oxidizes Nω-hydroxy-L-arginine to L-citrulline and NO.

All the NOS isoforms bind calmodulin. In nNOS and eNOS, calmodulin binding is induced by an increase in intracellular Ca^{2+} concentration. When calmodulin's affinity for NOS increases, it facilitates the flow of electrons from NADPH in the reductase domain to heme in the oxygenase domain. In inducible NOS (iNOS), calmodulin binds at already extremely low intracellular Ca^{2+} concentrations due to the different amino acid structure of the calmodulin binding site (Fig. 1 B and C). All NOS proteins contain a zinc-thiolate cluster formed by a zinc ion that is tetrahedrally coordinated to two CysXXXXCys motifs (by one of each monomer) at the boundary of the NOS dimer. Zinc in NOS performs a structural rather than catalytic function [21].

Endothelial NO synthase

An endogenous NO production, especially in the cardiovascular system, mainly depends on the activity of the enzyme endothelial NO synthase. The eNOS enzyme was originally identified and isolated from bovine aortic endothelial cells [22, 23]. Further studies showed that several types of non-endothelial cells, such as cardiomyocytes [24], platelets [25], and neurons [26], also express eNOS.

In humans, the eNOS gene is located in the region 7q35–7q36 of chromosome 7 [27]. This gene is present as a single copy in the haploid set of the human genome.

Although eNOS is expressed constitutively, many factors regulate eNOS at transcriptional, post-transcriptional, and post-translational levels. In this regard, variations in the eNOS gene affect the regulation of the eNOS enzyme and, accordingly, the NO production [28].

Under physiological conditions, eNOS is responsible for the production of most endothelial NO, and for this reason, it plays a key role in the regulation of the cardiovascular system. This fact is constantly confirmed by clinical and experimental studies. In particular, eNOS knockout gene mice rapidly develop hypertension and have a high risk of stroke or other cardiovascular disease compared to wild-type mice. Similar disorders are also reproduced by a pharmacological inhibition of eNOS.

The regulation mechanisms of the eNOS activity are extremely complex and can be divided into two levels: genetic and protein. At the genetic level, the expression and stability of eNOS genes are associated with its activity. The eNOS promoter contains a large number of transcription factor binding sites, including activator protein-1 (AP-1), activator protein-2 (AP-2), the endothelin family, the nuclear factor-kB (NF-kB, nuclear factor-kB), and neurofibromin 1 (NF-1). These transcription factor complexes can regulate the eNOS expression; and hypoxia, lipopolysaccharide, and several cytokines can induce it.

In quiescent endothelial cells, most of the expressed eNOS attaches to caveolae, pocket-like membrane invaginations rich in cholesterol and sphingolipids. The localization of eNOS in endothelial cell caveolae inactivates the enzyme as a result of the strong and direct interaction of eNOS with caveolin-1. This protein-protein interaction inhibits the eNOS activity mainly by interfering with the calmodulin binding site. In contrast, binding of calcium-activated calmodulin to eNOS displaces caveolin-1 and promotes the eNOS activation. Calmodulin interacts with a cognate binding site on eNOS located between the oxygenase and reductase domains. This binding shifts the adjacent autoinhibitory loop and allows NADPH-dependent electron flow to the enzyme heme. However, in the absence of bound calmodulin, the electron transfer is blocked and the eNOS catalytic activity is suppressed. In addition to caveolin-1, eNOS can interact with heat shock protein 90 (Hsp90), the protein involved in a multicomponent chaperone system that is responsible for the folding of several proteins. Hsp90 is involved in eNOS folding and can allosterically modulate the enzyme by inducing conformational changes or by stabilizing the dimeric form, thereby activating eNOS. It should be notified that the eNOS activity depends on the presence of a substrate (L-arginine) and cofactors (NADPH and BH4) [28, 29].

In addition to these mechanisms, eNOS can be also activated by stimuli that do not cause a sustained increase in intracellular Ca^{2+} but still result in the sustained NO release. The main stimulus is a shear stress eNOS

ISSN 2307-9266 e-ISSN 2413-2241
Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

can be phosphorylated by several serine (Ser), threonine (Thr), and tyrosine (Tyr) residues. Phosphorylation of Ser1177 stimulates the flow of electrons in the reductase domain, increases the sensitivity of the enzyme to Ca^{2+}, and represents an additional and independent mechanism of the eNOS activation [21].

Neuronal NO synthase

This type of NOS is expressed constitutively. The nNOS gene, located on chromosome 12, has a complex genomic organization. A cellular and tissue-specific regulation of nNOS is considered to be related to various transcription factors such as activator protein-2 (AP-2), transcription enhancer factor-1 (TEF-1)/MCAT binding factor, cAMP response element binding protein (CREB)/activating transcription factor/c-Fos, nuclear respiratory factor-1, Ets, nuclear factor-1 and NF-kappa B [30].

In addition, nNOS undergoes more than ten different alternative splicing options that potentially have unique structural features and catalytic properties. In the brain, the nNOSα form containing exon 2 is the main splicing variant, while nNOSμ is the predominant splicing variant in the skeletal muscle and the heart. nNOS-2, found in the mouse brain, acts as a dominant negative regulator of the nNOS activity due to the deletion in frame 105 of amino acid residues 504-608 involved in arginine binding. Both nNOSα and nNOSμ are attached to subcellular structures via the PDZ domain, while the other two forms, nNOSβ and nNOSγ, are cytoplasmic [31].

Post-translational modifications represent an additional level of the nNOS regulation activity and include phosphorylation, ubiquitination, and interactions with other structural and regulatory proteins, resulting in the formation of a functional complex.

The enzymatic activity of nNOS is modulated by phosphorylation at multiple sites and promotes the interaction between NO and other signaling pathways through various protein kinases. Phosphorylation of nNOS can occur at a variety of residues, including serine (Ser), threonine (The), and tyrosine (Tyr). This fact is associated with the activation/inactivation of the enzyme directly, or with the modulation of other regulatory domains present in nNOS.

In addition to phosphorylation, ubiquitination and sumoylation are two major post-translational regulatory mechanisms involved in levels and functions controlling of several neuronal proteins. Protein ubiquitination is a signal for the degradation of dysfunctional proteins.

In brain cells, nNOS is present in bound and soluble forms. The differential subcellular localization of nNOS explains the diversity of its functions [21, 32].

The regulation mechanisms of nNOS are similar to those of eNOS: the calcium-binding protein, calmodulin, acts as an allosteric activator and the nNOS activity is also regulated by heat shock proteins. Heat shock proteins (HSPs) comprise a family of molecular chaperones responsible for the proper folding and maturation of proteins. The Hsp90/Hsp70-based chaperone apparatus regulates the nNOS activity and turnover by modulating ligand-binding clefts [32].

The NOS of neurons contains a PDZ domain and can directly interact with similar domains of other proteins. These interactions determine the subcellular distribution and activity of the enzyme. The proteins containing the PDZ domain, such as syntrophin, PSD-95, or PSD-93, play an important role in the formation of multiprotein signaling complexes and are required for nNOS binding to various calcium sources. In skeletal muscles, nNOS is attached to the sarcolemma via syntrophin and dystrophin proteins. While in the CNS, nNOS connects to the cytoplasmic tail of NMDA-NR 2 via PSD95, which is responsible for calcium influx and the subsequent activation of nNOS. The subcellular fractionation of the brain tissue shows that about half of the nNOS in the brain is soluble, and the rest, via PSD95, is bound to the membrane fraction. PSD proteins are preferentially expressed at higher synaptic densities and interact with nNOS via PDZ domains [32, 33].

Neuronal NOS is involved in the modulation of physiological functions such as learning, memory, and neurogenesis. In the central nervous system, nNOS mediates the long-term regulation of synaptic transmission. NO formed in the CNS (under the action of nNOS) is involved in the central regulation of blood pressure [7]. In addition to the brain tissue, nNOS has been found in the spinal cord, sympathetic ganglia and adrenal glands, peripheral nerves, epithelial cells of various organs, nephrons, pancreatic islet cells, and vascular smooth muscle cells.

Inducible NO synthase

The iNOS gene is located on the 17th chromosome. The inducible isoform of NOS is mainly regulated at the level of expression. The mechanisms regulating the iNOS expression include the modulation of the promoter activity, the mRNA stability and translation, and finally protein. As with other enzymes, iNOS activity depends on the availability of the substrate. The stimuli and conditions that determine the iNOS expression depend on the functional state of the cell and its type. The inducers of the iNOS expression in one cell type may have no effect or even suppress an iNOS expression in another, even within the same organism [34].

Inducible NOS is not permanently expressed in cells, but its synthesis can be stimulated by bacterial lipopolysaccharide, pro-inflammatory cytokines (interferon-gamma, IL-1, IL-2, a tumor necrosis factor) or other agents,

DOI: 10.19163/2307-9266-2022-10-2-130-153

such as reactive oxygen species, as well as hormones that affect the synthesis of cyclic adenosine monophosphate (adrenaline, glucagon). Although iNOS is primarily identified in macrophages, the expression of the enzyme can be stimulated in practically any cell or tissue, provided the appropriate inducing agents have been identified. After the expression, iNOS is constantly active and is not regulated by intracellular Ca^{2+} concentrations [35].

Inducible in macrophages, NOS produces a large amount of NO, which is the basis of the cytotoxicity of these cells. Due to its affinity for protein-bound iron, NO is able to inhibit the key enzymes the catalytic centers of which contain it. These include iron-sulfur cluster-dependent enzymes (complexes I and II) involved in the mitochondrial electron transport, ribonucleotide reductase (an enzyme that limits the rate of DNA replication) and cisconitase (a key enzyme in the citric acid cycle). Higher concentrations of NO produced by induced macrophages can directly damage the DNA of the target cells and cause chains breaks and their fragmentation. The combination of these effects probably underlies the cytostatic and cytotoxic effects of NO on parasitic microorganisms and some tumor cells. Interestingly, non-immune cells can be also induced by cytokines to release enough NO to affect neighboring cells. For example, cytokine-activated endothelial cells have been shown to lyse tumor cells, while induced hepatocytes can use NO to kill malaria sporozoites. The iNOS activity is probably responsible for all these effects [21].

Unlike eNOS and nNOS, iNOS has a much higher affinity for calmodulin, so it can bind it at very low Ca^{2+} concentrations and thus its activity is not regulated by Ca^{2+}/calmodulin, but mainly at the transcriptional level. The kinetic parameters of iNOS determine the catalytic activity level, which generates a higher amount of NO than eNOS and nNOS, and is less sensitive to the NO-dependent autoinhibition which contributes to the interaction of large amounts of NO with reactive oxygen species (ROS) and the generation of more toxic nitrogen species, such as nitrate [36].

The activity of expressed iNOS is regulated by various factors, including the availability of its substrate and cofactors, the interaction with other proteins, and the autoinactivation (to a lesser extent).

Several proteins interact with iNOS and regulate its activity. In the central nervous system (brain) of lipopolysaccharide (LPS) injected mice, iNOS is found complexed with a cytosolic protein called caliririn (primarily caliririn-7). Kalirin interacts with iNOS monomers, but not with iNOS dimers. In the cells expressing kalirin, most of the iNOS exists as a heterodimer with kalirin.

There is little monomeric iNOS, indicating that kalirin inhibits the iNOS activity, preventing its homodimerization. A low expression of kalirin correlates with the increased iNOS activity in the hippocampus of patients with Alzheimer's disease. As the excess NO produced during inflammation is neurotoxic, calilirin may have a neuroprotective effect by inhibiting iNOS. In macrophages, iNOS interacts with 110 kDa protein called NOS-associated protein 110 (NAP110). NAP110 and iNOS complexes are found in the spleen and peritoneal macrophages of interferon-γ (IFN-γ) and LPS-treated mice. A co-expression of NAP110 and iNOS reduces the activity of the latter by 90%, although the amount of the iNOS protein does not change. Like kalirin, NAP110 inhibits the iNOS activity by interacting with iNOS monomers and preventing enzyme dimerization. Therefore, NAP110 may be a blocking regulator that prevents the increase in NO levels from toxicity [37].

NO can negatively regulate the iNOS activity. In stimulated mouse macrophages, the iNOS activity is increased by the addition of hemoglobin, an NO acceptor. The addition of NO donors S-nitrosoacetylpenicillamine or S-nitrosoglutathione significantly inhibits the iNOS activity, suggesting that NO may be involved in a negative feedback mechanism, but much weaker than that of other isoforms. In addition, the iNOS activity cannot be restored in activated cells without NO donors, indicating irreversible binding of NO to iNOS. A kinetic study showed that the main mechanism of iNOS autoinactivation is S-nitrosylation of the zinc tetrathiolate cluster, which causes a zinc loss, an irreversible dissociation of the iNOS dimer and a subsequent loss of its activity. Reducing agents may protect iNOS from this inactivation, suggesting that S-nitrosylation was indeed the main inactivation pathway [37].

Mitochondrial NO synthase

Although NO is a highly diffusible molecule that can cross membranes and reach mitochondria, there is also evidence that mitochondria produce NO [38] and that mitochondrial NOS exists. However, the identity of this mitochondrial NOS is still under debate [39, 40]. Several studies have shown that various tissues such as skeletal muscles [14], the heart [42], kidneys, the brain [42] and liver [41, 42], contain NOS (possibly classified as mitochondrial) with a positive immunoreactivity for antibodies against iNOS [41], eNOS and nNOS. In addition, Gao S. et. al. [43] were able to better determine the localization of eNOS on the cytoplasmic surface of the outer mitochondrial membrane in endothelial cells [43]. Elfering S.L. et. al. characterized the rat mitochondrial NOS sequence as a nNOSα isoform that can be modified by acylation, it is calcium sensitive, and has phosphorylation sites [44]. They hypothesized that these post-translational modifications might be responsible for targeting NOS at the mitochondrial inner membrane.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

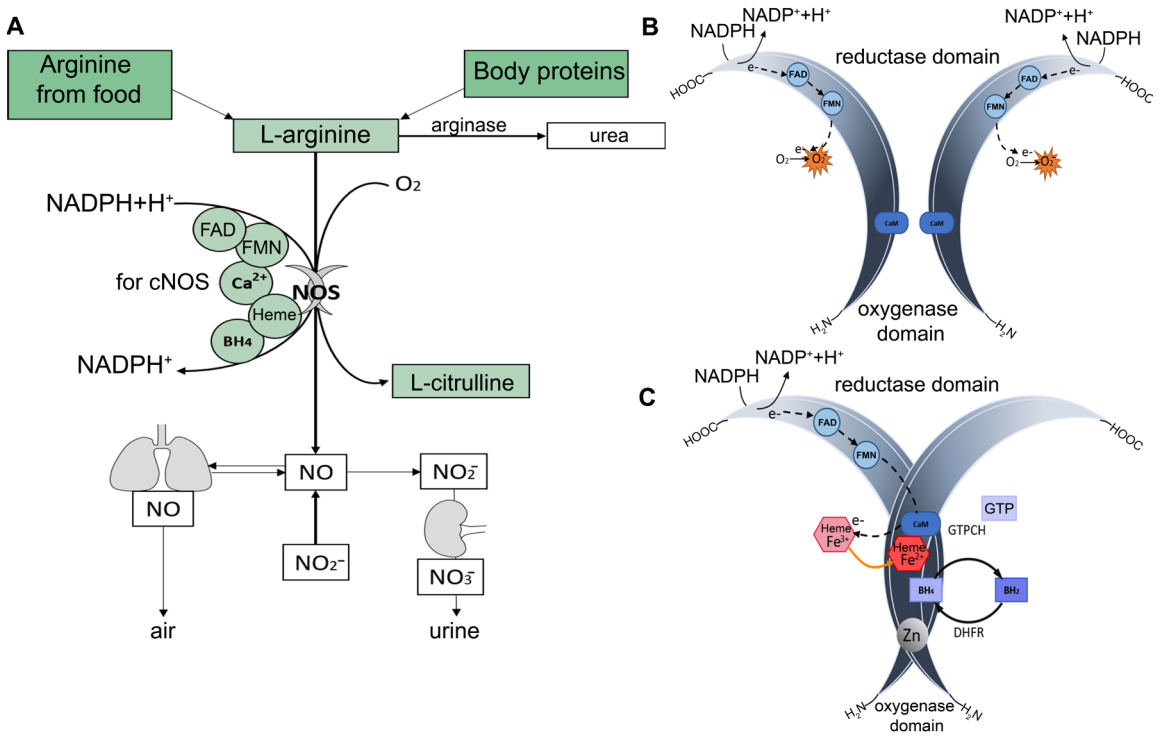

Figure 1 – Schematic pathway for NO synthesis, including enzymatic (via NOS; main pathway) and non-enzymatic pathways (A), structure and mechanism of NOS (B, C) action

Note. A) The important sources of NO include a NOS activity and a nitrite reduction (NO_2^-) under hypoxic and acidic conditions. NO reacts with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$). Superoxide can be obtained from several sources, including a mitochondrial activity and a NADPH oxidase (NOX) activity. When protonated or combined with carbon dioxide (CO_2), peroxynitrite produces a range of free radicals, including nitrogen dioxide (NO_2) as well as hydroxyl (OH) and carbonate (CO_3^-) radicals. NO reacts with metals such as iron (FeII) to form metal nitrosyls (FeIINO) such as the one found in soluble guanylate cyclase. NO also reacts with molecular oxygen to form nitrogen dioxide and nitrogen trioxide. Together, these particles can participate in oxidative (such as thiol oxidation) reactions, nitrosation (thiol nitrosation, RSNO) and nitration (tyrosine nitration, NO_2 Tyr) of biological targets; B) NOS monomers are able to transfer electrons from reduced NADPH to FAD and FMN and have a limited ability to reduce molecular oxygen to superoxide (O_2^-). Monomers and isolated reductase domains can bind calmodulin (CaM), which enhances the electron transfer within the reductase domain. NOS monomers are unable to bind the BH_4 cofactor or the substrate L-arginine and cannot catalyze the NO production; B) In the presence of heme, NOS can form a functional dimer. Heme is required for *a* cross-domain electron transfer from flavins to heme of the opposite monomer. Adapted from [17(a) и 21(b и c)].

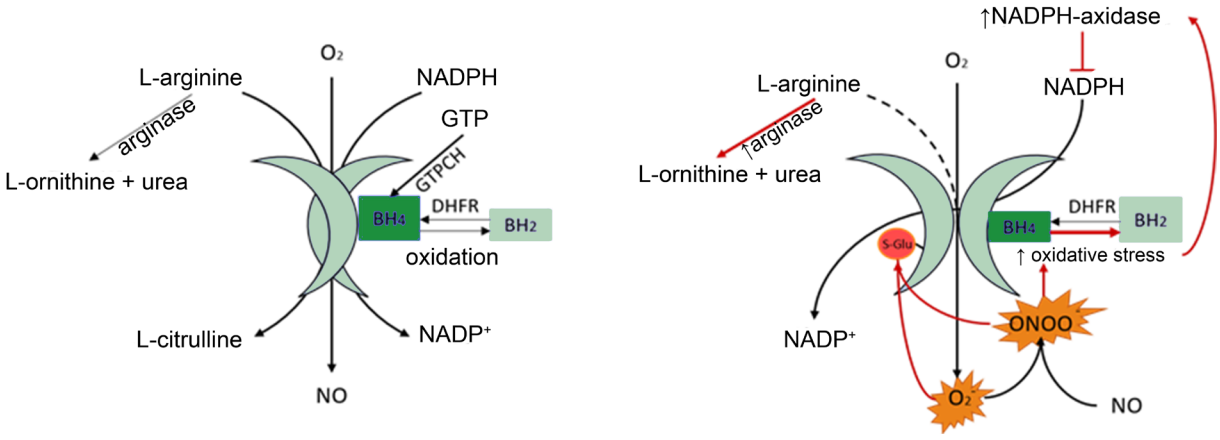

Figure 2 – Synthesis, recycling and oxidation of BH_4 as the determinant of NOS uncoupling

Note. On the left: Under normal conditions, BH_4 bioavailability is maintained by *de novo* synthesis from guanosine triphosphate (GTP) in which the rate limiting step is catalyzed by GTP cyclohydrolase (GTPCH) by dihydrofolate reductase (DHFR) mediated recycling of 7,8-dihydrobiopterin (BH_2), the primary product of a non-enzymatic oxidation of BH_4. On the right: "Unbound" NOS are characterized by the formation of superoxide (O_2^-). NOS cleavage is facilitated by a decrease in the bioavailability of BH_4 relative to the BH_2 or NOS protein. In turn, O_2^- formed by unbound NOS, reacts with NO to form peroxynitrite ($ONOO^-$), a highly reactive anion that rapidly oxidizes BH_4. Hence, the NOS uncoupling state is stabilized by the self-propagating oxidative stress. In addition to this primary BH_4-mediated cycle, additional mechanisms have been shown to promote uncoupling, including a reduced arginine bioavailability, high levels of oxidized glutathione (GSSG) compared to reduced glutathione (GSH), or elevated concentrations of endogenous NOS inhibitors LN-monomethylarginine (L-NMMA, monomethyl-L-arginine) and asymmetric dimethylarginine (ADMA). Adapted from [60].

DOI: 10.19163/2307-9266-2022-10-2-130-153

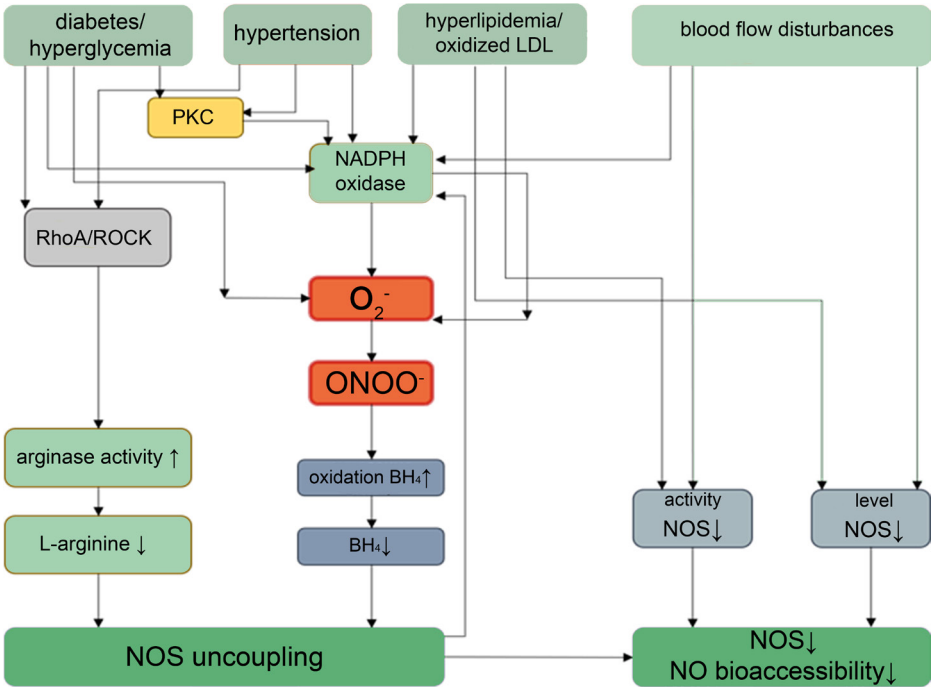

Figure 3 – Pathological conditions and mechanisms of NOS uncoupling

Note. Hypercholesterolemia, hypertension, smoking, and diabetes mellitus lead to the activation of NADPH (a reduced form of nicotinamide adenine dinucleotide phosphate) oxidase, partially by a protein kinase C (PKC)-dependent mechanism. Diabetes mellitus also stimulates the production of mitochondrial ROS, that then triggers the activation of NADPH oxidase, which can increase a mitochondrial production of superoxide (O_2-) and xanthine oxidase. The endothelial NO synthase (eNOS) enzyme can be uncoupled through two main mechanisms: deficiency of the BH_4 cofactor or the L-arginine substrate. O_2·- reacts with NO to form peroxynitrite ($ONOO^-$). ONOO- oxidizes BH_4 resulting in BH_4 deficiency. L-arginine deficiency is caused by an increase in the arginase expression and activity, partially through RhoA/ROCK-dependent mechanisms. Unbound eNOS produces superoxide, thereby increasing the oxidative stress. The eNOS uncoupling reduces the endothelial NO production, which is further exacerbated by a decrease in the eNOS expression and activity. In addition to the population-level risk factors, the impaired blood flow makes arterial bifurcations and lateral branches prone to atherosclerosis. The increased oxidative stress and reduced endothelial NO production contribute significantly to the increased atherosclerosis in these areas. Adapted from [58].

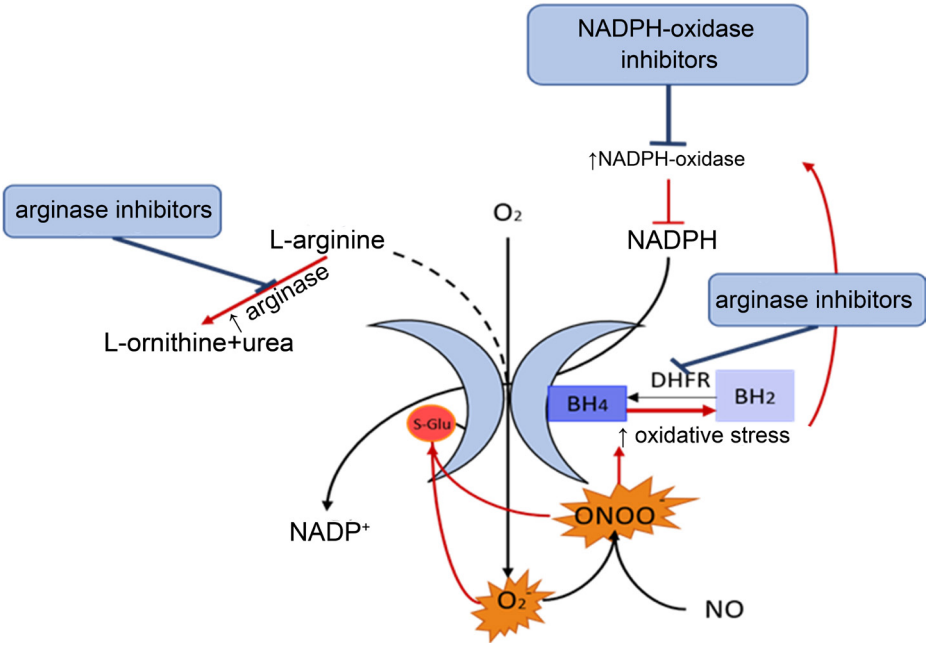

Figure 4 – Schematic representation of known causes and methods for correcting NOS uncoupling in the cardiovascular system

Note. Causes for NOS *uncoupling* are shown in red and treatments are shown in blue. Superoxide (O_2^-) or peroxynitrite ($ONOO^-$) can oxidize BH_4 to BH_2, which can be prevented by antioxidants or NADPH oxidase inhibitors, and interfere with s-glutathionylation (s-Glu). Folic acid can stimulate dihydrofolate reductase (DHFR) to reduce BH_2 to BH_4. Arginase inhibition can preserve L-arginine levels to make it available to the NOS enzime. Adapted from [59].

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

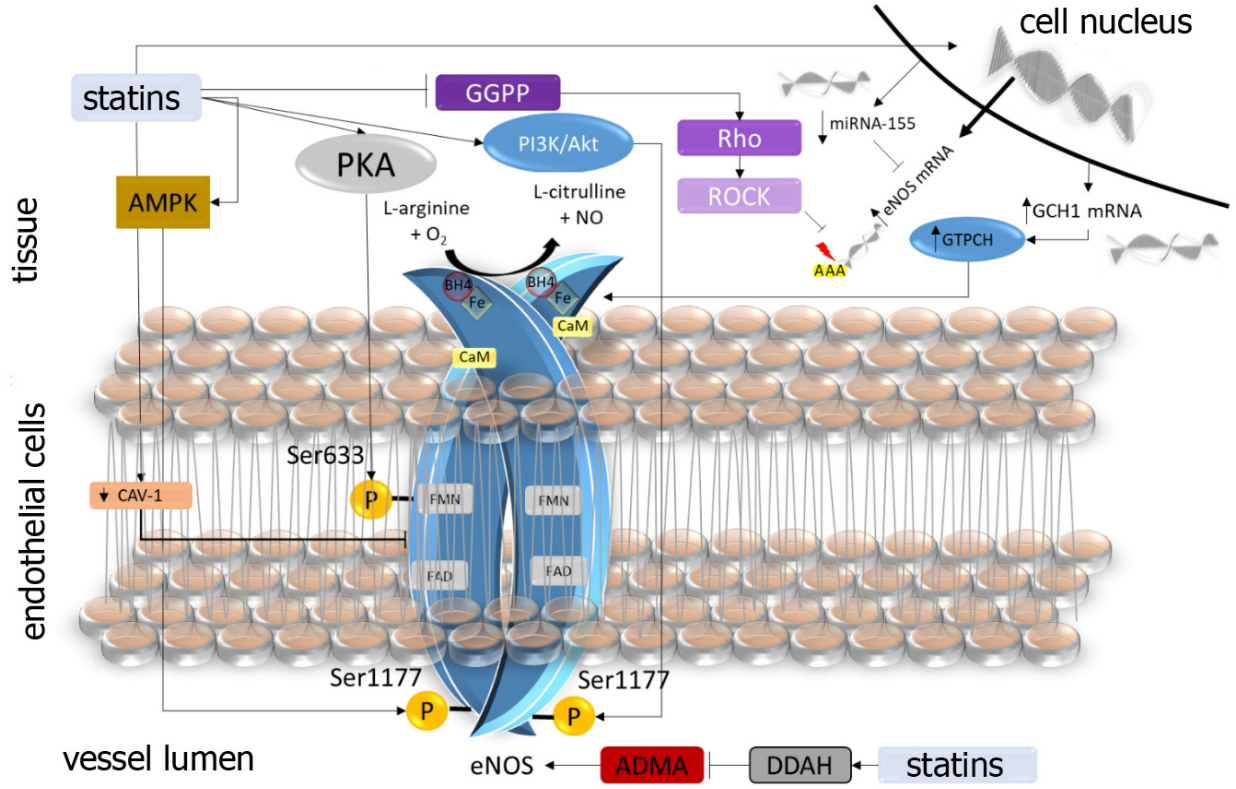

Figure 5 – Effect of statins on eNOS in endothelial cells

Note. By inhibiting mevalonate synthesis, statins reduce GGPP levels, prevent the Rho/ROCK activation and stabilize eNOS mRNA, thereby increasing the eNOS expression. By reducing caveolin-1 and circulating ADMA, as well as increasing eNOS phosphorylation at the Ser-633 and Ser-1177 activation sites via AMPK, Akt, and PKA, statins also increase the eNOS activity. Finally, by upregulating GTPCH, statins increase endothelial BH_4, the presence and restoration of eNOS binding. GGPP is geranylgeranyl pyrophosphate; ROCK – Rho-associated protein kinase; GTPCH – guanosine triphosphate cyclohydrolase; AMPK 2 adenosine monophosphate activated protein kinase; PKA, protein kinase A; PI3K – phosphatidylinositide-3-kinase; ADMA - asymmetric dimethylarginine. Adapted from [77].

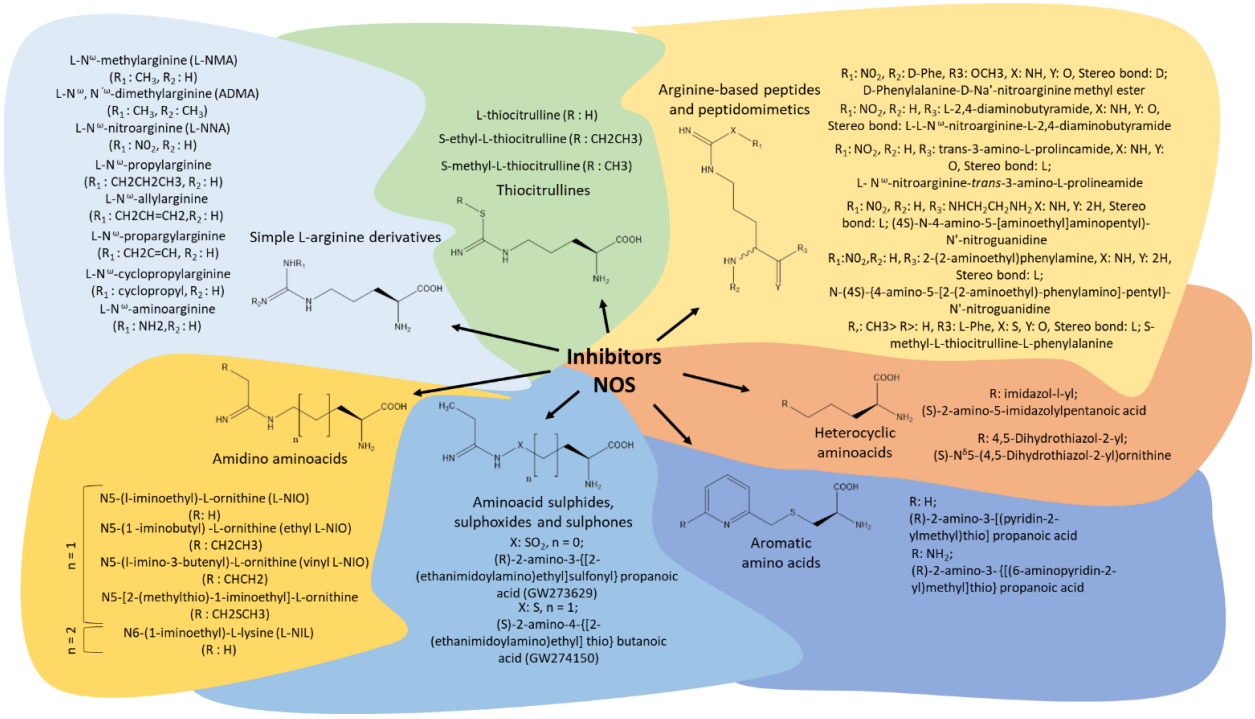

Figure 6 – List of the most important inhibitors based on arginine

Note. Adapted from [92].

DOI: 10.19163/2307-9266-2022-10-2-130-153

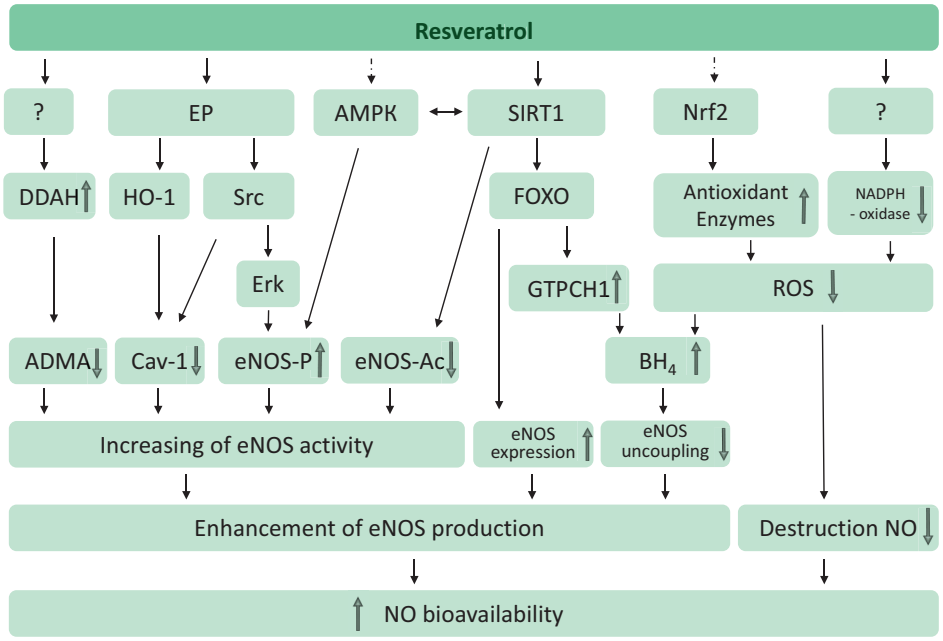

Figure 7 – Resveratrol targets to prevent NOS uncoupling (80 adapted)

Note. Resveratrol increases the NO production and prevents its breakdown. Resveratrol can activate sirtuin 1 (SIRT1) either directly (in a substrate-dependent manner) or indirectly (by inhibiting phosphodiesterases). SIRT1 stimulates endothelial NOS via deacetylation, enhances the eNOS expression via deacetylation of the forkhead box protein O1 (FOXO) transcription factor, and prevents the eNOS uncoupling by increasing the activity of GTP-cyclohydrolase I (GTPCH-I), a limiting factor in BH_4 biosynthesis. AMPK and nuclear factor 2 associated with Nrf2 are indirect targets of resveratrol. AMPK phosphorylates eNOS by serine 1177. eNOS can also be phosphorylated by Erk1/2, which is stimulated by a pathway involving the estrogen receptor (ER) and the Src family tyrosine kinase. Caveolin-1 (Cav-1) is a protein that negatively regulates the eNOS activity. ADMA is an endogenous eNOS inhibitor that is cleaved by DDAH. The targets for the activation of dimethylarginine dimethylaminohydrolase (DDAH) or inhibition of NADPH oxidase have not been identified yet. Adapted from [80].

Table 1 – Characteristics of various NO-synthase isoforms

Names	NOS-1 nNOS Neutral NOS NOSI	NOS-2 iNOS Induced NOS NOSII	NOS-3 eNOS Endothelial NOS NOSIII
Monomer size (person)	~160 кDa	~131 кDa	~133 кDa
Cofactors	NADPH; FMN; FAD; BH_4; Calmodulin; heme		
Inhibitors	N-methyl-L-arginine N-nitro-L-arginine 7-nitroindazole 1-(2-trifluoromethylphenyl)imidazole L-thiocitrulline S-methyl-L-thiocitrulline	Aminoguanidine S-benzylisothiourea 1-(2-trifluoromethylphenyl)imidazole L-N6-(1-iminoethyl)lysine 1400W	N-methyl-L-arginine N-nitro-L-arginine N-iminoethyl-L-ornithine 7-nitroindazole
Intracellular localization	Cytosol; Plasma membrane [99]; Mitochondria [100]; Caveoli [101].	Plasma membrane [102]; Phagosomes [103]; Golgi complex; Mitochondria.	Plasma membrane; Lipid rafts; Caveoli [104]; Cytosol [100].
Cells	Neurons [105]; Islets of the pancreas [106]; Endothelial cells; Smooth muscle cells [107].	Macrophages and other leukocytes [108]; Smooth muscle cells; Cardiomyocytes [35].	Endothelial cells [109]; Platelets [25].
Tissue expression	Central and peripheral nervous system [105]; Skeletal muscles [110]; Endometrium [111]; Macula densa [112].	Heart; Liver [113]; Smooth muscles; Endothelium [113].	Endothelium [114].
Functions	Neurotransmission [105]; Renal tubular glomerular interactions [115]; Intestinal peristalsis [116].	Bactericidal activity; Cytotoxicity; Inflammation [117]; Septic shock [118].	Vascular relaxation Decreased platelet adhesion [119, 120]; Angiogenesis [121].
Involvement in diseases	Renal diseases [115]; Affective disorders [122].	Inflammation	Hypertension; Endothelial dysfunction.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Confirming the results obtained by Elfering S.L. et al., Kanai A.J. et al. showed that mitochondria isolated from the hearts of mice in which the nNOSα isoform had been knocked out, did not produce NO, while eNOS-/- and iNOS-/- knocked out the mice, retaining the NO production in isolated mitochondria [45]. Lacza Z. et al. were able to detect the NO production, the NOS activity, and low levels of eNOS protein in mitochondria. However, the NOS activity was not impaired in the eNOS knockout mice [46]. In addition, the mitochondrial NO production was not affected by the nNOS and iNOS knockout mice.

Venkatakrishnan P. et al. were unable to detect the activity or expression of endothelial and neuronal NOS isoforms in rat liver mitochondria, casting doubt on the existence of mitochondrial NOS, at least in the liver [47].

Regardless of this controversy over the existence or identity of mitochondrial NOS, the recent studies show a stronger relationship between mitochondrial NOS and the respiratory chain. Parihar et al. suggested that respiratory chain complex I regulates the mitochondrial NOS activity, demonstrating the complex I-associated mitochondrial NOS activity in the isolated rat liver and brain mitochondria [48]. In addition, a study of the cardiac mitochondrial preparations demonstrated that complex I enriched fractions have higher mitochondrial NOS (nNOSα isoform), indicating that mitochondrial NOS is physically close to the complex I [49].

The conflicting results regarding the existence and identity of mtNOS can be explained by several methodological issues, which have been discussed in detail by Brookes P.S. [50]. Some of these problems include mitochondrial purity, the presence of other cell types in tissues (eg, vascular endothelial cells, inflammatory cells), the detection of the mitochondrial NO production, and the immunodetection of NOS isoforms. If mtNOS does exist despite methodological problems, the identity of mtNOS could also be related to the tissue of origin, which could explain the inconsistency in the identified isoform, for example, mtNOS could be an isoform of neurons in the brain, while iNOS could be in the brain and liver.

The existence of mtNOS can be confirmed by the relevance of NO in the control of mitochondrial respiration, since, when produced in mitochondria, NO will have a quick and easy access to its target, cytochrome oxidase, which is inhibited by NO [51]. However, it is also argued that, given the high diffusivity of NO, it is not necessary that it be produced near its site of action [52]. For example, NO can be produced by eNOS in vascular endothelial cells or by eNOS located on the surface of the cytosol of the outer mitochondrial membrane, then diffuse through other compartments reaching the respiratory chain [52].

Nitric oxide receptors

The most important physiological signaling pathway stimulated by NO, is the activation of soluble guanylate cyclase and the formation of cyclic guanosine monophosphate (cGMP). Soluble guanylate cyclase (sGC) is a key protein in the regulation of many physiological functions in mammals. This is a major NO receptor that plays a central role in the BP regulation, wound healing, memory formation, and other key physiological processes. The involvement of rHC is oftener and oftener found in various pathophysiological processes [53].

Since the rate constant of the NO association with heme centers is usually very high compared to other potential targets, even low, nanomolar, concentrations of NO are sufficient to activate sGCs. Rapid reactions between NO and targets with such kinetics limit alternative signaling processes that may otherwise be detrimental to cell functions. Upon the NO stimulation, sGC generates cyclic guanosine monophosphate from guanosine-5'triphosphate and activates protein kinase G-dependent signaling, required for various signaling cascades in several cell types. It is well known that dysregulation of sGC signaling leads to dysfunction of the cardiovascular, nervous, and gastrointestinal systems [54].

The physiologically significant mammalian sGC heterodimer consists of α1 and β1 subunits and is expressed in most cell and tissue types; however, two other sGC subunits, α2 and β2, have been identified, and mixed heterodimers have been discovered, in particular α2β1, which is expressed in the brain, placenta, spleen, and uterus.

Both α and β subunits contain the cyclase homology domain and belong to the class III cyclase family. Since the active site of sGC is located at the heterodimer boundary, two active centers are possible, but when subunits are connected, catalytic and pseudosymmetric pockets are formed [55].

Various physiological studies have shown that the β1 subunit of the heterodimer is necessary but not sufficient for proper sGC signaling; both α1 and α2 subunits can heterodimerize with the β1 subunit to form a functional protein. The conservative histidine on the β1 subunit was found to coordinate the heme moiety, which preferentially binds NO and carbon monoxide (CO) rather than oxygen (O_2). Structural changes caused by the NO binding, activate the catalytic domain of the sGC protein, which causes vasorelaxation [56].

Elucidation of the NOS role in physiological and pathophysiological processes in the body NOS uncoupling as a basis for pathophysiological conditions

Under physiological conditions, eNOS produces NO, which is a key vasoprotective factor in the endothelium.

DOI: 10.19163/2307-9266-2022-10-2-130-153

However, under pathological conditions associated with an oxidative stress, eNOS may cease to function. The oxidative stress contributes to endothelial dysfunction primarily due to the rapid inactivation of NO via oxidation by excess superoxide. At the second stage, a constant oxidative stress renders eNOS unbound, whereby superoxide is produced at the expense of NO. The deficiency of the eNOS cofactor tetrahydrobiopterin (BH_4), the deficiency of the eNOS substrate, L-arginine, and S-glutathionylation of eNOS are likely the main causes of the eNOS uncoupling. Peroxynitrite and superoxide can oxidize BH_4 leading to the BH_4 deficiency. The production of ROS from unbound eNOS has been shown in mouse models of atherosclerosis, as well as in patients with hypercholesterolemia, atherosclerosis, hypertension, diabetes mellitus, and in people who use nicotine for a long time [57].

Under conditions of atherosclerosis and vascular diseases, the bioavailability of NO in the vasculature decreases. All the known risk factors for cardiovascular diseases are accompanied by a decrease in NO bioavailability as a result of a decrease in its production and an increase in the superoxide inactivation.

Thus, a decrease in the NO production is not the result of a decrease in the eNOS expression. On the contrary, the eNOS expression is compensatory increased in vascular diseases. However, this compensation mechanism is often useless because the eNOS activity is inhibited or the eNOS enzyme becomes uncoupled (inoperative) [58].

Molecular mechanisms of NOS uncoupling

The most significant cause for NOS uncoupling is the loss of the critical cofactor BH_4. It can be synthesized in two ways: in a three-step process from guanazine triphosphate (GTP) to BH4 and the so-called “rescue route”, which recycles oxidized 7,8 dihydrobiopterin back to BH_4 using the recycling enzyme dihydrofolate reductase (DHFR, dihydrofolate reductase). An oxidative stress plays a significant role in the depletion of cellular BH_4 pools, leading to uncoupling. Superoxide can directly oxidize BH_4 to dihydrobiopterin (BH_2) and, therefore, destabilize the NOS dimer, leading to uncoupling. This appears to be the main reason for NOS uncoupling during hypertension and ischemic reperfusion, as increased superoxide levels correspond to a decrease in the BH_4/BH_2 ratio and subsequent NOS uncoupling. The oxidative stress can also affect the bioavailability of BH_4 not only through oxidation, but also through a direct decrease in the DHFR expression, thereby reducing the activity of the “rescue pathway” [59].

A direct oxidation of BH_4 by superoxide is relatively slow, but BH_4 is rapidly oxidized to BH_2 by peroxynitrite, a highly reactive anion formed by the reaction of superoxide with NO. Peroxynitrite also causes significant cell damage by nitrating the thiol and hydroxyl groups of the amino acid side chain, thereby impairing the enzymatic function.

The consequences of NOS uncoupling include: reduced the *de novo* NO formation; binding of bioactive NO by superoxide anions through the formation of peroxynitrite; cellular damage mediated by superoxide and peroxynitrite; the peroxynitrite mediated oxidation of BH_4 to BH_2 leading to a further propagation of NOS uncoupling.

Thus, NOS uncoupling is a self-reinforcing biochemical process driven by BH_4 oxidation. Due to the unbound eNOS-dependent oxidative stress, BH_4 oxidation has been observed both *in vitro* and *in vivo.* In these experiments, uncoupling was induced by increasing the eNOS expression under the conditions of a stable expression of GTP-cyclohydralase (GTPCH-I), effectively reducing the ratio of BH_4 to eNOS. If all other variables are held constant, the resulting oxidation of BH_4 to BH_2 can only be due to the formation of superoxide by the decoupled NOS. Cellular superoxide is also produced by a number of other mechanisms, including NADPH oxidase, xanthine oxidase, and redox processes. In pathology, the oxidative stress can induce BH_4 oxidation, thereby initiating or maintaining NOS uncoupling. Excess superoxide resulting from the initial BH_4 oxidation and NOS uncoupling will stimulate a cycle of cellular damage, a further BH_4 oxidation (BH_4 oxidation stabilizes unbound NOS subunits that continue to produce ROS and biochemical stabilization of the unbound NOS subunit activity). The interruption of this communication process is a promising strategy for the treatment of a wide range of cardiovascular diseases caused or exacerbated by the NOS uncoupling (Fig. 2) [60, 61].

Peroxynitrite leads to the NOS uncoupling. Although peroxynitrite can directly oxidize BH_4 *in vitro*, it can also directly affect the enzyme itself, which also leads to uncoupling: peroxynitrite releases zinc from the zinc-thiolate cluster and destroys the NOS dimer (Fig. 3) [59].

S-glutathionylation is a reversible post-translational modification that adds glutathione to reduced cysteine residues. This most often occurs during the oxidative stress, as eNOS acts as one of the main targets for S-glutathionylation. It has been found out that the eNOS-dependent production of NO and superoxide is modulated by its S-glutathionylation, indicating that intense S-glutathionylation correlates with a decrease in the NOS activity and an increase in the superoxide production. The increased oxidative stress causes a proportional increase in the amount of S-glutathionylated eNOS, which also indicates that the production of superoxide by the upregulation principle is a trigger for the eNOS uncoupling [59].

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Thus, S-glutathionylation uncouples eNOS, switching it from NO to the ROS formation, by attaching glutathione to cysteine residues between the FMN-binding and FAD-binding domains: this leads to enzyme uncoupling and the production of O_2- free radicals. The oxidative stress triggers S-glutathionylation of eNOS in endothelial cells and intact vessels. In addition, S-glutathionylation is increased during vasoconstriction, which reduces endothelium-dependent vasodilation. Given the importance of NO and eNOS mediated endothelial dysfunction in diseases such as heart attack, stroke, diabetes and cancer, the identification of this new redox signaling pathway provides new insights into therapeutic approaches to prevent or treat them [62].

Drugs for prevention of NOS uncoupling

As it has been noted above, NOS uncoupling is one of the main factors in the development of various diseases and, therefore, a promising target for the pharmacological intervention. Many approaches have been taken to try restoring NOS in various diseases, including: blocking the upstream of the ROS production, the BH_4 administration, the administration of folic acid to recycle BH_2 to BH_4, and the L-arginine administration (Fig. 4).

BH_4

A decreased BH_4 bioavailability followed by the eNOS uncoupling has a significant impact on the pathogenesis of endothelial dysfunction, which is a hallmark of vascular damage in cardiovascular pathologies, including hypertension, hyperlipidemia, and diabetes [63]. The intracellular ratios of BH_4/eNOS (and of BH_4 and other NOS isoforms) remain poorly defined, making it unclear whether the intracellular BH_4 deficiency is sufficient to induce the eNOS uncoupling. The BH_4/BH_2 ratio and the absolute molar concentration of BH_4 are key determinants of eNOS binding *in vivo*. The degree of BH_4 oxidation, BH_2 accumulation, and the superoxide production are directly correlated with the intracellular eNOS/BH_4 ratio. Through the mechanisms independent of eNOS, such as a direct uptake of reactive oxygen species, BH_4 can have a significant impact on the levels of the reactive oxygen species production by cells [64].

Thus, the replenishment of intracellular BH_4 can be considered a promising approach to stabilize NOS. However, there are reasons that limit the clinical application of this approach. The oral administration of excess BH_4 may be not effective if the NOS isoforms ratio is not balanced: too much BH_4 can activate iNOS under the conditions of inflammation, exacerbating the pathophysiology of this process. BH_4 administered p. o., is likely to oxidize and needs to be regenerated, but the attempts to enhance this re-regeneration process by combining BH_4 with antioxidants (vitamin C) have not resulted in the improved clinical efficacy of this combination. BH_4 has already been approved by the Food and Drug Administration (FDA, https://www.fda.gov/) as a therapeutic agent for the treatment of phenylketonuria, a phenylalanine hydroxylase deficiency that can be partially corrected by the administration of BH_4. Sapropterin dihydrochloride (Kuvan®, BioMarin, Tiburon, CA, www.vidal.ru) is a synthetic version of BH_4 currently used to treat patients with phenylketonuria [65, 66].

An analog of BH_4 is the compound 6-acetyl-7,7-dimethyl-5,6,7,8-tetrahydropterin (ADDP), a stable compound, soluble in both polar and organic solvents. ADDP can diffuse from plasma through cell membranes and cause vasodilation by stimulating the eNOS activity [67].

Metformin can attenuate the oxidative damage to endothelial cells by increasing the levels of GTP cyclohydralase-I (GTPCH-I) and BH_4, and thereby preventing the eNOS uncoupling. Metformin also inhibits the p47-phox subunit of NADPH oxidase, thereby reducing the ROS production and BH_4 oxidation. These protective effects of metformin are partially mediated by the activation of the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway [68].

Arginase inhibitors

The role of the arginase overexpression and/or activity and its downstream targets in cardiovascular dysfunction and injury, has been well established. Pharmacological effects on specific components of the arginase/ornithine pathway are promising as therapy for cardiovascular diseases [69].

Ureohydrolase arginase is a manganese-containing enzyme that catalyzes the final step of the ornithine cycle by converting L-arginine to L-ornithine and urea. The two isoforms of this enzyme have similar mechanisms of action and produce the same metabolites. The homology of their amino acid residues is more than 60%, and the regions important for the function of the enzyme are 100% homologous. In addition, both isoforms are involved in the dysregulation of the NOS function [70].

The arginase-induced uncoupling of eNOS leads to the increased ROS production, which contributes to the endothelial dysfunction and increased vascular stiffness. Increasing the level of arginase can reduce the concentration of L-arginine and has been shown to contribute to the pathophysiology of the circulatory system diseases [71].

Preclinical studies have shown that the addition of L-arginine can improve the erectile dysfunction, prevent or reduce the severity of the endothelial dysfunction. However, some studies on animals and humans have found out no benefit or increased adverse effects from

DOI: 10.19163/2307-9266-2022-10-2-130-153

the chronic administration of L-arginine. The resulting side effects are likely related to the counter-regulatory effects of L-arginine in the increasing arginase expression/activity, which may reduce the availability of L-arginine for NOS. Under the conditions of the excessive arginase activity, a competition with NOS for L-arginine causing the enzyme uncoupling, will occur. The increased formation of reactive oxygen species and key inflammatory mediators contributes to a pathological increase in the arginase activity [71].

Thus, several amino acids inhibit arginase, including: L-ornithine, L-leucine, L-valine, L-lysine, L-isoleucine, and L-norvaline. L-ornithine is the most potent of the above-listed. In addition, L-citrulline increases the NO production, but is also an allosteric inhibitor of arginase [71].

The research by El Bassossy H.M. et al. showed that the inhibition of arginase by citrulline, norvaline, or ornithine, alleviates hypertension associated with a metabolic syndrome by direct and indirect defense mechanisms. The direct mechanism is to maintain an endothelium-dependent relaxation and the NO generation, while the indirect mechanism is realized through the inhibition of insulin resistance and hypertriglyceridemia [72]. Ornithine inhibits the enzyme arginase by competing with its substrate (L-arginine) at the active sites of the enzyme. L-norvaline is a noncompetitive inhibitor of the enzyme arginase, and L-citrulline is its allosteric inhibitor.

L-norvaline (or 2-aminopentanoic acid) is a non-proteinogenic amino acid and an isoform of the common amino acid valine that has been extensively investigated in early enzymological studies. The structural similarity with L-ornithine gives the substance the ability to inhibit arginase by the negative feedback principle. In addition, the anti-inflammatory properties of L-norvaline have been demonstrated *in vitro* through the inhibition mechanism of ribosomal protein kinase S6-kinase β1. It should be notified that the arginase inhibition is promising for reducing the risk and frequency of cardiovascular diseases [73].

Statins inhibit HMG-CoA reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid, limiting cholesterol biosynthesis in the liver. In addition to inhibiting cholesterol synthesis, statins also block the synthesis of intermediate isoprenoids such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). FPP and GGPP serve as important lipid components for post-translational modification of various proteins, including heterotrimeric G proteins and small GTP-binding proteins belonging to the Ras, Rho, Rap, and Rab family of GTPases. Isoprenylation is critical for the intracellular transport and the function of small GTP-binding proteins. The modification with FPP is required for a proper localization of Ras family proteins, while GGPP is required for Rho, Rab and Rap family proteins. However, some Rho GTPases require both farnesylation and geranylgeranylation for proper functioning and the intracellular localization. By inhibiting the synthesis of mevalonate, statins inhibit the synthesis of intermediate isoprenoids, thereby preventing the isoprenylation of small GTPases, which leads to the inhibition of these signaling molecules. Interestingly, some of the cholesterol-independent or “pleiotropic” effects of statins may be related to the ability of statins to block the synthesis of intermediate isoprenoids [74, 75].

The ability of statins to increase the eNOS expression and activation may be an important mechanism by which statins improve the endothelial function and lower cholesterol levels (Fig. 5).

Geranylgeranylation of Rho GTPase has been shown to result in the downregulation of eNOS in endothelial cells; the administration of statins inhibited the formation of GGPP and therefore resulted in an increase in the eNOS expression, which was not observed during the co-incubation with GGPP. It has also been shown that an increased level of endogenous low-density lipoproteins (LDLs) negatively affects the expression of eNOS mRNA in human umbilical vein endothelial cells (HUVECs), and this effect, at the post-transcriptional level, was neutralized after the administration of simvastatin. The mechanism by which statins increase the eNOS mRNA stability is through an increased polyadenylation of eNOS mRNA due to the changes in the actin cytoskeleton caused by the Rho inhibition. In addition, the statin treatment of HUVECs induced by hydrogen peroxide (H_2O_2) activates the phosphatidylinositide 3-kinase (PI3K, phosphoinositide 3-kinase)/Akt pathway and enhances the eNOS expression. Tumor necrosis factor alpha (TNFα) is a powerful inflammatory stimulus that activates miR-155 miRNA and, accordingly, eNOS mRNA. It is important to notify, that its (TNFα) activation was attenuated by simvastatin, but the positive effect of the statin was abolished by co-incubation with mevalonate or GGPP [76].

In addition to increasing the eNOS expression at the transcriptional and post-transcriptional levels, at the post-translational level, statins enhance the activity of eNOS, which has multiple phosphorylation sites by serine/threonine residues. A HUVEC exposure to fluvastatin increases eNOS phosphorylation at the Ser-633 and Ser-1177 activation sites via protein kinase A (PKA) and the PI3K/Akt-mediated pathway, respectively. The statin-mediated induction of eNOS phosphorylation at Ser-1177 depends on the recruitment of Akt to the eNOS complex in endothelial cells (heat shock protein-90). In endothelial cells, atorvastatin increases eNOS phosphorylation

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

at Ser-633 via adenosine monophosphate-activated protein kinase (AMPK). Moreover, the *ex vivo* incubation of rat mesenteric resistant arteries with simvastatin, induces rapid AMPK-mediated eNOS phosphorylation at Ser-1177. In addition to modulating phosphorylation, statins also increase the eNOS activity at the post-translational level, causing it to dissociate from caveolin-1. Atorvastatin reduces the content of caveolin-1 and activates eNOS in EC, regardless of the presence or absence of LDL cholesterol [77].

Statins act at several levels, increasing both eNOS activity and stabilizing its dimers. The exposure of HUVEC to cerivastatin or fluvastatin results in the increased GTPCH expression and the increased BH_4 bioavailability, resulting in improved eNOS binding. In addition, statins prevent the oxidation of tetrahydrobiopterin to trihydrobiopterin (BH_4 to BH_3). The result of these processes is a decrease in the oxidative stress in endothelial cells and an improvement in the eNOS function [76].

Asymmetric dimethylarginine (ADMA) acts as an endogenous inhibitor of eNOS. ADMA is produced as a by-product of protein metabolism due to methylation of L-arginine residues, its production is significantly increased by LDL in endothelial cells, and it is metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH); under physiological conditions, it is excreted in the urine. Due to its close structural similarity to L-arginine, ADMA suppresses the eNOS activity either through the inhibition of the L-arginine cellular uptake or through a direct competitive inhibition of eNOS binding [78].

There are several hypotheses about the ways statins affect ADMA levels. One of them concerns the inhibition of the ADMA-induced inflammatory response modulated by mitogen-activated protein kinase (MAPK) in human endothelial cells. Statins activate the transcription factor of sterol response element binding protein (SREBP) by lowering membrane cholesterol. This factor specifically enhances the expression of more than 30 genes associated with the synthesis and absorption of fatty acids, phospholipids, cholesterol and triglycerides. One of its isoforms, a sterol regulatory element binding protein 2 (SREBP-2), increases the transcription of proprotein convertase subtilisin/kexin type 9 (PCSK9, proprotein convertase subtilisin/kexin type 9). Statins increase both PCSK9 mRNA and LDLR levels by activating sterol-mediated SREBP-2, an important activator of the transcription and DDAH activity (Fig. 6) [79].

Resveratrol

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a phytoalexin polyphenol found in various plant species including *Veratrum grandiflorum* (Maxim. ex Baker) O. Loes, *Polygonum cuspidatum* Siebold & Zucc., *Vitis vinifera* L., *Arachis hypogaea* L. and *Morus rubra* L. The name "resveratrol" comes from its origin; the compound is a resorcinol derivative from the species of the genus *Veratrum*.

Molecular targets of resveratrol include those which interact directly with it and the others that are modulated indirectly (e.g., through changes at the expression levels). For its effect on eNOS, the following targets are of particular importance: NAD+ – dependent histone deacetylase sirtuin 1 class III (SIRT1, silent information regulator 1); AMP-activated protein kinase (AMPK); nuclear factor-derived erythroid 2 – dependent factor-2 (Nrf2, nuclear factor E2-related factor 2); estrogen receptors (ER).

A part of the resveratrol effects on the cardiovascular system is mediated by endothelial NO. Resveratrol enhances the NO production through several mechanisms and prevents the NO breakdown by reducing the oxidative stress (Fig. 7).

Activators of various NOS isoforms

Calcium dobesilate (Doxium 1)

Calcium dobesilate (CaD) is widely used as an angioprotective agent for the treatment of vascular diseases, inhibits platelet aggregation, thrombus formation, increased capillary permeability, reduces their fragility, increased blood viscosity.

A specific property of dobesilate is a moderate increase in the eNOS activity with little or no effect on the iNOS activity, which indicates its future outlook for the treatment of vascular diseases, especially those caused by impaired NO secretion in the endothelium, including prolonged hyperglycemia [81].

The mechanism of calcium dobesilate action on the eNOS expression is realized in several ways.

As a result of the study of the molecular and cellular mechanisms underlying the protective effects of CaD carrird out by Zhou Y. et al. [84], the effect of calcium dobesilate on pentraxin 3 (PTX3) was established. This protein is an important component of humoral immunity and is secreted by various cells, including endothelial, mononuclear macrophages, fibroblasts, adipocytes, dendritic and smooth muscles after the stimulation of the inflammatory response. High plasma levels of PTX3 are associated with many diseases conjugated with endothelial dysfunction (diabetes mellitus, arterial hypertension, chronic kidney disease). PTX3 interferes with eNOS phosphorylation by serine 1177, reducing NO production, leading to endothelial dysfunction.

PTX3 induces dysfunction and morphological changes in the endothelial layer via the P-selectin/matrix metalloproteinase-1 pathway, which prevents the sep-

DOI: 10.19163/2307-9266-2022-10-2-130-153

aration of eNOS from caveolin-1, resulting in impaired NO signaling [82]. Another study also confirms that PTX3 inhibits the NOS/NO pathway by inhibiting eNOS phosphorylation and therefore not inducing a detachment from caveolin-1 required for activation [83].

Thus, calcium dobesilate can inhibit the PTX3 expression by altering the IKK/IKB/NF-κ B pathway, thereby improving endothelial dysfunction at the cellular level [84].

Endothelial cells also need a vascular endothelial growth factor A (VEGF-A) to maintain their various functions (differentiation, permeability, and the NOS expression) and survive [85], however, an excessive amount of this factor causes their damage, increased glomerular permeability and inflammation. An appropriate balance of the VEGF expression, binding, and signaling is required to maintain glomerular health and prevent glomerulosclerosis and rarefaction. Under pathological conditions, this balance is disturbed [86].

Despite high concentrations of VEGF in the vasculature, a reduced NO response was called "uncoupling" of eNOS and VEGF, which leads to the disruption of the cellular response and the intensification of the pathological process course. In addition to its positive effect on eNOS, at the molecular level, calcium dobesilate also reduces the oxidative stress and inhibits growth factors such as fibroblasts growth factor (FGF) and VEGF [87].

CavNOxin

CavNOxin is a synthetic peptide of 20 amino acids in the framework domain of caveolin with a triple alanine substitution fused to the peptide penetratin for a free entry into cells.

CavNOxin increases NO levels, decreases a vascular tone, and lowers mean BP in wild-type mice, but the effect is lost in gene or caveolin-1 knockout animals, indicating that it is caveolin-1 specific.

A chronic CavNOxin administration reduces the severity of atherosclerosis in mice with apolipoprotein E (ApoE, apolipoprotein E) knockout, fed a high-fat diet, and in mice with diabetes and atherosclerosis, while the loss of eNOS genetically reduces its effect. These results suggest that CavNOxin may reduce the inhibitory effect of caveolin on the eNOS function, allowing increased endogenous NO biosynthesis. The data from genetic studies using transgenic mice with the endothelial-specific expression of F92A CAV1 confirm that the regulated expression of modified caveolin is sufficient to increase the NO bioavailability, which leads to a significant decrease in BP [88].

NOS transcription enhancers

AVE3085 and AVE9488 are two small molecules that have been identified by high-throughput screening able of enhancing the eNOS67 transcription *in vitro* and *in vivo.*

A promoter analysis using the luciferase test showed that both compounds bind even the shortest fragment of the eNOS promoter (263 polynucleotides), but do not overlap with known eNOS transcription factors (GATA, Sp1/3-like, Elf-1, YY1, Sp1 or PEA3).

AVE3085 has a promoter binding cis element located in the proximal 263 base pairs of the promoter region.

In the experiments on the human internal mammary artery obtained after a coronary artery bypass surgery, a positive effect of AVE3085 was shown. It was expressed in the prevention of the damaging effect of homocysteine on the endothelium during the co-incubation with AVE3085, while an increase in the level of eNOS mRNA and an increase in the NO production was observed [89].

These NOS transcription enhancers have also been shown to increase vascular BH_4 levels, reduce a cuff-induced neointima formation, and improve the course of atherosclerosis in ApoE knockout animals after 12 weeks of diet. AVE9488 promotes cardiomyocyte remodeling in a model of myocardial infarction, which can be explained by a decrease in SMAD-mediated signaling [90].

Blockers of various NOS isoforms

NOS inhibitors can be classified from different points of view.

A classical enzymological approach makes it possible to distinguish reversible (subgroups: competitive, non-competitive and mixed type), irreversible, as well as reactive inhibitors, the action of which depends on the enzymatic reaction.

The most widely used classification of inhibitors is based on the site of their binding to the NOS enzyme, which makes it possible to recognize four different classes of inhibitors.

The first class, in fact the largest, interacts with the arginine-binding site. Some of the compounds belonging to this class are reaction-level inhibitors because they require an active enzyme and NADPH for a complete inhibition. The second class includes a set of compounds that mimic the BH_4 cofactor. The third class is represented by inhibitors that directly interact with heme. Some inhibitors belonging to this class bind to the heme of the monomeric form of the enzyme and prevent the formation of its active dimer. The fourth class covers NOS inhibitors interacting with calmodulin or flavin cofactors [91].

There is also a classification according to which NOS inhibitors can be divided into two groups: based on amino acids (derivatives and close analogues of arginine) and based on non-amino acids (a wide range of

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

compounds that do not have an arginine-like amino acid backbone). Arginine-based NOS inhibitors have generated a particular interest as this category includes a large number of compounds with potential experimental and clinical applications. Since these compounds are readily available and stable in the aqueous media, they serve as an excellent tool for an experimental inhibition of NOS [91].

Non-selective inhibitors

Even before crystal structures had been created, arginine-based compounds were found to inhibit NOS and exhibit neuroprotective properties. For example, NG-mono-methyl-L-arginine (2 L-NMMA) and NG-nitro-L-arginine methyl ester (3 L-NAME) protect brain neurons in a stroke and Parkinson's disease. However, these inhibitors also cause hypertensive effects, most likely due to the inhibition of eNOS.

The earlier studies have shown that L-arginine substrate analogs have inhibitory properties against three NOS isoforms. Among them, NG-monomethyl-L-arginine (L-NMMA) and NG-nitro-L-arginine (L-NNA) and its precursor NG-nitro-L-arginine methyl ester (L-NAME) have been characterized as common NOS inhibitors and continue to be widely used in research, especially L-NAME [92].

Endogenous ADMA and L-NMMA inhibitors

There are two endogenous NOS inhibitors. ADMA is a potent, non-competitive NOS inhibitor, while its affined L-NMMA is a less potent, competitive NOS inhibitor. Although ADMA has been shown to contribute to the development of the inflammatory syndrome and endothelial dysfunction observed in shock, the possibility of its clinical application requires a further study [91].

546C88 (N(G)-methyl-L-arginine hydrochloride)

Compound 546C88 (N(G)-methyl-L-arginine hydrochloride) is a non-selective NOS inhibitor that appears to rebalance a vasomotor tone in patients with septic shock, by reducing the concomitant need for norepinephrine. It has been studied in phase III clinical trials in Europe, North America, South America, South Africa and Australia. This study was terminated in advance due to an increased risk of death [91].

L-NAME

N-nitro-L-arginine methyl ester (L-NAME) and Ng-nitro-L-arginine (L-NArg) are synthetic, non-selective NOS inhibitors with implications for a substance abuse because they attenuate signs of opioid withdrawal in rats. L-NAME also seems promising for the treatment of a septic shock by maintaining adequate BP levels. A chronic administration of L-NAME reduces angiogenesis during *in vitro* migration and invasiveness, pointing to its possible future use as a tumor growth inhibitor [91].

VAS203

Pterin 4-amino-tetrahydrobiopterin (VAS203) is a BH_4 analogue that can inhibit all NOS proteins by replacing the BH_4 cofactor. VAS203 has shown efficacy in the treatment of the traumatic brain injury and has been used in phase II clinical trials [92].

Selective inhibitors of iNOS

N-[3-(aminomethyl)benzyl]acetamidine

N-[3-(aminomethyl)benzyl]acetamidine is a specific iNOS inhibitor. It remains one of the most widely used NOS inhibitors in the research due to its cell and tissue permeability. Its selectivity appears to be associated with an irreversible effect on the more rapidly responsive iNOS, while the inhibition of nNOS and eNOS is reversible. The similar effect is observed in N(5)-(1-iminoethyl)-1-ornithine (1-NIO) [92].

7-nitroindazole

One of the first highly selective compounds is 7-nitroindazole (7-NI). Initial crystallographic studies showed that 7-NI binds at the active site of eNOS and changes the Glu orientation of the active site, while 3-bromo-7-NI can bind at both the active and pterin sites [93]. 7-nitroindazole is a specific nNOS inhibitor and exhibits an anticonvulsant effect on models of seizures in rodents. It has also been shown to occasionally induce convulsions in rodents caused by cainite, nicotine, and soman. Although it has been shown that all three isoforms can bind 7-NI with very similar affinity, in vivo studies have shown inhibition of nNOS without significant effect on BP. This may be due to the fact that 7-NI can be taken up by cells in different ways and the permeability of endothelial cells for this compound is limited. Thus, although 7-NI cannot be accurately described as a specific inhibitor of nNOS, it may behave as such in in vivo models [92].

Aminoguanidine

Aminoguanidine is a selective iNOS inhibitor that attenuates the "graft versus host" disease by reducing hematopoietic parameters and a concomitant susceptibility to the bacterial infection in mice [94].

L-NIL

An exceptional compound among the first arginine-based NOS inhibitors developed is L-NIL. It has a moderate selectivity for iNOS, is well tolerated, and its prodrug (L-NIL-TA) can be administered orally [95].

DOI: 10.19163/2307-9266-2022-10-2-130-153

GW273629 и GW274150

Compounds GW273629 and GW274150 are sulphur-substituted acetamidine amino acids that are specific iNOS inhibitors. Having safer toxicity profiles than 1400W, GW273629 and GW274150 have been used in clinical trials for migraine therapy (NCT00242866; NCT00319137). The both compounds were found to be ineffective, possibly due to the peculiarities of pharmacokinetics [91, 92, 95].

eNOS

As mentioned above (see section "Endothelial NO synthase"), eNOS is posttranslationally palmitylated and transported to caveolae, where its activity is reduced due to binding to caveolin-1. Based on this mechanism, it seems possible to mimic the function of caveolin-1.

Based on this approach, cavtratin, a drug that inhibits eNOS, was developed. It is a caveolin scaffold domain fused to the cell-permeable peptide penetratin, which allows caveolin to pass through the plasma membrane. Cavtratin significantly targets at eNOS, reducing its activity and mimicking the inhibitory effect of caveolin-1, while not affecting the function of other NOS *in vivo*. Cavtratin reduces an increased microvascular permeability and blocks a tumor progression (shown in the model of HepG2 hepatocarcinoma cells transplanted into mice without thymus), restores tight junctions in brain microvascular endothelial cells treated with chemokine-2, inhibits matrix metalloproteinase-2/9 and cyclooxygenase-2, which can lead to the stabilization of atherosclerotic plaques and reduce the risk of intracerebral hemorrhage. Cavtratin also reduces the growth of glial cells and has a protective effect on the blood-brain barrier when exposed to VEGF-A in a mouse model of multiple sclerosis.

It remains unknown whether eNOS is the only target of cavtratin. However, most of the blocking effects of VEGF and the Transforming growth factor (TGF) were reversed when the experiments were performed in eNOS-deficient mice.

It is possible that cavtratin may increase blood pressure during a course administration. However, there is no evidence to support this potential effect. Furthermore, since CAV1 is believed to regulate many intracellular signaling pathways, cavtratin may have pleiotropic effects on many tissues, especially those lacking eNOS [90].

Other approaches to inhibition

Inhibition of downstream mediators

Methylene blue is a soluble guanylate cyclase (sGC) inhibitor. It is a dye that can be safely used in the people with a septic shock. It provides an effective protection in the TNFα-induced shock as well as in anaphylactic hypotension not corrected by other pharmacological agents. Methylene blue may be also useful in refractory cases of vasoplegia, a common complication of cardiopulmonary bypass, by alleviating an inflammation-mediated dysregulation of the endothelial function [96]. 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ) is a selective sGC inhibitor in rats that restores a basal ganglia function and improves motor symptoms in Parkinson's disease [94].

NO recycling

An NO-sensitive hydrogel, which demonstrated its fast response and sensitivity to NO, was developed by the research group of Park J. et al. That presented a potential for the application in biology and medicine [96]. There are projects to evaluate the ability of NO-cleavable cross-linkers (NOCCLs) to capture NO for the treatment of rheumatoid arthritis (RA). This is the first study on the possibility of using endogenous NO directly for the treatment of RA. By controlling the concentration of acrylamide as a polymer base and NOCCL as a crosslinking agent, a spherical nanogel was prepared by a solution polymerization to obtain an NO-neutralizing nanosized hydrogel (NO-Scv gel) for treating rheumatoid arthritis. As expected, the NO-Scv gel took up NO and reduced the levels of pro-inflammatory cytokines produced by activated macrophages in vitro. In addition, after an intra-articular injection of the nanogel into each paw of the mice with experimental RA, the therapeutic effects observed were superior to those notified with in vivo dexamethasone [98], which inhibits an iNOS-dependent NO formation by destabilizing iNOS mRNA by a mechanism requiring de novo protein synthesis. The effect of dexamethasone on the iNOS expression and the NO production, as well as on the degradation of iNOS mRNA, is to some extent abolished by type II interferon [97].

Therapeutic applications of activity modulators of various NO synthase isoforms

After various NOS isoforms had been identified, the search for their inhibitors progressed rapidly. As more and more active molecules were becoming available, it has become clear that the selectivity for NOS isoforms is a major problem. All 3 isoforms of human NOS (nNOS, eNOS, and iNOS) have nearly identical active site structures, making it difficult to design a selective inhibitor. It is especially important to avoid inhibition of eNOS due to its vital role in the cardiovascular system.

Experimental approaches using modulators of nitric oxide synthesis, including substrate manipulations, NO synthesis donors and inhibitors, will be useful to establish the relationship between NO systems and the cells of the cardiovascular and renal systems.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

As a vasodilator, NO may be a unique therapeutic agent for the treatment of hypertension and, as a result, renal failure and left ventricular hypertrophy. The inclusion of NO modulators in clinical practice may be useful not only as therapeutic agents for certain diseases, but also as means to slow the progression of diseases due to their influence on many systems of the mammalian body.

As a therapeutic approach, a modulation of the NOS activity can be implemented to treat endothelial dysfunction, which is the cause for many diseases. For example, the development of endothelial dysfunction is characteristic of diabetes mellitus: the course of this disease is characterized by a number of metabolic disorders, which ultimately lead to the disruption of functioning of various organs and systems, mainly cardiovascular. A summary of the characteristics of various NOS is presented in Table 1.

CONCLUSION

Enzymes of the NOS group became pharmacotherapeutic targets immediately after their key role in the NO biosynthesis had been established, and the interest in them is growing as new aspects of the pathogenesis of many acute and chronic diseases are being elucidated. Currently, almost all human diseases are to some extent associated with the endothelium and its dysfunction, and the restoration of the adequate vascular homeostasis is impossible without blood flow maintaining in the microvasculature at the physiological level. Increasingly, therapeutic strategies for many socially significant diseases (metabolic, cardiovascular, and neurometabolic) are endotheliocentric in nature and are aimed at preventing primary or secondary cardiovascular complications. Today, approaches to the stimulation of angiogenesis factors are being studied for the treatment of cardiovascular diseases, which show promising results in animals, but have not yet been implemented in the clinic. One important reason for this is the complexity and a large number of related molecular mechanisms that regulate ischemic vascular remodeling. Being responsible for the coordination and activity of vascular factors, the NO molecule can be such a regulator. Therefore, the NOS activity modulation may thus serve as an important factor for the operation of the complex network, contributing to a greater efficiency of angiogenesis.

FUNDING

The work was supported by the Russian Science Foundation (Project No. 20-75-10013).

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Denis V. Kurkin, Elizaveta E. Abrosimova, Dmitry A. Bakulin, Evgeniy I. Morkovin, Ivan N. Tyurenkov – conception, article planning, review of literary sources, collection of materials, writing and editing the article; Nikolai S. Kovalev, Marina A. Dubrovina, Aleksandr V. Borisov, Andrey V. Strygin – collecting materials, editing the article.

REFERENCES

1. Levine AB, Punihaole D, Levine TB. Characterization of the role of nitric oxide and its clinical applications. Cardiology. 2012;122(1):55–68. DOI: 10.1159/000338150.
2. Stuart-Smith K. Demystified. Nitric oxide. Mol Pathol. 2002; 55(6):360–6. DOI:10.1136/mp.55.6.360.
3. Fukuto JM. A recent history of nitroxyl chemistry, pharmacology and therapeutic potential. Br J Pharmacol. 2019 Jan;176(2):135–46. DOI: 10.1111/bph.14384.
4. Kourosh-Arami M, Hosseini N, Mohsenzadegan M, Komaki A, Joghataei MT. Neurophysiologic implications of neuronal nitric oxide synthase. Rev Neurosci. 2020 Aug 27;31(6):617–36. DOI: 10.1515/revneuro-2019-0111.
5. Hardingham N, Dachtler J, Fox K. The role of nitric oxide in pre-synaptic plasticity and homeostasis. Front Cell Neurosci. 2013 Oct 31;7:190. DOI: 10.3389/fncel.2013.00190.
6. Dubey H, Gulati K, Ray A. Alzheimer's Disease: A Contextual Link with Nitric Oxide Synthase. Curr Mol Med. 2020;20(7):505–15. DOI: 10.2174/1566524019666191129103117.
7. Liskova S. The organ-specific nitric oxide synthase activity in the interaction with sympathetic nerve activity: a hypothesis. Physiol Res. 2021 Apr 30;70(2):169-175. DOI: 10.33549/physiolres.934676.
8. Fender AC, Dobrev D. Nitric oxide as a fragile switch between cardioprotection and cardiac injury. Int J Cardiol. 2021 Nov 15;343:102–3. DOI: 10.1016/j.ijcard.2021.09.001.
9. Radziwon-Balicka A, Lesyk G, Back V, Fong T, Loredo-Calderon EL, Dong B, El-Sikhry H, El-Sherbeni AA, El-Kadi A, Ogg S, Siraki A, Seubert JM, Santos-Martinez MJ, Radomski MW, Velazquez-Martinez CA, Winship IR, Jurasz P. Differential eNOS-signalling by platelet subpopulations regulates adhesion and aggregation. Cardiovasc Res. 2017 Dec 1;113(14):1719–31. DOI: 10.1093/cvr/cvx179.
10. Infante T, Costa D, Napoli C. Novel Insights Regarding Nitric Oxide and Cardiovascular Diseases. Angiology. 2021 May;72(5):411–25. DOI: 10.1177/0003319720979243.
11. Idrizaj E, Traini C, Vannucchi MG, Baccari MC. Nitric Oxide: From Gastric Motility to Gastric Dysmotility. Int J Mol Sci. 2021 Sep 16;22(18):9990. DOI: 10.3390/ijms22189990.
12. Hong PP, Zhu XX, Yuan WJ, Niu GJ, Wang JX. Nitric Oxide Synthase Regulates Gut Microbiota Homeostasis by ERK-NF-κB Pathway in Shrimp. Front Immunol. 2021 Dec 3;12:778098. DOI: 10.3389/fimmu.2021.778098.

DOI: 10.19163/2307-9266-2022-10-2-130-153

13. Sibisi NC, Snyman C, Myburgh KH, Niesler CU. Evaluating the role of nitric oxide in myogenesis in vitro. Biochimie. 2021 Nov 26:S0300-9084(21)00269-8. DOI: 10.1016/j.biochi.2021.11.006.
14. Balke JE, Zhang L, Percival JM. Neuronal nitric oxide synthase (nNOS) splice variant function: Insights into nitric oxide signaling from skeletal muscle. Nitric Oxide. 2019 Jan 1;82:35–47. DOI: 10.1016/j.niox.2018.11.004.
15. Baig MS, Zaichick SV, Mao M, de Abreu AL, Bakhshi FR, Hart PC, Saqib U, Deng J, Chatterjee S, Block ML, Vogel SM, Malik AB, Consolaro ME, Christman JW, Minshall RD, Gantner BN, Bonini MG. NOS1-derived nitric oxide promotes NF-κB transcriptional activity through inhibition of suppressor of cytokine signaling-1. J Exp Med. 2015 Sep 21;212(10):1725–38. DOI: 10.1084/jem.20140654.
16. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent JL. Sepsis and septic shock. Nat Rev Dis Primers. 2016 Jun 30;2:16045. DOI: 10.1038/nrdp.2016.45.
17. Luiking YC, Engelen MP, Deutz NE. Regulation of nitric oxide production in health and disease. Curr Opin Clin Nutr Metab Care. 2010 Jan;13(1):97–104. DOI: 10.1097/MCO.0b013e328332f99d.
18. Bredt DS, Snyder SH. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. Proc Natl Acad Sci U S A. 1990 Jan;87(2):682–5. DOI: 10.1073/pnas.87.2.682.
19. Salvemini D, Kim SF, Mollace V. Reciprocal regulation of the nitric oxide and cyclooxygenase pathway in pathophysiology: relevance and clinical implications. Am J Physiol Regul Integr Comp Physiol. 2013 Apr 1;304(7):R473–87. DOI: 10.1152/ajpregu.00355.2012.
20. Palmieri EM, McGinity C, Wink DA, McVicar DW. Nitric Oxide in Macrophage Immunometabolism: Hiding in Plain Sight. Metabolites. 2020 Oct 26;10(11):429. DOI: 10.3390/metabo10110429.
21. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J. 2012 Apr;33(7):829-37, 837a–837d. DOI: 10.1093/eurheartj/ehr304.
22. Förstermann U, Pollock JS, Schmidt HH, Heller M, Murad F. Calmodulin-dependent endothelium-derived relaxing factor/nitric oxide synthase activity is present in the particulate and cytosolic fractions of bovine aortic endothelial cells. Proc Natl Acad Sci U S A. 1991 Mar 1;88(5):1788–92. DOI: 10.1073/pnas.88.5.1788.
23. Pollock JS, Förstermann U, Mitchell JA, Warner TD, Schmidt HH, Nakane M, Murad F. Purification and characterization of particulate endothelium-derived relaxing factor synthase from cultured and native bovine aortic endothelial cells. Proc Natl Acad Sci USA. 1991 Dec 1;88(23):10480–4. DOI: 10.1073/pnas.88.23.10480.
24. Balligand JL, Kelly RA, Marsden PA, Smith TW, Michel T. Control of cardiac muscle cell function by an endogenous nitric oxide signaling system. Proc Natl Acad Sci USA. 1993 Jan 1;90(1):347–51. DOI: 10.1073/pnas.90.1.347.
25. Sase K, Michel T. Expression of constitutive endothelial nitric oxide synthase in human blood platelets. Life Sci. 1995;57(22):2049–55. DOI: 10.1016/0024-3205(95)02191-k.
26. Dinerman JL, Dawson TM, Schell MJ, Snowman A, Snyder SH. Endothelial nitric oxide synthase localized to hippocampal pyramidal cells: implications for synaptic plasticity. Proceedings of the National Academy of Sciences of the United States of America. 1994 May;91(10):4214–8. DOI: 10.1073/pnas.91.10.4214.
27. Marsden PA, Heng HH, Scherer SW, Stewart RJ, Hall AV, Shi XM, Tsui LC, Schappert KT. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. J Biol Chem. 1993 Aug 15;268(23):17478-88.
28. Oliveira-Paula GH, Lacchini R, Tanus-Santos JE. Endothelial nitric oxide synthase: From biochemistry and gene structure to clinical implications of NOS3 polymorphisms. Gene. 2016 Jan 10;575(2 Pt 3):584–99. DOI: 10.1016/j.gene.2015.09.061.
29. Qian J, Fulton D. Post-translational regulation of endothelial nitric oxide synthase in vascular endothelium. Front Physiol. 2013 Dec 13;4:347. DOI: 10.3389/fphys.2013.00347.
30. Hall AV, Antoniou H, Wang Y, Cheung AH, Arbus AM, Olson SL, Lu WC, Kau CL, Marsden PA. Structural organization of the human neuronal nitric oxide synthase gene (NOS1). J Biol Chem. 1994 Dec 30;269(52):33082-90.
31. Eliasson MJ, Blackshaw S, Schell MJ, Snyder SH. Neuronal nitric oxide synthase alternatively spliced forms: prominent functional localizations in the brain. Proc Natl Acad Sci U S A. 1997 Apr 1;94(7):3396–401. DOI: 10.1073/pnas.94.7.3396.
32. Sharma NM, Patel KP. Post-translational regulation of neuronal nitric oxide synthase: implications for sympathoexcitatory states. Expert Opin Ther Targets. 2017 Jan;21(1):11–22. DOI: 10.1080/14728222.2017.1265505.
33. Zhang YH, Jin CZ, Jang JH, Wang Y. Molecular mechanisms of neuronal nitric oxide synthase in cardiac function and pathophysiology. J Physiol. 2014 Aug 1;592(15):3189–200. DOI: 10.1113/jphysiol.2013.270306.
34. Pautz A, Art J, Hahn S, Nowag S, Voss C, Kleinert H. Regulation of the expression of inducible nitric oxide synthase. Nitric Oxide. 2010 Sep 15;23(2):75–93. DOI: 10.1016/j.niox.2010.04.007.
35. Förstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I, Kleinert H. Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. Hypertension. 1994 Jun;23(6 Pt 2):1121–31. DOI: 10.1161/01.hyp.23.6.1121.
36. Tejero J, Shiva S, Gladwin MT. Sources of Vascular Nitric Oxide and Reactive Oxygen Species and Their Regulation. Physiol Rev. 2019 Jan 1;99(1):311–79. DOI: 10.1152/physrev.00036.2017.
37. Cinelli MA, Do HT, Miley GP, Silverman RB. Inducible nitric oxide synthase: Regulation, structure, and inhibition. Med Res Rev. 2020 Jan;40(1):158–89. DOI: 10.1002/med.21599.
38. Giulivi C, Poderoso JJ, Boveris A. Production of nitric oxide by mitochondria. J Biol Chem. 1998 May 1;273(18):11038–43. DOI: 10.1074/jbc.273.18.11038.
39. Tengan CH, Rodrigues GS, Godinho RO. Nitric oxide in skeletal muscle: role on mitochondrial biogenesis and function. Int J Mol Sci. 2012 Dec 14;13(12):17160–84. DOI: 10.3390/ijms131217160.
40. Figueira TR, Barros MH, Camargo AA, Castilho RF, Ferreira JC, Kowaltowski AJ, Sluse FE, Souza-Pinto NC, Vercesi AE. Mitochondria as a source of reactive oxygen and nitrogen species: from molecular mechanisms to human health. Antioxid Redox Signal. 2013 Jun 1;18(16):2029–74. DOI: 10.1089/ars.2012.4729.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

41. Tatoyan A, Giulivi C. Purification and characterization of a nitric-oxide synthase from rat liver mitochondria. J Biol Chem. 1998 May 1;273(18):11044–8. DOI: 10.1074/jbc.273.18.11044.
42. Bates TE, Loesch A, Burnstock G, Clark JB. Immunocytochemical evidence for a mitochondrially located nitric oxide synthase in brain and liver. Biochem Biophys Res Commun. 1995 Aug 24;213(3):896–900. DOI: 10.1006/bbrc.1995.2213.
43. Gao S, Chen J, Brodsky SV, Huang H, Adler S, Lee JH, Dhadwal N, Cohen-Gould L, Gross SS, Goligorsky MS. Docking of endothelial nitric oxide synthase (eNOS) to the mitochondrial outer membrane: a pentabasic amino acid sequence in the autoinhibitory domain of eNOS targets a proteinase K-cleavable peptide on the cytoplasmic face of mitochondria. J Biol Chem. 2004 Apr 16;279(16):15968–74. DOI: 10.1074/jbc.M308504200.
44. Elfering SL, Sarkela TM, Giulivi C. Biochemistry of mitochondrial nitric-oxide synthase. J Biol Chem. 2002 Oct 11;277(41):38079–86. DOI: 10.1074/jbc.M205256200.
45. Kanai AJ, Pearce LL, Clemens PR, Birder LA, VanBibber MM, Choi SY, de Groat WC, Peterson J. Identification of a neuronal nitric oxide synthase in isolated cardiac mitochondria using electrochemical detection. Proc Natl Acad Sci U S A. 2001 Nov 20;98(24):14126–31. DOI: 10.1073/pnas.241380298.
46. Lacza Z, Snipes JA, Zhang J, Horváth EM, Figueroa JP, Szabó C, Busija DW. Mitochondrial nitric oxide synthase is not eNOS, nNOS or iNOS. Free Radic Biol Med. 2003 Nov 15;35(10):1217–28. DOI: 10.1016/s0891-5849(03)00510-0.
47. Venkatakrishnan P, Nakayasu ES, Almeida IC, Miller RT. Absence of nitric-oxide synthase in sequentially purified rat liver mitochondria. J Biol Chem. 2009 Jul 24;284(30):19843–55. DOI: 10.1074/jbc.M109.003301.
48. Parihar MS, Nazarewicz RR, Kincaid E, Bringold U, Ghafourifar P. Association of mitochondrial nitric oxide synthase activity with respiratory chain complex I. Biochem Biophys Res Commun. 2008 Feb 1;366(1):23–8. DOI: 10.1016/j.bbrc.2007.11.056.
49. Bombicino SS, Iglesias DE, Zaobornyj T, Boveris A, Valdez LB. Mitochondrial nitric oxide production supported by reverse electron transfer. Arch Biochem Biophys. 2016 Oct 1;607:8–19. DOI: 10.1016/j.abb.2016.08.010.
50. Lacza Z, Pankotai E, Busija DW. Mitochondrial nitric oxide synthase: current concepts and controversies. Front Biosci (Landmark Ed). 2009 Jan 1;14(12):4436–43. DOI: 10.2741/3539.
51. Giulivi C. Characterization and function of mitochondrial nitric-oxide synthase. Free Radic Biol Med. 2003 Feb 15;34(4):397–408. DOI: 10.1016/s0891-5849(02)01298-4.
52. Tengan CH, Moraes CT. NO control of mitochondrial function in normal and transformed cells. Biochim Biophys Acta Bioenerg. 2017 Aug;1858(8):573–81. DOI: 10.1016/j.bbabio.2017.02.009.
53. Shah RC, Sanker S, Wood KC, Durgin BG, Straub AC. Redox regulation of soluble guanylyl cyclase. Nitric Oxide. 2018 Jun 1;76:97–104. DOI: 10.1016/j.niox.2018.03.013.
54. Sharin VG, Mujoo K, Kots AY, Martin E, Murad F, Sharina IG. Nitric oxide receptor soluble guanylyl cyclase undergoes splicing regulation in differentiating human embryonic cells. Stem Cells Dev. 2011 Jul;20(7):1287–93. DOI: 10.1089/scd.2010.0411.
55. Derbyshire ER, Marletta MA. Structure and regulation of soluble guanylate cyclase. Annu Rev Biochem. 2012;81:533–59. DOI: 10.1146/annurev-biochem-050410-100030.
56. Montfort WR, Wales JA, Weichsel A. Structure and Activation of Soluble Guanylyl Cyclase, the Nitric Oxide Sensor. Antioxid Redox Signal. 2017 Jan 20;26(3):107–21. DOI: 10.1089/ars.2016.6693.
57. Förstermann U, Xia N, Li H. Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. Circ Res. 2017 Feb 17;120(4):713-735. DOI: 10.1161/CIRCRESAHA.116.309326.
58. Li H, Förstermann U. Uncoupling of endothelial NO synthase in atherosclerosis and vascular disease. Curr Opin Pharmacol. 2013 Apr;13(2):161-7. DOI: 10.1016/j.coph.2013.01.006.
59. Roe ND, Ren J. Nitric oxide synthase uncoupling: a therapeutic target in cardiovascular diseases. Vascul Pharmacol. 2012 Nov-Dec;57(5-6):168–72. DOI: 10.1016/j.vph.2012.02.004.
60. Alkaitis MS, Crabtree MJ. Recoupling the cardiac nitric oxide synthases: tetrahydrobiopterin synthesis and recycling. Curr Heart Fail Rep. 2012 Sep;9(3):200–10. DOI: 10.1007/s11897-012-0097-5.
61. Crabtree MJ, Brixey R, Batchelor H, Hale AB, Channon KM. Integrated redox sensor and effector functions for tetrahydrobiopterin- and glutathionylation-dependent endothelial nitric-oxide synthase uncoupling. J Biol Chem. 2013 Jan 4;288(1):561–9. DOI: 10.1074/jbc.M112.415992.
62. Chen CA, Wang TY, Varadharaj S, Reyes LA, Hemann C, Talukder MA, Chen YR, Druhan LJ, Zweier JL. S-glutathionylation uncouples eNOS and regulates its cellular and vascular function. Nature. 2010 Dec 23;468(7327):1115–8. DOI: 10.1038/nature09599.
63. Chen DD, Chen LY, Xie JB, Shu C, Yang T, Zhou S, Yuan H, Chen AF. Tetrahydrobiopterin regulation of eNOS redox function. Curr Pharm Des. 2014;20(22):3554–62. DOI: 10.2174/13816128113196660747.
64. Crabtree MJ, Tatham AL, Hale AB, Alp NJ, Channon KM. Critical role for tetrahydrobiopterin recycling by dihydrofolate reductase in regulation of endothelial nitric-oxide synthase coupling: relative importance of the de novo biopterin synthesis versus salvage pathways. J Biol Chem. 2009 Oct 9;284(41):28128–36. DOI: 10.1074/jbc.M109.041483.
65. Kietadisorn R, Kietselaer BL, Schmidt HH, Moens AL. Role of tetrahydrobiopterin (BH4) in hyperhomocysteinemia-induced endothelial dysfuction: new indication for this orphan-drug? Am J Physiol Endocrinol Metab. 2011 Jun;300(6):E1176; author reply E1177-8. DOI: 10.1152/ajpendo.00084.2011.
66. Dikalova A, Aschner JL, Kaplowitz MR, Summar M, Fike CD. Tetrahydrobiopterin oral therapy recouples eNOS and ameliorates chronic hypoxia-induced pulmonary hypertension in newborn pigs. Am J Physiol Lung Cell Mol Physiol. 2016 Oct 1;311(4):L743–L753. DOI: 10.1152/ajplung.00238.2016.
67. Suckling CJ, Gibson CL, Huggan JK, Morthala RR, Clarke B, Kununthur S, Wadsworth RM, Daff S, Papale D. 6-Acetyl-7,7-dimethyl-5,6,7,8-tetrahydropterin is an activator of nitric oxide synthases. Bioorg Med Chem Lett. 2008 Mar 1;18(5):1563–6. DOI: 10.1016/j.bmcl.2008.01.079.

68. An H, Wei R, Ke J, Yang J, Liu Y, Wang X, Wang G, Hong T. Metformin attenuates fluctuating glucose-induced endothelial dysfunction through enhancing GTPCH1-mediated eNOS recoupling and inhibiting NADPH oxidase. J Diabetes Complications. 2016 Aug;30(6):1017–24. DOI: 10.1016/j.jdiacomp.2016.04.018.
69. Caldwell RB, Toque HA, Narayanan SP, Caldwell RW. Arginase: an old enzyme with new tricks. Trends Pharmacol Sci. 2015 Jun;36(6):395–405. DOI: 10.1016/j.tips.2015.03.006.
70. Caldwell RW, Rodriguez PC, Toque HA, Narayanan SP, Caldwell RB. Arginase: A Multifaceted Enzyme Important in Health and Disease. Physiol Rev. 2018 Apr 1;98(2):641–65. DOI: 10.1152/physrev.00037.2016.
71. Kim JH, Bugaj LJ, Oh YJ, Bivalacqua TJ, Ryoo S, Soucy KG, Santhanam L, Webb A, Camara A, Sikka G, Nyhan D, Shoukas AA, Ilies M, Christianson DW, Champion HC, Berkowitz DE. Arginase inhibition restores NOS coupling and reverses endothelial dysfunction and vascular stiffness in old rats. J Appl Physiol (1985). 2009 Oct;107(4):1249–57. DOI: 10.1152/japplphysiol.91393.2008.
72. El-Bassossy HM, El-Fawal R, Fahmy A, Watson ML. Arginase inhibition alleviates hypertension in the metabolic syndrome. Br J Pharmacol. 2013 Jun;169(3):693–703. DOI: 10.1111/bph.12144.
73. Polis B, Srikanth KD, Gurevich V, Gil-Henn H, Samson AO. L-Norvaline, a new therapeutic agent against Alzheimer's disease. Neural Regen Res. 2019 Sep;14(9):1562–72. DOI: 10.4103/1673-5374.255980.
74. Rikitake Y, Liao JK. Rho GTPases, statins, and nitric oxide. Circ Res. 2005 Dec 9;97(12):1232–5. DOI: 10.1161/01.RES.0000196564.18314.23.
75. Rohilla A, Rohilla S, Kumar A, Khan MU, Deep A. Pleiotropic effects of statins: A boulevard to cardioprotection. Arab J Chem. 2016; 9. – P. S21–S27. DOI: 10.1016/j.arabjc.2011.06.025.
76. Gorabi AM, Kiaie N, Hajighasemi S, Banach M, Penson PE, Jamialahmadi T, Sahebkar A. Statin-Induced Nitric Oxide Signaling: Mechanisms and Therapeutic Implications. J Clin Med. 2019 Nov 22;8(12):2051. DOI: 10.3390/jcm8122051.
77. Margaritis M, Channon KM, Antoniades C. Statins as regulators of redox state in the vascular endothelium: beyond lipid lowering. Antioxid Redox Signal. 2014 Mar 10;20(8):1198–215. DOI: 10.1089/ars.2013.5430.
78. Antoniades C, Shirodaria C, Leeson P, Antonopoulos A, Warrick N, Van-Assche T, Cunnington C, Tousoulis D, Pillai R, Ratnatunga C, Stefanadis C, Channon KM. Association of plasma asymmetrical dimethylarginine (ADMA) with elevated vascular superoxide production and endothelial nitric oxide synthase uncoupling: implications for endothelial function in human atherosclerosis. Eur Heart J. 2009 May;30(9):1142–50. DOI: 10.1093/eurheartj/ehp061.
79. Serban C, Sahebkar A, Ursoniu S, Mikhailidis DP, Rizzo M, Lip GY, Kees Hovingh G, Kastelein JJ, Kalinowski L, Rysz J, Banach MA. A systematic review and meta-analysis of the effect of statins on plasma asymmetric dimethylarginine concentrations. Sci Rep. 2015; 5(1): 9902. DOI: 10.1038/srep09902.
80. Xia N, Förstermann U, Li H. Resveratrol and endothelial nitric oxide. Molecules. 2014 Oct 9;19(10):16102–21. DOI: 10.3390/molecules191016102.
81. Suschek C, Kolb H, Kolb-Bachofen V. Dobesilate enhances endothelial nitric oxide synthase-activity in macro- and microvascular endothelial cells. Br J Pharmacol. 1997 Dec;122(7):1502–8. DOI: 10.1038/sj.bjp.0701512.
82. Carrizzo A, Lenzi P, Procaccini C, Damato A, Biagioni F, Ambrosio M, Amodio G, Remondelli P, Del Giudice C, Izzo R, Malovini A, Formisano L, Gigantino V, Madonna M, Puca AA, Trimarco B, Matarese G, Fornai F, Vecchione C. Pentraxin 3 Induces Vascular Endothelial Dysfunction Through a P-selectin/Matrix Metalloproteinase-1 Pathway. Circulation. 2015 Apr 28;131(17):1495–505. DOI: 10.1161/CIRCULATIONAHA.114.014822.
83. Leung WK, Gao L, Siu PM, Lai CW. Diabetic nephropathy and endothelial dysfunction: Current and future therapies, and emerging of vascular imaging for preclinical renal-kinetic study. Life Sci. 2016 Dec 1;166:121–30. DOI: 10.1016/j.lfs.2016.10.015.
84. Zhou Y, Qi C, Li S, Shao X, Ni Z. Investigation of the Mechanism Underlying Calcium Dobesilate-Mediated Improvement of Endothelial Dysfunction and Inflammation Caused by High Glucose. Mediators Inflamm. 2019; 2019: 9893682. DOI: 10.1155/2019/9893682.
85. Sison K, Eremina V, Baelde H, Min W, Hirashima M, Fantus IG, Quaggin SE. Glomerular structure and function require paracrine, not autocrine, VEGF-VEGFR-2 signaling. J Am Soc Nephrol. 2010 Oct;21(10):1691–701. DOI: 10.1681/ASN.2010030295.
86. Baelde HJ, Eikmans M, Lappin DW, Doran PP, Hohenadel D, Brinkkoetter PT, van der Woude FJ, Waldherr R, Rabelink TJ, de Heer E, Bruijn JA. Reduction of VEGF-A and CTGF expression in diabetic nephropathy is associated with podocyte loss. Kidney Int. 2007 Apr;71(7):637–45. DOI: 10.1038/sj.ki.5002101.
87. Haller H, Ji L, Stahl K, Bertram A, Menne J. Molecular Mechanisms and Treatment Strategies in Diabetic Nephropathy: New Avenues for Calcium Dobesilate-Free Radical Scavenger and Growth Factor Inhibition. Biomed Res Int. 2017;2017:1909258. DOI: 10.1155/2017/1909258.
88. Kraehling JR, Sessa WC. Contemporary Approaches to Modulating the Nitric Oxide-cGMP Pathway in Cardiovascular Disease. Circ Res. 2017 Mar 31;120(7):1174–82. DOI: 10.1161/CIRCRESAHA.117.303776.
89. Hou HT, Wang J, Zhang X, Wang ZQ, Chen TN, Zhang JL, Yang Q, He GW. Endothelial nitric oxide synthase enhancer AVE3085 reverses endothelial dysfunction induced by homocysteine in human internal mammary arteries. Nitric Oxide. 2018; 81:21–7. DOI: 10.1016/j.niox.2018.10.001.
90. White AR, Ryoo S, Bugaj L, Attarzadeh DO, Thiyagarajan S, Chen K, Attwater S, Abbot B, Li D, Champion HC, Shoukas AA, Nyhan D, Hare JM, Berkowitz DE, Tuday EC. Early changes in vasoreactivity after simulated microgravity are due to an upregulation of the endothelium-dependent nitric oxide/cGMP pathway. Eur J Appl Physiol. 2010 Sep;110(2):395–404. DOI: 10.1007/s00421-010-1514-7.
91. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. Biochem J. 2001 Aug 1;357(Pt 3):593–615. DOI: 10.1042/0264-6021:3570593.
92. Schulman IH, Hare JM. Regulation of cardiovascular cellular processes by S-nitrosylation. Biochim Biophys Acta. 2012 Jun;1820(6):752–62. DOI: 10.1016/j.bbagen.2011.04.002.
93. Poulos TL, Li H. Nitric oxide synthase and structure-based inhibitor design. Nitric Oxide. 2017 Feb 28;63:68–77. DOI: 10.1016/j.niox.2016.11.004.

ISSN 2307-9266 e-ISSN 2413-2241

94. Wong VW, Lerner E. Nitric oxide inhibition strategies. Future Sci OA. 2015; 1(1): FSO35. DOI: 10.4155/fso.15.35.
95. Víteček J, Lojek A, Valacchi G, Kubala L. Arginine-based inhibitors of nitric oxide synthase: therapeutic potential and challenges. Mediators Inflamm. 2012;2012:318087. DOI: 10.1155/2012/318087.
96. Park J, Pramanick S, Park D, Yeo J, Lee J, Lee H, Kim WJ. Therapeutic-Gas-Responsive Hydrogel. Adv Mater. 2017 Nov;29(44). DOI: 10.1002/adma.201702859.
97. Korhonen R, Lahti A, Hämäläinen M, Kankaanranta H, Moilanen E. Dexamethasone inhibits inducible nitric-oxide synthase expression and nitric oxide production by destabilizing mRNA in lipopolysaccharide-treated macrophages. Mol Pharmacol. 2002 Sep;62(3):698–704. DOI: 10.1124/mol.62.3.698.
98. Yeo J, Lee YM, Lee J, Park D, Kim K, Kim J, Park J, Kim WJ. Nitric Oxide-Scavenging Nanogel for Treating Rheumatoid Arthritis. Nano Lett. 2019 Oct 9;19(10):6716–24. DOI: 10.1021/acs.nanolett.9b00496.
99. Putzke J, Seidel B, Huang PL, Wolf G. Differential expression of alternatively spliced isoforms of neuronal nitric oxide synthase (nNOS) and N-methyl-D-aspartate receptors (NMDAR) in knockout mice deficient in nNOS alpha (nNOS alpha(Delta/Delta) mice). Brain Res Mol Brain Res. 2000 Dec 28;85(1-2):13–23. DOI: 10.1016/s0169-328x(00)00220-5.
100. Frandsen U, Lopez-Figueroa M, Hellsten Y. Localization of nitric oxide synthase in human skeletal muscle. Biochem Biophys Res Commun. 1996 Oct 3;227(1):88–93. DOI: 10.1006/bbrc.1996.1472.
101. El-Yazbi AF, Cho WJ, Cena J, Schulz R, Daniel EE. Smooth muscle NOS, colocalized with caveolin-1, modulates contraction in mouse small intestine. J Cell Mol Med. 2008 Aug;12(4):1404–15. DOI: 10.1111/j.1582-4934.2008.00335.x.
102. Saluja R, Saini R, Mitra K, Bajpai VK, Dikshit M. Ultrastructural immunogold localization of nitric oxide synthase isoforms in rat and human eosinophils. Cell Tissue Res. 2010 May;340(2):381–8. DOI: 10.1007/s00441-010-0947-y.
103. Saini R, Singh S. Inducible nitric oxide synthase: An asset to neutrophils. J Leukoc Biol. 2019 Jan;105(1):49–61. DOI: 10.1002/JLB.4RU0418-161R.
104. Feron O, Dessy C, Moniotte S, Desager JP, Balligand JL. Hypercholesterolemia decreases nitric oxide production by promoting the interaction of caveolin and endothelial nitric oxide synthase. J Clin Invest. 1999 Mar;103(6):897–905. DOI: 10.1172/JCI4829.
105. Garthwaite J. Concepts of neural nitric oxide-mediated transmission. Eur J Neurosci. 2008 Jun;27(11):2783–802. DOI: 10.1111/j.1460-9568.2008.06285.x.
106. Lajoix AD, Reggio H, Chardès T, Péraldi-Roux S, Tribillac F, Roye M, Dietz S, Broca C, Manteghetti M, Ribes G, Wollheim CB, Gross R. A neuronal isoform of nitric oxide synthase expressed in pancreatic beta-cells controls insulin secretion. Diabetes. 2001 Jun;50(6):1311–23. DOI: 10.2337/diabetes.50.6.1311.
107. Schwarz PM, Kleinert H, Förstermann U. Potential functional significance of brain-type and muscle-type nitric oxide synthase I expressed in adventitia and media of rat aorta. Arterioscler Thromb Vasc Biol. 1999 Nov;19(11):2584–90. DOI: 10.1161/01.atv.19.11.2584.
108. Kleinert H, Pautz A, Linker K, Schwarz PM. Regulation of the expression of inducible nitric oxide synthase. Eur J Pharmacol. 2004 Oct 1;500(1-3):255–66. DOI: 10.1016/j.ejphar.2004.07.030.
109. Förstermann U, Schmidt HH, Pollock JS, Sheng H, Mitchell JA, Warner TD, Nakane M, Murad F. Isoforms of nitric oxide synthase. Characterization and purification from different cell types. Biochem Pharmacol. 1991 Oct 24;42(10):1849–57. DOI: 10.1016/0006-2952(91)90581-o.
110. Dombernowsky NW, Ölmestig JNE, Witting N, Kruuse C. Role of neuronal nitric oxide synthase (nNOS) in Duchenne and Becker muscular dystrophies – Still a possible treatment modality? Neuromuscul Disord. 2018 Nov;28(11):914–26. DOI: 10.1016/j.nmd.2018.09.001.
111. Mörlin B, Andersson E, Byström B, Hammarström M. Nitric oxide induces endometrial secretion at implantation time. Acta Obstet Gynecol Scand. 2005 Nov;84(11):1029–34. DOI: 10.1111/j.0001-6349.2005.00804.x.
112. Lu D, Fu Y, Lopez-Ruiz A, Zhang R, Juncos R, Liu H, Manning RD Jr, Juncos LA, Liu R. Salt-sensitive splice variant of nNOS expressed in the macula densa cells. Am J Physiol Renal Physiol. 2010 Jun;298(6):F1465–71. DOI: 10.1152/ajprenal.00650.2009.
113. Förstermann U, Li H. Therapeutic effect of enhancing endothelial nitric oxide synthase (eNOS) expression and preventing eNOS uncoupling. Br J Pharmacol. 2011 Sep;164(2):213–23. DOI: 10.1111/j.1476-5381.2010.01196.x.
114. Moncada S. Nitric oxide: discovery and impact on clinical medicine. J R Soc Med. 1999 Apr;92(4):164–9. DOI: 10.1177/014107689909200402.
115. Tojo A, Onozato ML, Fujita T. Role of macula densa neuronal nitric oxide synthase in renal diseases. Med Mol Morphol. 2006 Mar;39(1):2–7. DOI: 10.1007/s00795-006-0310-2.
116. Takahashi T. Pathophysiological significance of neuronal nitric oxide synthase in the gastrointestinal tract. J Gastroenterol. 2003;38(5):421–30. DOI: 10.1007/s00535-003-1094-y.
117. Xu L, Xie K, Fidler IJ. Therapy of human ovarian cancer by transfection with the murine interferon beta gene: role of macrophage-inducible nitric oxide synthase. Hum Gene Ther. 1998 Dec 10;9(18):2699–708. DOI: 10.1089/hum.1998.9.18-2699.
118. Kröncke KD, Fehsel K, Kolb-Bachofen V. Inducible nitric oxide synthase and its product nitric oxide, a small molecule with complex biological activities. Biol Chem Hoppe Seyler. 1995 Jun;376(6):327–43. DOI: 10.1515/bchm3.1995.376.6.327.
119. Moncada S, Higgs EA. Endogenous nitric oxide: physiology, pathology and clinical relevance. Eur J Clin Invest. 1991 Aug;21(4):361–74. DOI: 10.1111/j.1365-2362.1991.tb01383.x.
120. Langrehr JM, Hoffman RA, Lancaster JR Jr, Simmons RL. Nitric oxide--a new endogenous immunomodulator. Transplantation. 1993 Jun;55(6):1205–12. DOI: 10.1097/00007890-199306000-00001.
121. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, Zeiher AM, Dimmeler S. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. Nat Med. 2003 Nov;9(11):1370–6. DOI: 10.1038/nm948.
122. Zhou QG, Zhu XH, Nemes AD, Zhu DY. Neuronal nitric oxide synthase and affective disorders. IBRO Rep. 2018 Nov 17;5:116–32. DOI: 10.1016/j.ibror.2018.11.004.

DOI: 10.19163/2307-9266-2022-10-2-130-153

AUTHORS

Denis V. Kurkin – Doctor of Sciences (Pharmacy), Associate Professor, Professor of the Department of Clinical Pharmacology and Intensive Care; First Deputy Director of the Research Center of Innovative Pharma Products, Volgograd State Medical University. ORCID ID: 0000-0002-1116-3425. E-mail: strannik986@mail.ru

Elizaveta E. Abrosimova – post-graduate student of the Department of Pharmacology and Pharmacy, Institute of Continuing Medical and Pharmaceutical Education, Volgograd State Medical University. ORCID ID: 0000-0002-6472-6906. E-mail: abrosimova.volgmed@gmail.com

Dmitry A. Bakulin – Candidate of Sciences (Medicine), Senior Researcher, Laboratory of Pharmacology of Cardiovascular Drugs, Research Center of Innovative Pharma Products, Volgograd State Medical University. ORCID ID: 0000-0003-4694-3066. E-mail: mbfdoc@gmail.com

Nikolai S. Kovalev – post-graduate student of the Department of Pharmacology and Pharmacy, Research Center of Innovative Pharma Products, Volgograd State Medical University. ORCID ID: 0000-0002-1116-3425. E-mail: kovalev.volgmed@gmail.com

Marina A. Dubrovina – post-graduate student of the Department of Pharmacology and Pharmacy, Research Center of Innovative Pharma Products, Volgograd State Medical University. ORCID ID: 0000-0003-1903-8589. E-mail: dubrovina.volgmed@gmail.com

Aleksandr V. Borisov – Researcher, Laboratory of Pharmacology of Cardiovascular Drugs, Research Center of Innovative Pharma Products, Volgograd State Medical University. ORCID ID: 0000-0003-0202-0008. E-mail: borissow1978@rambler.ru

Andrey V. Strygin – Candidate of Sciences (Medicine), Associate Professor, Deputy Director of the Research Center of Innovative Pharma Products, Volgograd State Medical University. ORCID ID: 0000-0002-6997-1601. E-mail: drumsav@mail.ru

Evgeniy I. Morkovin – Candidate of Sciences (Medicine), Associate Professor, Head of the Laboratory of Neuropsychopharmacology, Research Center of Innovative Pharma Products, Volgograd State Medical University. ORCID ID: 0000-0002-7119-3546. E-mail: e.i.morkovin@gmail.com

Ivan N. Tyurenkov – Doctor of Sciences (Medicine), Professor, Corresponding Member of the Russian Academy of Sciences, Head of the Laboratory of Pharmacology of Cardiovascular Drugs, Research Center of Innovative Pharma Products; Head of the Department of Pharmacology and Pharmacy, Institute of Continuing Medical and Pharmaceutical Education, Volgograd State Medical University. ORCID ID: 0000-0001-7574-3923. E-mail: fibfuv@mail.ru

REVIEWS, LECTURES

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

MINIMISATION OF RISKS ASSOCIATED WITH THE USE OF POLLEN-BASED MEDICINES, AT THE STAGE OF POLLEN COLLECTION

A.A. Taube[1], T.A. Buyanova[2], E.I. Sakanyan[3]

[1] Scientific Centre for Expert Evaluation of Medicinal Products
Bld. 2, 8, Petrovsky Blvd, Moscow, Russia, 127051
[2] St. Petersburg Chemical and Pharmaceutical University
Bld. A, 14, Prof. Popov Str., St. Petersburg, Russia, 197376
[3] Joint-Stock Company "Scientific and Production Association "Microgen"
Bld. 2, 15, 1st Dubovskaya Str., Moscow, Russia, 115088

E-mail: Aleksandra.taube@pharminnotech.com

Received 08 Jan 2022 After peer review 25 Feb 2022 Accepted 30 March 2022

The aim of the study is the elaboration of Rules for Harvesting/Collecting of Pollen to minimize the risks associated with the use of pollen-based medicinal products.

Materials and methods. The following electronic resources were used in the study: PubMed, Medline, ScienceDirect, Web of Science, Scopus, Google Scholar, eLibrary, World Allergy Organization, Cochrane Database, Stallergenesgreer, Allergen-scienceandconsulting, Pharmacopoeia, Fda.gov, fs.usda.gov, Ema.europa.eu. The analysis covered the period from January 1, 2010 until December 31, 2021.

Results. Currently, there are some general requirements for the quality of pollen in Russia, but there are no controls or standardised procedures for harvesting, drying, and purification of pollen. The USA and EU also lack established qualification programmes for pollen-collecting companies and/or individual pollen collectors. Regulatory authorities establish requirements only for visual control of raw materials or delegate responsibility to the manufacturer. The analysis of the existing regulatory documentation revealed lack of requirements for collection, storage, and processing of pollen used as the raw material for the production of allergen products. This calls for the elaboration of appropriate regulatory documents. The authors have compiled the Rules for Harvesting/Collection of Pollen, which include 6 parts. The Rules are intended for individuals directly involved in harvesting/collection of pollen, and contain requirements for pollen collectors, the process of pollen collection, documentation, storage, and transportation.

Conclusion. The authors have prepared the Rules for Harvesting/Collecting of Pollen, which include 6 parts. The Rules cover the whole process of pollen collection and all related processes. The implementation of this document will improve the process of pollen collection, thus reducing the risks associated with the use of pollen-based medicines. Further studies will assess the impact of the pollen quality on the safety of medicinal products.

Keywords: allergen-specific immunotherapy; herbal substances; safe use of medicinal products; good collection practice; adverse drug reactions; pollen; allergen extract

Abbreviations: ASI – allergen-specific immunotherapy; GM – general monograph; EU – European Union; USA – the United States of America; SP Rus. XIV ed. – State Pharmacopeia (Russia) XIV edition; PPE – personal protective equipment.

For citation: A.A. Taube, T.A. Buyanova, E.I. Sakanyan. Minimisation of risks associated with the use of pollen-based medicines, at the stage of pollen collection. *Pharmacy & Pharmacology.* 2022;10(2): 154-163. **DOI:** 10.19163/2307-9266-2022-10-2-154-163

Для цитирования: А.А. Таубэ, Т.А. Буянова, Е.И. Саканян. Минимизация рисков применения лекарственных препаратов на основе пыльцы на стадии заготовки сырья. *Фармация и фармакология.* 2022;10(2): 154-163. **DOI:** 10.19163/2307-9266-2022-10-2-154-163

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОБЗОРЫ, ЛЕКЦИИ

DOI: 10.19163/2307-9266-2022-10-2-154-163

МИНИМИЗАЦИЯ РИСКОВ ПРИМЕНЕНИЯ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ НА ОСНОВЕ ПЫЛЬЦЫ НА СТАДИИ ЗАГОТОВКИ СЫРЬЯ

А.А. Таубэ[1], Т.А. Буянова[2], Е.И. Саканян[3]

[1] Федеральное государственное бюджетное учреждение «Научный центр экспертизы средств медицинского применения» Министерства здравоохранения Российской Федерации
127051, Россия, Москва, Петровский б-р, д. 8, стр. 2
[2] Федеральное государственное бюджетное образовательное учреждение высшего образования Санкт-Петербургский химико-фармацевтический университет» Министерства здравоохранения Российской Федерации
197376, Россия, Санкт-Петербург, ул. Профессора Попова, д. 14, лит. А
[3] Акционерное общество «Научно-производственное объединение «Микроген»
115088, Россия, Москва, 1-я Дубовская ул., д. 15, стр. 2

E-mail: Aleksandra.taube@pharminnotech.com

Получена 08.01.2022 После рецензирования 25.02.2022 Принята к печати 30.03.2022

Цель. Подготовка проекта Правил заготовки (сбора) растительной пыльцы для минимизации рисков при применении лекарственных препаратов на основе пыльцы.

Материалы и методы. В исследовании использовали следующие электронные ресурсы: PubMed, Medline, ScienceDirect, Web of Science, Scopus, Google Scholar, eLibrary, World Allergy Organization, Cochrane Database, Stallergenesgreer, Allergenscienceandconsulting, Pharmacopoeia, Fda.gov, fs.usda.gov, Ema.europa.eu. Поиск осуществляли за период с 1 января 201 по 31 декабря 2021.

Результаты. В настоящее время в России существуют общие требования к качеству пыльцевого материала, однако какой-либо контроль и стандартизация процесса заготовки, сушки и очистки отсутствует. В США и ЕС также отсутствуют установленные программы квалификации организаций по сбору пыльцы и/или индивидуальных сборщиков. Регуляторные органы ограничиваются визуальными требованиями к исходному сырью или возлагают ответственность на усмотрение производителя. В ходе анализа действующих нормативных документов было выявлено отсутствие требований в отношении сбора, хранения и обработки пыльцы, используемой в качестве сырья для производства лекарственных препаратов аллергенов. В связи с этим существует необходимость разработки нормативных документов. Составлены «Правила заготовки (сбора) пыльцы», в которых выделены 6 разделов. Правила предназначены для лиц, непосредственно осуществляющих заготовку (сбор) пыльцы, и содержат требования к заготовителям, к процессу сбора пыльцы, документации, хранению и транспортированию пыльцы.

Заключение. Составлены «Правила заготовки (сбора) пыльцы», в которых выделены 6 разделов. Составленные Правила полностью регламентируют процесс сбора пыльцы и все сопутствующие ему процессы. Внедрение документа позволит повысить качество сбора пыльцы, тем самым уменьшить риски применения лекарственных препаратов на ее основе. Планируется изучение влияния качества пыльцевого сырья на безопасность применения лекарственных препаратов.

Ключевые слова: аллергенспецифическая иммунотерапия; лекарственное растительное сырье; безопасность применения лекарственных препаратов; надлежащая практика сбора; нежелательные реакции; пыльца; экстракт аллергена

Список сокращений: АСИТ – аллерген специфическая иммунтерапия; ЛП- лекарственный препарат; ОФС – общая фармакопейная статья; ЕС – Европейский Союз; США – Соединенные Штаты Америки; ЛРС – лекарственное растительное сырье; ГФ РФ XIV издания – Государственная фармакопея XIV издания; СИЗ – средства индивидуальной защиты.

INTRODUCTION

A significant part of the population is affected by allergic diseases [1, 2]. According to some estimates, about 20–30% of the world's population [3] suffer from allergies, including 1 to 5% of the ones who suffer from pollen-associated food allergies, which may not necessarily include allergic rhinitis symptoms [4].

The study of the allergic diseases mechanism and pathogenesis resulted in the development of a modern treatment method—allergen-specific immunotherapy (ASI) [5–7]. The ASI principle consists in the administration of gradually increasing doses of pollen-based allergen extract which induces an allergic response, to a patient [8, 9]. The ASI method is the only evidence-based etiotropic therapy for allergic diseases [10–13]. However, there are still topical problems regarding clinical efficacy and tolerability of the therapy [14–16]. Different dosage forms of allergens and allergoids are manufac-

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

tured for the ASI method that uses pollen's ability to induce an allergic response [17]. Pollen is a herbal substance used for the production of allergen extracts, i.e. active pharmaceutical ingredients (herbal preparations) which, in their turn, are used for the production of allergen and allergoid products [15]. Herbal substances are highly diverse and variable in terms of bioactive substance composition [18–20], which depends not only on the geographical range and weather conditions, but also on the period and procedure of collecting/harvesting [21, 22], and this makes it difficult to establish uniform standards [23, 24].

Pollen, as a morphological group, has a number of specific features that are not taken into account in current regulatory documents. Pollen is a delicate material that is rather difficult to collect. The main difference between pollen and other herbal preparations is that much of pollen is harvested in the natural habitat, which has a significant impact on the quality and uniformity of the final product [25]. Pollen composition is affected by genetic and environmental factors, such as the harvested plants, weather conditions, air and soil pollution during the plant growth [26,27], which makes it difficult to assess the products' safety profile [28]. A potential development of adverse events, as well as a medicinal product efficacy in ASI, depend not only on the administered dose, but also on the biochemical characteristics of the administered allergen product [29-33]. The first stage of the study included the elaboration of a controlled physicochemical parameters list, qualitative and quantitative characteristics (including macro- and microscopic species-specific characteristics) to be included in the regulatory documentation for pollen [34].

The efficacy and safety of medicinal products are affected by pollen harvesting and storage conditions. To prevent pollen contamination, including the one with pollen from other plants, the harvesting/collecting of raw materials should be regulated by relevant documents. In this area, the following regulations are in force in the Russian Federation: Rules of Good Manufacturing Practice of the Eurasian Economic Union adopted by Resolution No. 77 of the Council of the Eurasian Economic Commission dated November 3, 2016 (hereinafter Resolution No. 77)[1]; Resolution No. 15 of the Council of the Eurasian Economic Commission On Adoption of the Rules of Good Practice of Cultivation, Collection, Processing, and Storage of Starting Materials of Herbal Origin (hereinafter Resolution No. 15)[2]; Recommendations of the Board of the Eurasian Economic Commission dated May 10, 2018, No. 6[3].

Pollen collectors must follow the Rules of Good Practice of Cultivation, Collection, Processing, and Storage of Starting Materials of Herbal Origin adopted by Resolution No. 15 of the Council of the Eurasian Economic Commission dated January 26, 2018[4]. Chapter 3 "Personnel" of Resolution No. 15 states: "If the harvesters/collectors do not have knowledge of the harvested medicinal plants, they should receive training, their work should be supervised, and appropriate records should be kept". At the same time, there are no established criteria and requirements for pollen harvesting/collecting. The environmental issues should also be given consideration[5]. All of the above listed factors support the need for the elaboration of rules for harvesting/collecting of pollen as a morphological group of herbal substances.

THE AIM of the study is the elaboration of Rules for Harvesting/Collecting of Pollen to minimize the risks associated with the use of pollen-based medicinal products.

MATERIALS AND METHODS

The following electronic resources were used in the study: PubMed, Medline, ScienceDirect, Web of Science, Scopus, Google Scholar, eLibrary, World Allergy Organization, Cochrane Database, Stallergenesgreer, Allergenscienceandconsulting, Pharmacopoeia, Fda.gov, fs.usda.gov, Ema.europa.eu. The queries were made using the following English and Russian keywords combinations: allergen specific immunotherapy; pollen; pollen collection; major and minor, Allergy, grass allergens; molecular diagnosis; safety; Allergen extract; Allergen standardisation. The analysis covered the period from January 1, 2010 until December 31, 2021.

Russian and foreign laws and regulations were used as study materials. The rules were elaborated based on the following documents: State Pharmacopoeia of the Russian Federation, XIV edition (SP Rus., XIV ed.)[6]; Rules of Good Practice of Cultivation, Collection, Processing, and Storage of Starting Materials of Herbal Origin[7]; Russian National Standard GOST 24027.0-80 Herbal Sub-

[1] Rules of Good Manufacturing Practice of the Eurasian Economic Union adopted by Resolution No. 77 of the Council of the Eurasian Economic Commission of November 3, 2016 (as amended on July 14, 2021)

[2] Rules of Good Practice of Cultivation, Collection, Processing, and Storage of Starting Materials of Herbal Origin adopted by Resolution No.15 of the Council of the Eurasian Economic Commission of January 26, 2018

[3] Recommendations of the Board of the Eurasian Economic Commission On the Guideline for the Quality of Herbal Medicinal Products of May 10, 2018, No.6.

[4] Order of the Ministry of Natural Resources of Russia of July 28, 2020 No. 494 On Adoption of the Rules for Harvesting Forest Plants for the Food Industry, and Medicinal Plants (Registered in the Ministry of Justice of Russia on December 14, 2020, registration No.61428).

[5] Forestry Code of the Russian Federation of December 4, 2006 No.200-FZ (as amended on July 31, 2020). Available from: http://www.consultant.ru/document/cons_doc_LAW_64299/.

[6] State Pharmacopeia of the Russian Federation XIV edition. Vol. I–IV; M., 2018. available from: https://femb.ru/record/pharmacopea14.

[7] Rules of Good Practice of Cultivation, Collection, Processing, and Storage of Starting Materials of Herbal Origin adopted by Resolution No.15 of the Council of the Eurasian Economic Commission of January 26, 2018.

DOI: 10.19163/2307-9266-2022-10-2-154-163

stances. Rules for Acceptance, and Sampling Methods[8]; Russian National Standard GOST 24027.1-80 Herbal Substances. Identification, Detection of Pest Contamination, Testing of Impurities and Degree of Fineness[9]; Russian National Standard GOST 24027.2-80 Herbal Substances. Determination of Water Content, Ash, Extractives, Tannins, and Essential Oil[10]; Committee for Medicinal Products for Human Use. Guideline on Allergen Products: Production and Quality Issues. European Medicines Agency. –London. 2008[11]; European Pharmacopoeia. 10th ed. European Department for the Quality of Medicines[12]; US Food and Drug Administration. Compliance Program Guidance Manual, Chapter 45, Biological Drug Products, Inspection of Biological Drug Products (CBER) 7345.848[13]; Code of Federal Regulations Food and Drug Administration (21 680.1). Allergenic Products. V.7[14].

RESULTS AND DISCUSSION

Like other herbal substances, pollen is usually harvested from wild and cultivated plants. General Pharmacopoeia Monograph (GPM).1.7.1.0001.15 "Allergens"[15] states that in the case of cultivated plants, the maturation of pollen has to take place in pollinaria – specially equipped facilities with continuous indoor climate monitoring. Such methods of obtaining raw materials are used in the EU and the USA. Several large manufacturers, such as Stallergenes (France), have their own pollinaria[16]. This is a new and fairly uncommon strategy that allows obtaining homogeneous materials and minimizing man-caused impacts [26].

There are several methods of pollen collection: manual, vacuum-assisted, and water-assisted. Any of these methods may be used for pollen collection from a wide variety of plants. The choice of the method will depend on the experience of a particular collector, available resources, an area size, and the required amount of raw materials[17]. Each method has its benefits and drawbacks. The most common method in Russia is a manual collection by an open method, which is very laborious, but not very efficient. The water method is more efficient. It consists in placing the plant parts with mature anthers in trays filled with water. The anthers open up, and the pollen grains stick to the tray surface. The main advantage of this method is that it helps to obtain high-purity materials. However, this method is also very laborious, and the collected pollen is exposed to moisture, which subsequently stimulates the growth of microorganisms, including pathogenic ones [26].

The vacuum method is recommended for pollen collection from the plants that grow as a ground cover, like most weeds. Before starting the collection process, the area is carefully inspected for any related or unrelated plants that could by collected by mistake. The collection is carried out with the help of special devices in the form of reservoirs fitted with air tubes working similar to a vacuum cleaner. The main advantage of this method is that it is relatively simple. The main disadvantage of the method is that it is impossible to remove all unwanted plants from the collection area, and the final product may contain a lot of pollen grains from other plants, which cannot be isolated. Mechanical sieving is the easiest and most popular method of pollen purification, as sieving through different micronic mesh sizes helps to remove biological contaminants that are different in size from pollen grains. The purification by air allows removal of those impurities that are also different in mass. However, the air-assisted purification equipment is expensive and often results in the damage to pollen, which makes it difficult to assess its allergenic properties [26].

Based on the above, it is recommended to include three main manual collection methods into the Rules[18]. The first collection method uses cyclone dust collectors and is the most effective of all the three methods. Raw materials are collected into special bags and can be processed immediately after the collection. The second method is more problematic, because pollen is collected together with flowers. The collected flowers have to be dried in special convection dryers. Next, the flowers are cut to separate the pollen, and the pollen is sieved. The advantage of this method is that it produces dried materials, already ready for storage. However, a major disadvantage of the method is the likelihood of finding parts of the cut flowers in the harvested materials. The third method is the least effective of all. Inflorescences are placed in a plastic bag, without cutting them from the

[8] Russian National Standard GOST 24027.0-80 Herbal substances. Rules for Acceptance, and Sampling Methods. Available from: https://docs.cntd.ru/document/1200022938.

[9] Russian National Standard GOST 24027.1-80 Herbal Substances. Identification, Detection of Pest Contamination, Testing of Impurities and Degree of Fineness. Available from: https://rags.ru/gosts/gost/14034/.

[10] Russian National Standard GOST 24027.2-80 Herbal Substances. Determination of Water Content, Ash, Extractives, Tannins, and Essential Oil. Available from: https://rags.ru/gosts/gost/30604/.

[11] Guideline on Allergen Products: Production and Quality Issues. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-allergen-products-production-quality-issues_en.pdf.

[12] European Pharmacopoeia. 10th ed. Available from: https://www.edqm.eu/en/web/edqm/european-pharmacopoeia-ph-eur-10th-edition.

[13] US Food and Drug Administration. Compliance Program Guidance Manual, Chapter 45, Biological Drug Products. Available from: https://www.fda.gov/media/73834/download.

[14] Code of Federal Regulations Food and Drug Administration (21 680.1). Allergenic Products. V.7. Available from: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-F/part-680/section-680.1.

[15] State Pharmacopeia of Russian Federation XIV ed. GMP.1.7.1.0001.15 Allergens. M.; 2018. Available from: http://pharmacopoeia.ru/ofs-1-7-1-0001-15-allergeny. Russian

[16] Stallergenes Greer. Allergen Extracts: Derived from Natural Sources. Available from: https://www.stallergenesgreer.com/manufacturing.

[17] Copes DL, Vance NC, Randall WK, Jasumback A, Hallman R. Vacuum collection of Douglas-fir pollen for supplemental mass pollinations. Res. Note PNW-RN-503. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 1991: 9 p.

[18] Encyclopaedia of Beekeeping. Collection and Storage of Pollen. Available from: http://paseka.pp.ru/pchela-i-zdorove-cheloveka/614-sbor-i-xranenie-pylczy-i-pergi.html. Russian

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

plant, and are shaken vigorously. This method is more appropriate for large inflorescences, as it is less likely to damage the flowers.

The choice of the method should be governed by the type of the harvested plant. The composition and quality of pollen depend on a variety of factors, therefore, no major biologically active components have been determined yet that could be used for pollen qualification or standardisation.

General Pharmacopoeia Monograph.1.7.1.0001.15 "Allergens"[19] (SP Rus., XIV ed.); provides the general requirements for the quality of pollen: a harvesting period; a residual moisture; morphological characteristics; impurities; heavy metals (sulfated ash); pest contamination. Nevertheless, the general monograph does not contain requirements for the methods of pollen collection.

The most relevant current regulations include the following ones: Russian National Standards GOST 24027.0-80, GOST 24027.1-80, and GOST 24027.2-80, Rules of Good Manufacturing Practice of the Eurasian Economic Union adopted by Resolution No. 77. The analysis of the regulatory documents has revealed no mention of pollen in them. Resolution No.15 applies to the production of herbal substances by the agricultural industry and covers the organic material production and obtaining herbal substances from wild-growing plants. However, the collection rules do not cover specific aspects of pollen collection from wild-growing and cultivated plants. At the same time, Resolution No. 77 states that the requirements for raw materials also apply to pollen.

Thus, there are some general requirements for the pollen quality ~~of~~ in Russia, but there are no controls or standardized procedures for harvesting, drying, and purification of pollen. The USA and EU also lack specific qualification programmes for pollen-collecting companies and/or individual pollen collectors[20]. Regulatory authorities have established requirements for the allergen extract quality, but when it comes to the raw materials, they mainly rely on the requirements for visual control of raw materials or suggest delegating the responsibility to the manufacturer. Therefore, allergen extract manufacturers usually provide a list of their own requirements for the companies supplying pollen and/or for individual collectors [36]. Table 1 summarizes quality control parameters for some medicinal products by different manufacturers, which are authorized in Russia [37]. The standardisation performed is based on the major protein [38-40].

The pollen composition has an effect on both efficacy and safety of ~~the~~ pollen-based herbal medicinal products. The allergenic composition of pollen depends on genetic factors, weather and climate conditions, man-made factors, a period and methods of pollen collection, its procession, and storage [35]. For instance, rains damage the exine of pollen grains, and that affects the allergenicity of the obtained pollen [41]. Therefore, collection of pollen during rains may affect the allergen profile of pollen. The qualitative and quantitative composition of allergens contained in pollen, is determined not only by specific weather or soil conditions, but also by intrinsic genetic properties of different varieties of plants [42]. If the raw material contains pollen from different plant genera and species, this may result in the presence of cross-reacting allergens and, consequently, increase the risks of adverse drug reactions due to the changes in the dosages of allergens in herbal medicinal products [43], or reduce the treatment efficacy due to the allergen incompatibility [44]. The risks of adverse reactions to immunotherapy depend on each patient's individual model of sensitization to plant allergens [45]. In general, ASI is regarded a safe kind of therapy. The studies suggest that the most frequent adverse reactions are of a local type (64%) [45]. No systemic adverse reactions have been observed in pre-clinical [46] and clinical [47] studies.

A proper pollen storage is critical for ensuring the quality of raw materials and preserving their protein composition[21]. Harvesting, processing, storage, and a quality control of pollen are performed with due regard to anatomical and diagnostic properties of plants.

Elaboration of regulatory document setting requirements for pollen collection as raw material for medicines production

The authors of this paper suggest an approach to the elaboration of a document on harvesting/collecting, storage, and processing of pollen (hereinafter referred to as the Rules) (Fig. 1). These Rules apply to harvesting/collecting of pollen from wild and cultivated plants, to be used as a raw material for the industrial-scale production of medicinal products.

The Rules are intended for the individuals who are directly involved in harvesting/collecting of pollen, i.e. individual collectors and self-employed businessmen who have an appropriate certificate of training.

Personnel – pollen collectors

1. In order to be admitted to pollen harvesting/collecting, a Collector must be checked for infectious diseases, and if any infection is detected, the Collector must be suspended.

2. The Collector must meet all the requirements set forth in the Rules of Good Practice of Cultivation, Collection, Processing, and Storage of Starting Materials of Herbal Origin.

[19] State Pharmacopeia of Russian Federation XIV ed. GMP.1.7.1.0001.15 Allergens. M.; 2018.

[20] US Food and Drug Administration. Compliance Program Guidance Manual, Chapter 45, Biological Drug Products. Inspection of Biological Drug Products (CBER) 7345.848. Available from: https://www.fda.gov/media/73834/download.

[21] Allergen Science and Consulting. Storage of Allergenic Raw Materials. Available from: http://allergenscienceandconsulting.com/allergenic-raw-materials-storage/.

DOI: 10.19163/2307-9266-2022-10-2-154-163

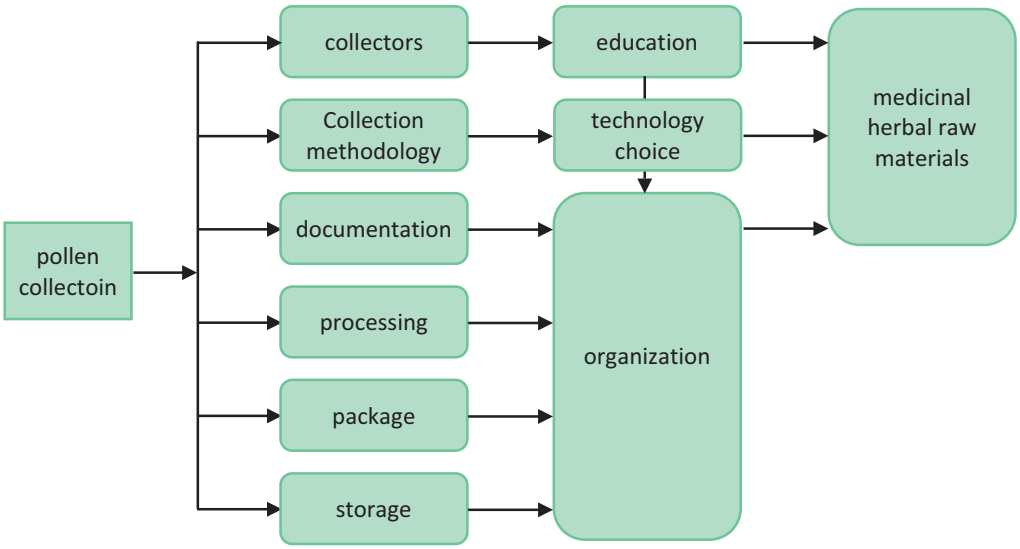

Figure 1 – Diagram of proper pollen harvesting for medicines production (A.A. Taube, 2021)

Table 1—Principles of standardization of medicinal products for ASI

Parameter	Allergens ("Seva Pharma", Czech Republic)	Staloral, Oralair (Stellergen, France)	LAIS (Lofarma, Italy)
Molecular structure	Polymer	Polymer	Monomer (allergoid)
Standardization	Protein nitrogen content	Reactivity index (characterises the major antigen)	Allergenic unit (characterises the antigenic determinant)
Administration route	Sublingual (drops, tablets)	Sublingual (drops, tablets)	Sublingual (tablets)

3. Prior to harvesting, the Collector must be informed about the principles of handling rare and protected medicinal plants, and a sustainable use of natural resources in accordance with the Forestry Code of the Russian Federation[22].

4. The Collector must have sufficient knowledge of the harvested wild and cultivated plants.

5. Pollen is a highly allergenic product; therefore the Collector must be protected from its harmful effect.

6. Collectors must be provided with adequate sanitary-hygienic labour conditions, working clothes, and appropriate personal protective equipment (PPE).

Technical requirements for pollen harvesting/collecting

The next stage of the study involved the elaboration of technical requirements for pollen collection methods.

The authors have carefully studied the process of pollen collection and formulated the following requirements.

1. To minimize the content of heavy metals and other ecotoxicants in pollen, it is forbidden to carry out harvesting near roads and manufacturing facilities.

2. Harvested medicinal plants should not be damaged by bacteria or insects, only healthy plants should be harvested.

3. A harvesting period should coincide with the flowering period of the plant, which may vary depending on the type of the plant and its vegetation area. For example, flowering of birch takes place from the end of April till the end of May, while flowering of cereals takes place much later – from the end of May till the end of July.

4. Pollen collection should not be carried out during or after the rain and in the morning, if there is dew. Damp or wet plants/trees should not be harvested. A high humidity increases the risk of mould and bacteria that are damaging for the pollen allergen. Pollen collection should take place in the daytime or in the evening A more specific time of pollen collection depends on the genus and species of the harvested plant. The collection time may also be influenced by the region where the collection is taking place.

5. Pollen collection should be carried out in such a way as to minimize particulate contaminations (e.g., from the soil).

6. Contact of raw materials with other types of pollen must be avoided in order to prevent cross-contaminations.

7. Pollen collection may be carried out by manual

[22] Forestry Code of the Russian Federation of December 4, 2006 No. 200-FZ (as amended on July 31, 2020). Available from: http://www.consultant.ru/document/cons_doc_LAW_64299/. Russian

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

or vacuum methods. The choice of the method must be justified for each type of the plant. A proper collection helps to increase the efficiency of the process and avoid contaminations of raw materials.

8. The equipment should be made from high-quality materials preventing cross-contaminations. The machine parts that are in direct contact with the raw materials, must be carefully cleaned to avoid contaminations from previously collected pollen and from residual amounts of detergents.

9. During harvesting, raw material must be protected from the exposure to the substances used in the equipment operation, such as lubricants and fuel.

10. In order to obtain pollen of good quality, the processing time should be stuck to. The Collector must deliver the harvested raw materials to the customer within 2–3 hours after the collection. If this is not feasible, the raw materials must be dried. Drying is carried out in convection dryers at 35–40°C [48]. Before drying, the pollen is spread out in a thin layer on greaseproof paper. This helps to prevent mould formation during storage. After drying, plant parts, fungal spores, and insect fragments are removed from the pollen. The two main purification methods are mechanical sieving and air purification.

11. The storage of pollen until its delivery to the manufacturer is carried out according to the Rules of Good Practice of Cultivation, Collection, Processing, and Storage of Starting Materials of Herbal Origin.

12. Accompanying documentation is prepared according to the Rules of Good Practice of Cultivation, Collection, Processing, and Storage of Starting Materials of Herbal Origin.

13. It is prohibited to harvest endangered plant species (Article 85 of the Administrative Offence Code of the Russian Federation dated December 30, 2001 No.195-FZ[23]).

14. Repeated harvesting of medicinal plants in the same area is carried out in accordance with the Order of the Ministry of Natural Resources of Russia dated July 28, 2020 No.494[24]. Pollen is an above-ground organ of the plant, therefore the collection interval is once in 2 years for annual plants and once in 4–6 years for perennial plants. This is an essential requirement for prevention of medicinal plant extinction.

15. The harvesting/collection method is developed for each species on a case-by-case basis.

16. Values of organoleptic properties should be determined by dedicated experts for each individual type of a herbal substance. However, there are some visual control parameters, which are common for all types of herbal substances: evident impurities, such as soil or parts of plants; contaminations with pathogenic microorganisms, mould, larvae, etc. These problems may arise when pollen is collected from unhealthy plants, or when the conditions of raw materials storage are not observed [49].

[23] Administrative Offence Code of the Russian Federation of December 30, 2001 No. 195-FZ (as amended on April 20, 2021).

[24] Order of the Ministry of Natural Resources of Russia of July 28, 2020 No. 494 On Adoption of the Rules for Harvesting Forest Plants for the Food Industry, and Medicinal Plants (Registered in the Ministry of Justice of Russia on December 14, 2020, registration No. 61428). Russian

Documentation

At the next stage of the study, the authors elaborated the Documentation part of the Good Practice. All activities performed with raw materials must be documented. The authors elaborated a Pollen Collection standard form, which is given in the annex to the Rules.

The Pollen Collection form was compiled, based on the form for harvesting herbal substances given in the Rules of Good Practice of Cultivation, Collection, Processing, and Storage of Starting Materials of Herbal Origin. The form contains the following fields to be filled:

1. Latin name of the harvested plant (genus, species, subspecies, variety);
2. Russian name of the plant;
3. Place of collection (district / region / country / geographical bearings if possible);
4. Date and time of the collection to make sure that the collection time is chosen correctly;
5. Collector's data, i.e. his/her full name;
6. Collector's location. For legal entities—the address of the company, for the individuals registered as self-employed businessmen – residential addresses, to enable a communication with the supplier, if necessary;
7. Collection/weather conditions, since collection is forbidden in rainy weather;
8. Nearby plants – to know which pollen impurities may be present in the harvested material;
9. Insects that may be found on nearby plants—to perform tests for potential contaminations of pollen;
10. Collection method – in order to know which impurities should be tested and which tests should be performed;
11. The amount of collected pollen;
12. Additional information which may include the growing conditions if known, and the fertilizers used;
13. Whether the harvested material was dried after the collection, and if so, which equipment and conditions were used;
14. The circumstances that can affect the quality.

The delivery of the harvested material by the Collector to the customer must be based on a commercial contract, which stipulates the provision of accompanying documents confirming that the collection of pollen was performed in accordance with the applicable requirements.

Storage and transportation

Pollen must be stored under well-defined conditions. Requirements for storage and transportation are

DOI: 10.19163/2307-9266-2022-10-2-154-163

established in the SP Rus., XIV ed. and in the Rules of Good Practice of Cultivation, Collection, Processing, and Storage of Starting Materials of Herbal Origin. Individual storage conditions may be considered for pollen of some particular plants.

The main requirements are:

1. Pollen and packaging materials must be stored in dry, well-aerated facilities, protected from rodents, birds, and insects.

2. Pollen must be packaged in dry, clean, thick bags.

3. Each package must be labelled in accordance with the Decision of the Council of the Eurasian Economic Commission dated November 3, 2016 No. 76[25]. The following has to be specified:

– raw materials names;
– names of suppliers/collectors;
– lot/batch number;
– year and month of harvesting/collecting;
– delivery dates;
– shelf life.

4. Different types of raw materials must be stored in tight packages to prevent contamination.

5. Packaged raw materials must be stored on trays to avoid wetting.

6. Pollen herbal substances must be stored and transported frozen (at a relative humidity of 3±0.5%);

7. Frozen herbal substances should be stored at temperatures below –18°C, and below –20°C in case of long-term storage.

CONCLUSION

Based on the results of the performed analysis, the authors prepared the Rules for Harvesting/Collecting of Pollen, which include 6 parts. The Rules cover the whole process of pollen collection and all related processes. The implementation of this document as an independent part of the Rules of Good Practice of Cultivation, Collection, Processing, and Storage of Starting Materials of Herbal Origin will make it possible to collect pollen of wild and cultivated plants and obtain high-quality raw materials (with minimum impurities/heavy metals/contaminations with pathogenic microorganisms/mould, etc.), without detriment to the plant population.

The availability of a regulatory document establishing requirements for the process of harvesting, storage, and processing of pollen herbal substances will make it possible to improve the process of pollen collection, thus reducing risks associated with the use of pollen-based medicines. The focus of further studies could be on establishing the correlation between the quality of pollen and development of adverse drug reactions.

FUNDING

The study reported in this publication was carried out as a part of a publicly funded research project No. 056-00001-22-00 and was supported by the Federal State Budgetary Institution “Scientific Centre for Expert Evaluation of Medicinal Products” of the Ministry of Health of the Russian Federation (R&D public accounting No. 121021800098-4).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS’ CONTRIBUTIONS

Aleksandra A. Taube – formulation of the study concept, choice of the study methods, analysis and interpretation of the obtained results, direct involvement in the study, carrying responsibility for all aspects of the study; Tatyana A. Buyanova – analysis of scientific literature and guidelines, carrying responsibility for all aspects of the study related to data reliability; Elena I. Sakanyan – editing and revision of the text, final approval of the paper, carrying responsibility for all aspects of the study.

REFERENCES

1. Bastl K, Bastl M, Bergmann K-C, Berger M, Berger U. Translating the Burden of Pollen Allergy Into Numbers Using Electronically Generated Symptom Data From the Patient’s Hayfever Diary in Austria and Germany: 10-Year Observational Study. J Med Internet Res. 2020; 22(2):e16767. DOI: 10.2196/16767.
2. Kiguchi T, Yamamoto-Hanada K, Saito-Abe M, Sato M, Irahara M, Ogita H, Miyagi Y, Inuzuka Y, Toyokuni K, Nishimura K, Ishikawa F, Miyaji Y, Kabashima S, Fukuie T, Narita M, Ohya Y. Pollen-food allergy syndrome and component sensitization in adolescents: A Japanese population-based study. PLoS One. 2021 Apr 14;16(4):e0249649. DOI: 10.1371/journal.pone.0249649.
3. Ta V, Scott DR, Chin WK, Wineinger NE, Kelso JM, White AA. Differential skin test reactivity to pollens in pollen food allergy syndrome versus allergic rhinitis. Allergy Asthma Proc. 2015;36(5):379–85. DOI: 10.2500/aap.2015.36.3862.
4. Poncet P, Sénéchal H, Charpin D. Expert Update on pollen-food allergy syndrome. Rev Clin Immunol. 2020;16(6):561–78. DOI: 10.1080/1744666X.2020.1774366.
5. Yagami A, Ebisawa M. New findings, pathophysiology, and antigen analysis in pollen-food allergy syndrome. Curr Opin Allergy Clin Immunol. 2019;199(3):218-23. DOI: 10.1097/ACI.0000000000000533.

[25] Decision of the Council of the Eurasian Economic Commission of November 3, 2016 No.76 On Adoption of the Requirements for Labelling of Medicinal Products for Human Use and Veterinary Medicinal Products.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

6. Petrova SYu, Berzhets VM, Petrova NS, Hrulyova VA, Emel'yanov OYu, Hlgatyan SV, Koreneva EA. Perspektivy razvitiya lechebnyh form allergenov. Ot abstraktnyh problem k konkretnym resheniyam. [Immunopatologiya, allergologiya, infektologiya]. Journal of immunopatologiya, allergologiya, infektologiya. 2018;1:40–7. DOI: 10.14427/jipai.2018.1.40. Russian
7. Fortescue R, Kew KM, Tsun Leung MS. Sublingual immunotherapy for asthma. Cochrane Database Syst Rev. 2020;9(9):CD011293. DOI: 10.1002/14651858.CD011293.pub3.
8. Asero R, Gianni Mistrello G, Amato S. Detection of pan-allergens in commercial pollen extracts for allergen immunotherapy. Ann Allergy Asthma Immunol. Aug;117(2):180–5. DOI: 10.1016/j.anai.2016.05.010.
9. Kleine-Tebbe J, Matricardi PM, Hamilton R.G. Allergy Work-Up Including Component-Resolved Diagnosis: How to Make Allergen-Specific Immunotherapy More Specific. Immunol Allergy Clin North Am. 2016;36(1):191–203. DOI: 10.1016/j.iac.2015.08.012.
10. El-Qutob D, Letrán A, Matheu V, Fernandez-Caldas E. Cancer: Still a contraindication for allergen immunotherapy?: Specific immunotherapy and cancer. World Allergy Organization Journal. 2021;14(10): 100597 DOI: 10.1016/j.waojou.2021.100597.
11. Calderón MA, Bacharier LB. Controversies in Allergy: A Pro/Con Review of Sublingual Allergen Immunotherapy and Allergic Asthma. J Allergy Clin Immunol Pract. 2021;9(5):1818–25. DOI: 10.1016/j.jaip.2021.02.029.
12. Mailhol C, Didier A. Allergen-specific immunotherapy in the treatment of pollen allergy. Rev Mal Respir. 2013;30(2):142–51. DOI: 10.1016/j.rmr.2012.07.005.
13. Cox L. Sublingual immunotherapy for aeroallergens: Status in the United States. Allergy Asthma Proc. 2014;35(1):34–42. DOI: 10.2500/aap.2014.35.3708.
14. Kinaciyan T, Nagl B, Faustmann S, Frommlet F., Kopp S, Wolkersdorfer M, Wöhrl S, Bastl K, Huber H, Berger U, Bohle B. Efficacy and safety of 4 months of sublingual immunotherapy with recombinant Mal d 1 and Bet v 1 in patients with birch pollen – related apple allergy. J Allergy Clin Immunol. 2018; 141(3):1002–08. DOI: 10.1016/j.jaci.2017.07.036.
15. Asero R, Mistrello G, Amato S. Detection of pan-allergens in commercial pollen extracts for allergen immunotherapy. Ann Allergy Asthma Immunol. 2016; 117(2):180–5. DOI: 10.1016/j.anai.2016.05.010.
16. Rodríguez Del Río P, Díaz-Perales A, Sánchez-García S, Escudero C, Ibáñez MD, Méndez-Brea P, Barber D. Profilin, a Change in the Paradigm. J Investig Allergol Clin Immunol. 2018;28(1):1-12. DOI: 10.18176/jiaci.0193.
17. Codina R, Lockey RF. Pollen used to produce allergen extracts. Ann Allergy Asthma Immunol. 2017; 118(2):148–53. DOI: 10.1016/j.anai.2016.02.005.
18. Wang L, Zhang X, Hu J. Proteomic analysis and identification of possible allergenic proteins in mature pollen of populus tomentosa. Int. J. Mol. Sci. 2018;19(1):250 DOI: 10.3390/ijms19010250.
19. Brier S, Le Mignon M, Jain K, Lebrun C, Peurois F, Kellenberger C, Bordas-Le Floch V, Mascarell L, Nony E, Moingeon P. Characterization of epitope specificities of reference antibodies used for the quantification of the birch pollen allergen Bet v 1. Allergy. 2018;73:1032–40. DOI: 10.1111/all.13364.
20. Costa J, Bavaro SL, Benedé S, Diaz-Perales A, Bueno-Diaz C, Gelencser E, Klueber J, Larré C, Lozano-Ojalvo D, Lupi R, Mafra I, Mazzucchelli G, Molina E, Monaci L, Martín-Pedraza L, Piras C, Rodrigues PM, Roncada P, Schrama D, Cirkovic-Velickovic T, Verhoeckx K, Villa C, Kuehn A, Hoffmann-Sommergruber K, Holzhauser T. Are Physicochemical Properties Shaping the Allergenic Potency of Plant Allergens? Clin Rev Allergy Immunol. 2022;62(1):37–63. DOI: 10.1007/s12016-020-08810-9.
21. Kailaivasan T, Davies JM. The molecular allergology of subtropical grass pollen. Mol Immunol. 2018;100:126–35. DOI: 10.1016/j.molimm.2018.03.012.
22. Klimek L., Brehler R., Hamelmann E., Kopp M., Ring J., Treudler R., Jakob T., Worm M., Pfaar O. Development of subcutaneous allergen immunotherapy (part 2): preventive aspects and innovations. Allergo J Int. 2019;28:107–19. DOI: 10.1007/s40629-019-0097-z.
23. Zimmer J, Bridgewater J, Ferreira F, van Ree R, Rabin RL, Vieths S. The History, Present and Future of Allergen Standardization in the United States and Europe. Front Immunol. 2021;12:725831. DOI: 10.3389/fimmu.2021.725831.
24. Nelson HS. Allergy immunotherapy: Future directions for the 2020s. Allergy Asthma Proc. 2020 Sep 1;41(5):314–25 DOI: 10.2500/aap.2020.41.200041
25. Ignatov AA, Ramenskaya GV, Smirnov VV. Sovremennye tendencii v standartizacii preparatov allergenov. [Farmakokinetika i farmakodinamika]. Pharmacokinetics and pharmacodynamics. 2015; 1:16–20 Russian
26. Codina R, Crenshaw RC, Lockey RF. Considerations About Pollen Used for the Production of Allergen Extracts. J Allergy Clin Immunol Pract. 2015; 3 (5):676–82. DOI: 10.1016/j.jaip.2015.04.003
27. Amato D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, Liccardi G, Popov T, van Cauwenberge P. Allergenic pollen and pollen allergy in Europe. Allergy. 2007;62(9):976–90. DOI: 10.1111/j.1398-9995.2007.01393.x.
28. Larsen JM, Bang-Berthelsen CH, Qvortrup K, Sancho AI, Hansen AH, Andersen KIH, Thacker SSN, Eiwegger T, Upton J, Bøgh KL. Production of allergen-specific immunotherapeutic agents for the treatment of food allergy. Crit Rev Biotechnol. 2020;40(6):881–94. DOI: 10.1080/07388551.2020.1772194.
29. Rossi RE, Monasterolo G, Coco G, Operti D. Possible relationship between systemic side effects and sensitization to rPar j 2 in allergic patients submitted to an ultra-rush (20 min) sublingual immunotherapy and selected by component resolved diagnosis. Int Arch Allergy Immunol. 2005;138(2):105–10. DOI: 10.1159/000088431
30. Petrova SYu, Hlgatyan SV, Berzhec VM, Vasil'eva AV. Allergensoderzhashchie vakciny dlya specificheskoj immunoterapii. [ZHurnal mikrobiologii, epidemiologii i immunobiologii]. Journal of Microbiology, Epidemiology and Immunobiology. 2021;98(1):104–12. DOI: 10.36233/0372-9311-11 Russian
31. Starchenka S, Heath MD, Lineberry A, Higenbottam T, Skinner MA.Transcriptome analysis and safety profile of the early-phase clinical response to an adjuvanted grass allergoid immunotherapy. World Allergy Org J. 2019; 12(11):100087. DOI: 10.1016/j.waojou.2019.100087.
32. Cudowska B, Kapingidza AB, Pawłowicz M, Pampuch A, Hyduke N, Pote S, Schlachter CR, Lebensztejn DM, Chruszcz M, Kowal K. Production and Use of Recombinant

DOI: 10.19163/2307-9266-2022-10-2-154-163

Profilins Amb a 8, Art v 4, Bet v 2, and Phl p 12 for Allergenic Sensitization Studies. Molecules. 2020; 25(2):369. DOI: 10.3390/molecules25020369.
33. O'Malley A, Kapingidza AB, Hyduke N, Dolamore C, Kowal K, Chruszcz M. Crystal structure of timothy grass allergen Phl p 12.0101 reveals an unusual profilin dimer. Acta Biochim Pol. 2021;68(1):15–22. DOI: 10.18388/abp.2020_5587.
34. Taube AA, Gilyok MT, Sakanyan EI Methodological Approaches to Improving the Quality of Medicinal Plant Raw Materials of the Pollen Morphology Group. Pharm Chem J. 2022;55(12): 1407–11. DOI: 10.1007/s11094-022-02589-z.
35. Park J-W, Jeong K-Y. Allergen standardization. Allergy Asthma Respir Dis. 2018;6(4):191–6. DOI: 10.4168/aard.2018.6.4.191
36. Soldatov AA, Medunicyn NV, Avdeeva ZHI, Bondarev VP, Mironov AN. Preparaty lechebnyh allergenov: problemy i puti povysheniya kachestva, bezopasnosti i effektivnosti. [Vedomosti Nauchnogo tsentra ekspertizy sredstv meditsinskogo primeneniya. Regulyatornye issledovaniya i ekspertiza lekarstvennykh sredstv]. Bull Scie Centre Expert Eval Med Prod. 2013; 4:31–7. Russian
37. Fedorov AS, Litvinova LS, But-Gusaim VI, Litvinenko SN. Allergen-specificheskaya immunoterapiya: terapevticheskie vakciny dlya allergicheskih zabolevanij. [Medicinskaya immunologiya]. Medic Immunol. 2015;17(5):407–22. Russian
38. Yang YSh, Xu ZQ, Zhu W, Zhu DX, Jiao YX, Zhang LSh, Hou YB, Wei JF, Sun JL. Molecular and immunochemical characterization of profilin as major allergen from Platanus acerifolia pollen. Int Immunopharmacology. 2022;106:108601. DOI: 10.1016/j.intimp.2022.108601.
39. Caraballo L, Valenta R, Acevedo Nand Zakzuk J Are the TermsMajor and Minor Allergens Useful for Precision Allergology? Front Immunol. 2021;12:651500. DOI: 10.3389/fimmu.2021.651500.
40. Landa-Pineda CM, Arroyo-Becerra A, Rosas-Alvarado A, Terán LM, Garcia-Cruz ML, Marchat LA, Reyes-López CA. Major allergen from Amaranthus palmeri pollen is a profilin: Isolation, partial characterisation and IgE recognition // Allergol Immunopathol (Madr). 2006;449(2):160–6. DOI: 10.1016/j.aller.2015.05.002
41. D'Amato G. The effects of climate change on respiratory allergy and asthma induced by pollen and mold allergens. Allergy. 2020;75(9):2219–28. DOI: 10.1111/all.14476.
42. Fernández-Caldas E, Carnés J, Iraola V, Casanovas M. Comparison of the allergenicity and Ole e 1 content of 6 varieties of Olea europaea pollen collected during 5 consecutive years. Ann Allergy Asthma Immunol. 2007 May;98(5):464–70. DOI: 10.1016/S1081-1206(10)60761-2.
43. Weber RW. Cross-reactivity of plant and animal allergens. Clin Rev Allergy Immunol. 2001 Oct;21(2–3):153–202. DOI: 10.1385/CRIAI:21:2-3:153.
44. Daigle BJ, Rekkerth DJ. Practical recommendations for mixing allergy immunotherapy extracts. Allergy Rhinol (Providence). 2015 Jan;6(1):1–7. DOI: 10.2500/ar.2015.6.0111.
45. Sastre J, Rodríguez F, Campo P, Laffond E, Marín A, Alonso MD. Adverse reactions to immunotherapy are associated with different patterns of sensitization to grass allergens. Allergy. 2015;70(5):598–600. DOI: 10.1111/all.12575.
46. Mitobe Y, Yokomoto Y, Ohashi-Doi K. Safety evaluation of standardized allergen extract of Japanese cedar pollen for sublingual immunotherapy. Regul Toxicol Pharmacol. 2015 April;71(3):529–40. DOI: 10.1016/j.yrtph.2015.02.009
47. Canonica GW, Cox L, Pawankar R, Baena-Cagnani CE, Blaiss M, Bonini S, Bousquet J, Calderón M, Compalati E, Durham S.R, van Wijk R.G, Larenas-Linnemann D, Nelson H, Passalacqua G, Pfaar O, Rosário N, Ryan D, Rosenwasser L, Schmid-Grendelmeier P, Senna G, Valovirta E, Van Bever H, Vichyanond P, Wahn U, Yusuf O. Sublingual Immunotherapy: World Allergy Organization Position Paper. 2013 update. World Allergy Organ J. 2014;7(6):4–52. DOI:10.1186/1939-4551-7-6
48. Bokov DO, Smirnov VV, Demina NB. Proizvodstvo i standartizaciya pyl'cevyh allergennyh ekstraktov // Razrabotka i registraciya lekarstvennyh sredstv=Drug development & registration. 2014;4(9): 68–83.
49. Kostić AZ, Milinčić DD,Petrović TS, Krnjaja VS, Stanojević SP, Barać MB, Tešić ZL, Pešić MB. Mycotoxins and Mycotoxin Producing Fungi in Pollen: Review. Toxins (Basel). 2019 Jan 24; 24(11(2)):64. DOI: 10.3390 / toxins11020064.

AUTHORS

Aleksandra A. Taube – Candidate of Sciences (Pharmacy), Leading Researcher at the Center for Planning and Coordination of Research Works of the Scientific Centre for Expert Evaluation of Medicinal Products. ORCID ID: 0000-0001-5594-4859. E-mail: Aleksandra.taube@pharminnotech.com

Tatyana A. Buyanova – master's student of the Department of Economics and Management of St. Petersburg Chemical and Pharmaceutical University. ORCID ID: 0000-0001-7033-424X. E-mail: buyanovatanya1@gmail.com

Elena I. Sakanyan – Doctor of Sciences (Pharmacy), Director of Research, Joint-Stock Company "Scientific and Production Association "Microgen". ORCID ID: 0000-0001-8536-4804. E-mail: sakanjan@mail.ru

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

COMPARATIVE PHARMACOECONOMIC ANALYSIS OF LONG-ACTING LANREOTIDE USED IN ACROMEGALY THERAPY WITHIN CONDITIONS OF THE RUSSIAN FEDERATION HEALTH CARE SYSTEM

I.S. Krysanov[1,2,3], E.V. Makarova[3,4], V.Yu. Ermakova[2,5]

[1] Moscow State University of Food Production
11, Volokolamsk Hwy., Moscow, Russia, 125080
[2] LLC "Institute of Clinical and Economic Expertise and Pharmacoeconomics"
Bld. 6, 21, Novomytishchinsky Ave., Mytishchi, Moscow region, Russia, 141008
[3] A.I. Yevdokimov Moscow State University of Medicine and Dentistry
Bld. 1, 20, Delegatskaya Str., Moscow, Russia, 127473
[4] National Scientific Research Institute of Public Health
Bld. 1, 12, Vorontsovo Pole Str., Moscow, Russia, 105064
[5] Sechenov First Moscow State Medical University
Bld. 4, 2, Bolshaya Pirogovskaya Str., Moscow, Russia, 119435

E-mail: krysanov-ivan@mail.ru

Received 05 Feb 2022 After peer review 07 Apr 2022 Accepted 25 Apr 2022

The aim of this study is to conduct a comprehensive pharmacoeconomic evaluation of the use of long-acting subcutaneous lanreotide gel compared to alternative drugs, for the treatment of acromegaly.

Materials and methods. Based on the literature data, a treatment model with a 1-year outlook was developed, a cost-effectiveness analysis (CEA) in pharmacoeconomics and a sensitivity analysis of changes in the model parameters were carried out. Direct medical costs for the annual therapy course were calculated. The data on the medicines costs were taken from the register of marginal prices of the State Register of Medicines.

Results. According to the unified Russian registry of the pituitary-hypothalamic tumors area, the achievement of remission in the acromegaly patients using lanreotide, a long-acting gel for a subcutaneous administration, compared to the long-acting octreotide, is 51% vs 24%. During the first year of treatment with octreotide, the total pharmacotherapy costs were lower than with lanreotide (RUB 225,496.07 vs RUB 574,451.84). According to the results of the cost-effectiveness analysis for one achieved case of remission, the advantage of using lanreotide over long-acting octreotide was revealed (RUB 1,251,870.56 versus RUB 1,431,005.31). The sensitivity analysis demonstrated the model's stability to increases in the lanreotide price (up to +18%), decreases in the octreotide prices (up to –22%), increases in the transsphenoidal adenomectomy prices (up to +59%), and decreased lanreotide remission rates (up to –12%).

Conclusion. Although the treatment costs analysis showed lower total per year costs of the treatment with long-acting octreotide compared to lanreotide, the calculation of the cost-effectiveness ratio per remission showed that lanreotide had been superior to long-acting octreotide.

Keywords: acromegaly; lanreotide; octreotide; cost-effectiveness analysis; treatment costs

Abbreviations: MP – medicinal product; SSAs, somatostatin analogues; IGF-1 – insulin-like growth factor-1; QOL – quality of life; STH – somatotropic hormone; VAT – value added tax; TM – trade margin; RCT – randomized clinical trial; CT – clinical trial; HTMC – high-tech medical care; AEs – adverse effects; RR – relative risk; CI – confidence interval.

For citation: I.S. Krysanov, E.V. Makarova, V.Yu. Ermakova. Comparative pharmacoeconomic analysis of long-acting lanreotide used in acromegaly therapy within conditions of the Russian Federation health care system. *Pharmacy & Pharmacology.* 2022;10(2):164-173. **DOI:** 10.19163/2307-9266-2022-10-2-164-173

Для цитирования: И.С. Крысанов, Е.В. Макарова, В.Ю. Ермакова. Сравнительный фармакоэкономический анализ применения ланреотида пролонгированного действия для терапии акромегалии в условиях системы здравоохранения Российской Федерации. *Фармация и фармакология.* 2022;10(2):164-173. **DOI:** 10.19163/2307-9266-2022-10-2-164-173

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2022-10-2-164-173

СРАВНИТЕЛЬНЫЙ ФАРМАКОЭКОНОМИЧЕСКИЙ АНАЛИЗ ПРИМЕНЕНИЯ ЛАНРЕОТИДА ПРОЛОНГИРОВАННОГО ДЕЙСТВИЯ ДЛЯ ТЕРАПИИ АКРОМЕГАЛИИ В УСЛОВИЯХ СИСТЕМЫ ЗДРАВООХРАНЕНИЯ РОССИЙСКОЙ ФЕДЕРАЦИИ

И.С. Крысанов[1,2,3], Е.В. Макарова[3,4], В.Ю. Ермакова[2,5]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Московский государственный университет пищевых производств»
125080, Россия, г. Москва, Волоколамское шоссе, д. 11
[2] ООО «Институт клинико-экономической экспертизы и фармакоэкономики»
141008, Россия, Московская область, г. Мытищи, Новомытищинский проспект, д. 21/6
[3] Федеральное государственное бюджетное образовательное учреждение высшего образования «Московский государственный медико-стоматологический университет имени А.И. Евдокимова» Министерства здравоохранения Российской Федерации
127473, Россия, г. Москва, Делегатская ул., 20/1
[4] Федеральное государственное бюджетное учреждение «Национальный научно-исследовательский институт общественного здоровья» Российской академии медицинских наук
105064, Россия, г. Москва, ул. Воронцово Поле, д. 12, стр. 1
[5] Федеральное государственное автономное образовательное учреждение высшего образования «Первый Московский государственный медицинский университет имени И.М. Сеченова» Министерства здравоохранения Российской Федерации (Сеченовский университет)
119435, Россия, г. Москва, Большая Пироговская ул., 2, стр. 4

E-mail: krysanov-ivan@mail.ru

Получена 05.02.2022 После рецензирования 07.04.2022 Принята к печати 25.04.2022

Цель. Проведение комплексной фармакоэкономической оценки применения препарата ланреотид гель для подкожного введения пролонгированного действия по сравнению с альтернативными препаратами для терапии акромегалии.
Материалы и методы. На основании данных литературы была построена модель лечения с горизонтом 1 год, проведен фармакоэкономический анализ затраты-эффективность (CEA) и анализ чувствительности к изменениям параметров модели. Прямые медицинские затраты были рассчитаны на годовой курс терапии. Данные о стоимости лекарственных препаратов были взяты из реестра предельных цен Государственного реестра лекарственных средств.
Результаты. По данным единого Российского регистра опухолей гипоталамо-гипофизарной области, достижение ремиссии у пациентов с акромегалией при использовании ланреотид, гель для подкожного введения пролонгированного действия в сравнении с октреотидом пролонгированного действия составляет 51% против 24%. Общие затраты на фармакотерапию в течение первого года при использовании октреотида были ниже, чем при использовании ланреотида (225496,07 руб. против 574451,84 руб.). По результатам анализа «затраты-эффективность» на один достигнутый случай ремиссии было выявлено преимущество применения ланреотида перед октреотидом пролонгированного действия (1251870,56 руб. против 1431005,31 руб.). Анализ чувствительности продемонстрировал устойчивость модели к увеличению цены на ланреотид (до +18%), уменьшению цены на октреотид (до –22%), увеличению цены на транссфеноидальную аденомэктомию (до +59%) и снижению частоты ремиссий на ланреотид (до –12%).
Заключение. Несмотря на то, что анализ затрат на лекарственные препараты показал меньшие общие затраты на годовой курс терапии октреотидом пролонгированного действия в сравнении с ланреотидом, при расчёте коэффициента «затраты-эффективность» на один достигнутый случай ремиссии было выявлено преимущество применения ланреотида перед октреотидом пролонгированного действия.
Ключевые слова: акромегалия; ланреотид; октреотид; анализ затраты-эффективность; затраты на лечение
Список сокращений: ЛП – лекарственный препарат; АСС – аналоги соматостатина; ИФР-1 – инсулиноподобный фактор роста-1; КЖ – качество жизни; СТГ – соматотропный гормон; НДС – налог на добавленную стоимость; ТН – торговая наценка; РКИ – рандомизированное клиническое исследование; КИ – клиническое исследование; ВМП – высокотехнологическая медицинская помощь; ИФР-1 – инсулиноподобный фактор роста-1; НЯ – нежелательные явления; ОР – относительный риск; ДИ – доверительный интервал.

INTRODUCTION

Acromegaly is a severe neuroendocrine disease caused by a chronic hyperproduction of a somatotropic hormone (STH) in individuals with a completed physiological growth [1]. Due to the development of various complications, the disease is accompanied by a progressive disability, a reduced life expectancy, and an increased mortality risk [2].

Somatostatin analogues (SSAs) are the most common pharmacological treatment option for acrome-

galy. According to the current clinical guidelines [1], the main indication for the SSAs prescription is the adjunctive therapy while maintaining a disease activity at the end of the surgery [3, 4]. SSAs are also used as the first therapy line for small adenomas. In this group, the most common drugs are octreotide and lanreotide [4]

The problem of the therapy resistance in the acromegaly patients is widespread in the clinical practice [5]. To date, about 50% of the acromegaly patients do not achieve biochemical remission when using standard SSAs doses [3]. One of the possible ways to overcome the resistance is to increase the dose or reduce the interval between the injections.

To improve the therapy effectiveness, Russian and international experts also recommend an intragroup replacement of octreotide with lanreotide [5]. In the SSAs group, octreotide is older, while lanreotide is more modern. A number of studies note its better tolerability, a higher percentage of successful injections and, as a result, the achievement of a better control over acromegaly [4].

Lanreotide is a natural somatostatin analogue, able of suppressing the growth hormone secretion due to its pronounced tropism for human somatostatin receptors SSTR [6, 7]. It exists in the usual form (Somatulin®, injections once per 14 days, practically not used at the moment), and in a more convenient prolonged form (Somatulin® Autogel®, injections once per 28 days) [7].

A significant price of SSAs and lanreotide, in particular, is worth notifying. In the study published by Lesén E. et al., in 2013, the annual costs of the SSAs therapy in Sweden were valued at 13,500 euros [8]. In the article by Orlewska E. et al., the annual average costs of therapy with lanreotide (Autogel, 120 mg) in Poland were valued at 12,187 euros [9]. In the analysis by Biermasz N.L. et al., the costs of the SSAs therapy in the Netherlands were reported as €16,500 for octreotide (Sandostatin® LAR, the mean dose of 24 mg/4 weeks) and €23,000 for lanreotide Autogel [10].

No similar studies have been conducted in the Russian Federation. To carry out a competent and reasonable distribution of the healthcare budget, this fact highlights the importance of a comprehensive assessment of different options for the acromegaly treatment.

THE AIM of this study is to conduct a comprehensive pharmacoeconomic evaluation of the use of long-acting subcutaneous lanreotide gel compared to alternative drugs, for the treatment of acromegaly.

To achieve the aim, the following tasks were set:

1. A comparative review of the clinical efficacy and safety of lanreotide, a long-acting subcutaneous gel, for the acromegaly treatment, vs long-acting octreotide.

2. Direct costs calculations for the acromegaly treatment with lanreotide and octreotide within the framework of the Russian healthcare system, as well as for the surgical treatment (transsphenoidal adenomectomy) in case of a failure to achieve remissions.

3. Developing a pharmacoeconomic model to assess the relationship between the prescription of lanreotide and the costs of acromegaly therapy using mathematical modeling.

4. The sensitivity analysis of the selected model to changes in the primary parameters.

MATERIALS AND METHODS

In order to analyze the effectiveness and safety, a search for the information on randomized clinical trials in the following electronic resources – Cochrane Library; Medline eLIBRARY – was made.

The search was performed on September 28, 2021. It included publications with a statute of 10-year limitations with the following keywords: “lanreotide” OR “octreotide” AND “clinical effectiveness” OR “clinicaloutcomes” AND “acromegaly”; “lanreotide” OR “octreotide” AND “clinical efficacy” OR “outcomes” and “acromegaly”.

According to the primary search query, 902 publications were found in the Medline database; 0 in the Cochrane Library database; 12 – in the eLibrary. A further selection was carried out according to the PRISMA criteria (Fig. 1). When reviewing the literature, the following publications were selected:

1) Review by Qiao N. et al., 2020 [11]; 2) Review and meta-analysis by Leonart L.P. et al., 2018 [12]; 3) Review by Abu Dabrh A.M. et al., 2014) [13]; 4) Review by Marko N.F. et al., 2012 [14]; 5) RCT by Bernabeu I. et al., 2020 [15]; 6) RCT by Guistina A. et al., 2017 [6]; 7) RCT by Annamalai A.K. et al., 2013 [16]; 8) RCT by Gariani K. et al., 2013 [17]; 9) RCT by Li Q.Z. et al., 2012 [18]; 10) RCT by Carmichael J.D. et al., 2012 [19]; 11) RCT by Tutuncu Y. et al., 2012 [20]; 12) CT by Salvatori R. et al., 2017 [21]; 13) CT by LANTERN, An Z et al., 2017 [22]; 14) CT by PRIMARYS, Caron P.J. et al., 2016 [23]; 15) Data from the Moscow Register, Antsiferov M.B. et al., 2020 [24]; 16) Data from the unified Russian registry of tumors of the pituitary-hypothalamic system, Belaya Zh.E. et al., 2020 [2].

The original somatulin medicinal preparation – Somatulin® Autogel® (Ipsen Pharma, France) – gel for a subcutaneous (s/c) administration of the long-acting drug in the doses of 60 mg, 90 mg and 120 mg, was chosen as the study drug (SD).

For a comparative analysis, octreotide preparations that are most commonly used in acromegaly patients in clinical practice were selected: Sandostatin-LAR® original preparation (Novartis, Switzerland), generic preparations Octreotide-depot® (Pharmsynthesis, Russia) and Octreotide-long® (Nativa, Russia) in the doses of 10 mg, 20 mg and 30 mg.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-164-173

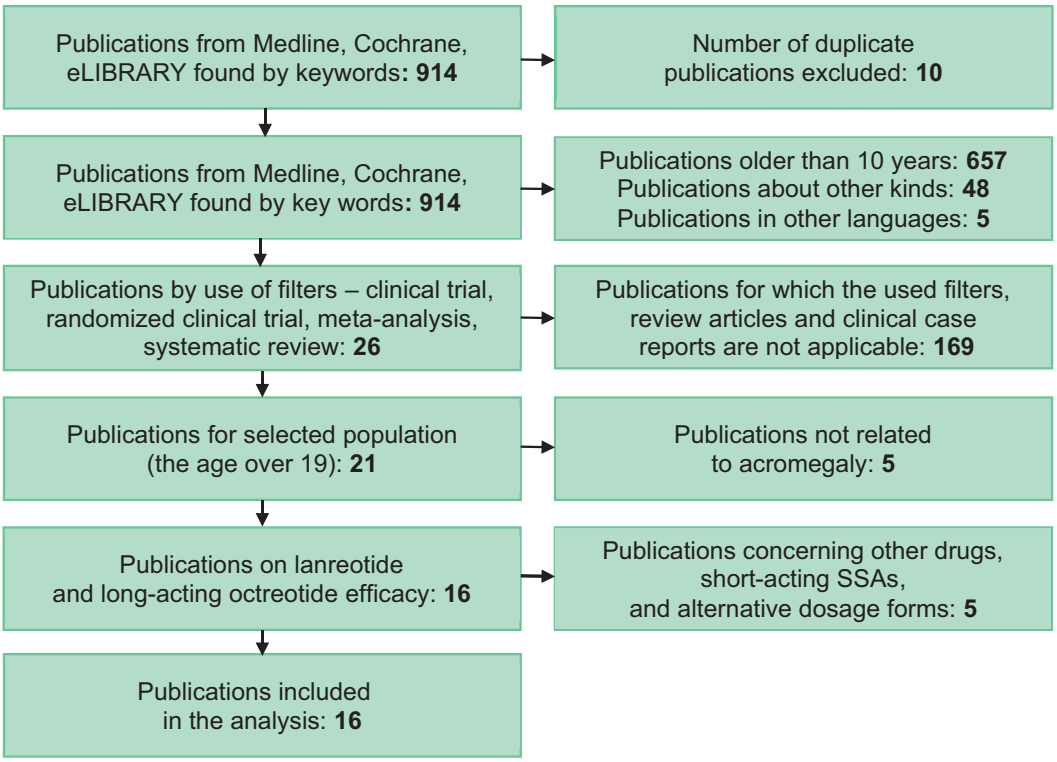

Figure 1 – Study selection scheme

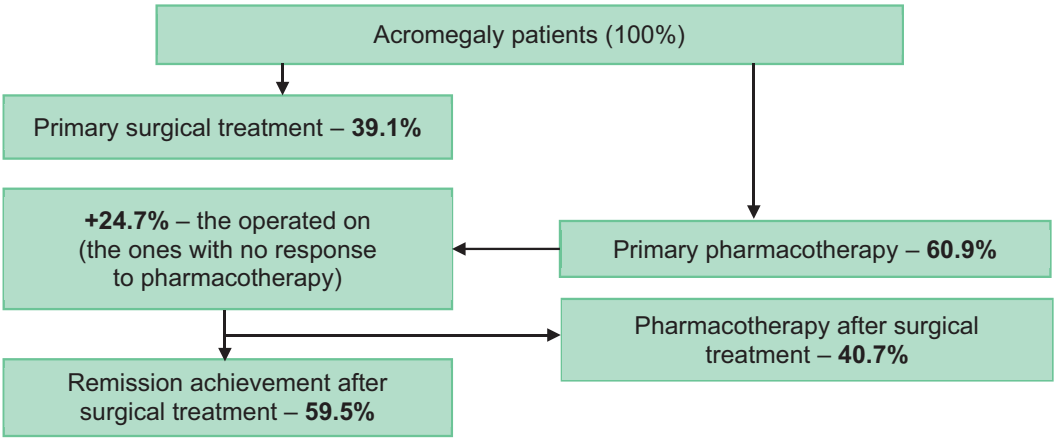

Figure 2 – Route of acromegaly patients according to Belaya Z.E. et al, 2020 [3]

Table 1 – Lanreotide titration scheme of long-acting gel for s/c administration in acromegaly patients in the first year of therapy according to Chanson P. et al., 2008 [26]

Therapy term	Dose	Frequency
Therapy start (1–3 mths)	90 mg – 100%	Once per 28 days
4–6 mths	60 mg – 17,4%; 90 mg – 12,8%;120 mg – 69,8%	Once per 28 days
7–12 mths	60 mg – 14,0%; 90 mg – 13,0%; 120 mg – 73,0%	Once per 28 days

Table 2 – Octreotide titration scheme in acromegaly patients in the first year of therapy according to Dreval A.V. et al., 2014 [5]

Therapy term	Dose	Frequency
Therapy start (1–3 mths)	20 mg – 100%	Once per 28 days
4–6 mths	10 mg – 5,6%; 20 mg – 58,22%; 30 mg – 53,16%	Once per 28 days
7–12 mths	10 mg – 5,1%; 20 mg – 41,8%; 30 mg – 13,9%; 40 mg – 39,2%	Once per 28 days

Table 3 – Median prices for different doses of long-acting somatostatin analogues

MP (INN)	Dose (mg)	Median price (rubles)	Median price +VAT+TM (rubles)	Median price +VAT+TM (USD)*
Lanreotide	60	30,830.85	37,931.04	523.1
Lanreotide	90	42,246.28	56,896.57	784.78
Lanreotide	120	61,661.70	75,862.08	1,046.37
Octreotide	10	7,949.95	9,780.78	134.9
Octreotide	20	15,899.91	19,561.58	269.81
Octreotide	30	23,849.85	29,342.35	404.72

Note: dollar exchange rate dated 05.10.21: 1 $ = 72.5 rubles.

Table 4 – Costs calculation of Somatulin® Autogel® annual course per one acromegaly patient (with prices in rubles, including VAT and TM)

Therapy term	Dosing regimen	Frequency of administration	Quantity of units	Cost per 1 unit	Cost per period
1–3 therapy mths.	90 mg, once per 28 days, s/c	1.00	3.0	56,896.57	170,689.70
4–6 therapy mths.	60 mg, once per 28 days, s/c	0.174	3.0	37,931.04	19,800.00
	90 mg, once per 28 days, s/c	0.128	3.0	56,896.57	21,848.28
	120 mg, once per 28 days, s/c	0.698	3.0	75862.08	158,855.20
7–12 therapy mths.	60 mg, once per 28 days, s/c	0.14	3.0	37,931.04	15,931.04
	90 mg, once per 28 days, s/c	0.13	3.0	56,896.57	22,189.66
	120 mg, once per 28 days, s/c	0.73	3.0	75,862.08	166,137.96
Cost per treatment course	575,451.84 rubles or 7,937.26 $				

Table 5 – Costs calculation of long-acting octreotide annual course per one acromegaly patient (with prices in rubles, including VAT and TM)

Therapy term	Dosing regimen	Frequency of administration	Quantity of units	Cost per 1 unit	Cost per period
1–3 therapy mths.	20 mg, once per 28 days, s/c	1.00	3.0	19,561.58	58684.73
4–6 therapy mths.	10 mg, once per 28 days, s/c	0.056	3.0	9,780.78	1643.17
	20 mg, once per 28 days, s/c	0.582	3.0	19,561.58	34154.51
	30 mg, once per 28 days, s/c	0.531	3.0	29,342.35	46742.36
7–12 therapy mths.	10 mg, once per 28 days, s/c	0.051	3.0	9,780.78	1496.45
	20 mg, once per 28 days, s/c	0.418	3.0	19,561.58	24530.22
	30 mg, once per 28 days, s/c	0.139	3.0	29,342.35	12235.76
	40 mg, once per 28 days, s/c	0.392	6.0	19,561.58	46008.83
Cost per treatment course	225,496.07 руб. or 3,110.29 $				

Table 6 – Cost-effectiveness ratios

Parameter	Long-acting lanreotide	Long-acting octreotide
Costs of pharmacotherapy for the first year (rubles/patient)	575,451.84	225,496.07
Costs of therapy for transsphenoidal adenomectomy (rubles)	240,358.0	240,358.0
Only need for surgery	15.7%	15.7%
Need for pharmacotherapy	36.2%	36.2%
Both surgery and pharmacotherapy	48,1%	48.1%
Remission rate*	0,51	0.24
Total costs per person per year, rubles	638,453.99	343,441.27
Cost-effectiveness ratio, rubles / case of achieved remission	1,251,870.56	1,431,005.31
Difference	179,134.74 rubles or 2,470.82 $	

Note: * – based on the data of the Russian Register [3]

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-164-173

The study estimated direct medical costs for a one-year treatment course. The data on the medicines costs were taken from the register of marginal prices of the State Register of Medicines (SRMs)[1] as on September 2, 2021. The value added tax (VAT) of 10% and the weighted average trade margin (TM) of 11.845%[2] were also taken into account. To convert the price into US dollars, the current exchange rate as of 10/05/21[3] was used 1 $ = 72.5 rubles.

To calculate the therapy costs, the research data describing the routine use of the studied drugs in real clinical practice, were used [25].

In the study by Chanson P. et al. [26], conducted in Germany, an analysis of the dose titration of Somatulin® Autogel® in acromegaly patients, during a year of therapy (Table 1) is presented. Under the conditions of the Russian healthcare system, Dreval A.V. et al. [5] conducted a dose titration study of octreotide preparations in acromegaly patients (Table 2).

The analysis of direct medical costs [25] took into account the main pharmacotherapy and the cost of a surgical intervention (transsphenoidal adenomectomy) in case of a failure, to achieve remission within a year. The cost was calculated on the basis of the Decree of the Government of the Russian Federation dated December 28, 2020 No. 2299[4] "On the Program of State Guarantees of Free Medical Care for Citizens for 2021 and for the planning period of 2022 and 2023." According to this document, microsurgical and endoscopic interventions for pituitary adenomas belong to the class of high-tech medical care (HTMC) and, depending on the complexity of the operation, are estimated at 169,754 rubles. (HTMC 1), or – 310,961 rubles. (HTMC 2). The median cost was considered to amount to 240,358 rubles.

Based on the data of the Register [3], published in the article by Belaya Zh.E. et al., there are several route options in acromegaly patients (Fig. 2).

– Group 1. They undergo surgical treatment and achieve remission without pharmacotherapy prescription.

– Group 2. Acromegaly patients undergo surgical treatment, but require subsequent pharmacotherapy.

– Group 3. Pharmacotherapy is primarily prescribed, and then a patient undergoes surgical treatment.

– Group 4. Primarily pharmacotherapy is prescribed, against this background, there is either remission achieved and the patient remains on maintenance doses of drugs, or the dose of drugs is increased.

Based on the research hypothesis, the method of "cost-effectiveness" as a clinical and economic analysis was chosen [25]. This method implies the correlation of costs with the results obtained and the comparison of two or more alternative medical technologies by this indicator. At the same time, the results are presented in the form of "natural" indicators of clinical effectiveness, the number of the years of saved lives, or other objective criteria that are significant for a particular pathology[5]. In the case of the SSAs therapy for acromegaly, the percentage of patients who had achieved remission, was chosen as an efficiency criterion.

In this analysis, only direct costs were taken into account, so the final formula was as follows [25]:

$$CER = DC/Ef$$

where: CER (a cost-effectiveness ratio) shows the costs per unit of efficiency; DC denotes direct costs; Ef is the effectiveness of a medical technology application.

At the final stage of the study, a sensitivity analysis was performed, its purpose was to determine the model sensitivity to changes in the initial parameters – drug prices and changes in the model, taking into account the real clinical practice of using octreotide and lanreotide in acromegaly patients [25].

RESULTS

Efficacy and safety analysis

Octreotide, including a long-acting one, was registered for use in acromegaly patients long ago and has a large evidence base [4, 5]. Lanreotide is a more modern drug. Its prolonged form was registered only in 2014 (Somatulin® Autogel®), however, the effectiveness of its use was confirmed in a number of studies and meta-analyses [12-23

The PRIMARYS protocol [20] was an open-label, one-year CT of a long-acting lanreotide efficacy in the standard regimen (120 mg once per 28 days) for the control of acromegaly symptoms. The number of participants was 90 people with a newly diagnosed disease. A clinical presentation was assessed using the Acromegaly Symptom Assessment Questionnaire (PASQ) as a part of European Network for Patient Safety and Quality of Care: PaSQ; and a quality of life (QoL) was assessed using the AcroQoL questionnaire. Acromegaly symptoms and QoL improved significantly from weeks 12 to 48, with a weak correlation between the total PASQ score ($R= –0.55$, $p<0.0001$) and the total AcroQoL score, and a physical condition score ($R= –0.67$, $p<0.0001$) [20] during week 48.

Similar results on the Somatulin® Autogel® effectiveness are presented in the work by Antsiferov M.B. et al., which provides the data from the Moscow Register of Patients. A statistical analysis showed that the

[1] State register of maximum selling prices, 2021. Available from: https: //minzdrav.gov.ru/opendata/7707778246-gosreestrpredelnyhotpusknyhcen/visual. Russian

[2] Omelyanovsky VV, Avksentieva MV, Sura MV, Khachatryan GR, Gerasimova KV, Ivakhnenko OI, Dzanaeva AV. Guidelines for conducting a comparative clinical and economic evaluation of a medicinal product. Approved by order of the Center for Healthcare Quality Assessment and Control of the Ministry of Health of the Russian Federation dated December 29, 2018 No.242, Moscow, 2018. – 46 p. Russian

[3] Bank of Russia. Available from: https://www.cbr.ru/eng/about_br/irp/.

[4] Decree of the Government of the Russian Federation of December 28, 2020 N 2299 "On the Program of State Guarantees of Free Provision of Medical Care to Citizens for 2021 and for the Planning Period of 2022 and 2023" (with amendments and additions). Russian

[5] State register of maximum selling prices, 2021. Russian

hormones level after 12 weeks of treatment (GH <1.2 mcg / l and an insulin-like growth factor-1 (IGF-1), which corresponds to <110% of the upper age limit) is the effectiveness marker of a 12-month treatment course. A decrease in IGF-1 below 110 and 125% of the upper age norm, respectively, after 12 weeks, was a predictor of an adequate and stable hormonal control [24].

Clinical data on the use of long-acting lanreotide based on a multicenter SODA study are presented by Salvatori R et al., 2017 [12]. In this work, a two-year efficacy and safety of lanreotide, a long-acting s/c gel, was studied. The study included 241 acromegaly patients treated with lanreotide. IGF-1 levels below the upper reference value were achieved in 71.2% after 1 year of treatment and in 74.4% after 2 years. The most frequent adverse events (AEs) value ≤2.5 μg/l was recorded in 83.3% after 1 year and in 80.0% after 2 years; STH <1.0 μg/l – in 61.7% after 1 year and in 61.4% after 2 years. The most common adverse event (AE) was arthralgia (25.7%). 10 of 106 serious AEs reported by 42 patients, were treatment related [21].

In the recent network meta-analysis by Leonart L.P. et al. [12], a clinical efficacy of different drugs for the acromegaly treatment were compared. The study included 2059 articles. The results showed that pegvisomant and long-acting lanreotide were statistically superior to placebo: a relative risk (RR), a 95% confidence interval (CI) were 0.06 (0.00–0.55) and 0.09 (0.0-0. 88), respectively. No significant differences were found out; however, when comparing lanreotide and long-acting octreotide, there was a trend towards the advantage of the first one (RR 95%, CI was 0.74 (0.06–7.91). No serious side effects of treatment were notified.

Among the Russian publications, the Russian Registry of Pituitary-Hypothalamic tumors data should be notified, where a large sample demonstrated a clinical advantage of lanreotide in achieving remission of the disease – 51% vs 24% for long-acting octreotide. Thus, based on the analyzed data, the cost-effectiveness method was chosen for a further analysis, and the remission rates were included in the pharmacoeconomic model as an efficiency criterion [3].

Cost analysis and model developing

The prices for the drugs under study were included in the cost analysis. The price for each formulation of lanreotide (Somatulin® Autogel® 60 mg, 90 mg and 120 mg) was taken into account separately. According to the recommendations of the Federal State Budgetary Institution "Centre for Expertise and Quality Control in Health Care" (the Ministry of Health of Russia)[6], when calculating a price for long-acting octreotide preparations, a median price was considered (Table 3).

[6] Omelyanovsky VV, Avksentieva MV, Sura MV, Khachatryan GR, Gerasimova KV, Ivakhnenko OI, Dzanaeva AV. Guidelines for conducting a comparative clinical and economic evaluation of a medicinal product. Russian

Based on current clinical guidelines [1], works by Chanson P. et al. [26] and Dreval A.V. et al. [5], one patient's treatment models with lanreotide or long-acting octreotide in the first year of therapy were developed. The results of the cost analysis for a treatment course are presented in Tables 4 and 5.

The total costs for pharmacotherapy in adult patients with acromegaly during the first year using long-acting octreotide was significantly lower than the costs for lanreotide: 225,496.07 rubles vs 575,451.84 rubles ($3,110.29 vs. $7,937.26), but achieving remission was much less common.

Results of cost-effectiveness analysis

At the next stage of the study, a comparative CEA analysis of the lanreotide and long-acting octreotide use in acromegaly patients during the first year of therapy was carried out. The cost of transsphenoidal adenomectomy, as well as the need for different types of treatment in acromegaly patients in the first year of treatment, were taken into account: the frequency of surgery, the appointments of pharmacotherapy, and their combinations. The calculation results of the cost-effectiveness ratios are presented in Table 6.

Despite the fact that the drug costs analysis showed lower total costs for the annual therapy course with long-acting octreotide compared to lanreotide, when calculating the cost-effectiveness ratio for one achieved remission case, the advantage of using lanreotide over long-acting octreotide was revealed: 1,251,870.56 RUB vs 1,431,005.31 RUB ($17,267.18 vs. $19,738.0).

The use of lanreotide in acromegaly patients makes it possible to reduce the total costs during the year per 1 patient in remission by 179,134.74 rubles. ($2,470.82) compared to long-acting octreotide.

Sensitivity Analysis Results

A sensitivity analysis demonstrated the stability of the model to change: prices for lanreotide – up to +18%; prices for octreotide – up to –22%; prices for transsphenoidal adenomectomy – up to + 59%; a reduction in the remissions frequency when using lanreotide – up to –12%.

DISCUSSION

The data obtained have also been confirmed by the international studies by Marco N.F. et al. [14]; Marty R. et al. [27]; Neggers S.J. et al. [28]; Orlewska E. et al. [9].

Marco N.F. et al. calculated the annual treatment costs with long-acting somatostatin analogs. For the calculation, the average dose of lanreotide was taken – 103 mg / 4 weeks, and octreotide – 24 mg / 4 weeks. The median annual cost of octreotide was $43,526 and the median annual cost of lanreotide was $41,216. The costs of lifelong treatment with octreotide, lanreotide, pegvisomant, or SSAs + pegvisomant were $1,667,052; 1,578,567; 2620833; and $2,573,339, respectively [14].

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-164-173

In the study by Marty R. et al., Somatulin® Autogel® (90 mg) and Sandostatin Lar® (20 mg) were chosen to calculate annual cost savings per patient. The results were as follows: 356.4 euros, 929.5 euros and 1457.5 euros for France, Germany and the UK, respectively. It was concluded that annual cost savings for the healthcare system could be as high as €948,236, €3,176,618 and €3,645,213 in France, Germany, and the UK, respectively [27].

Annual costs for lanreotide and octreotide were calculated by Neggers S.J. et al. to be $41,216 and $43,526, respectively. Somatulin® Autogel® was cheaper to use, with the same 60% efficacy for the both drugs. The authors calculated that on average, the disease is diagnosed in a patient aged 40 years and, if successfully treated, does not shorten the patient's life expectancy. Assuming a life expectancy in the US of 78.3 years, the median treatment time per patient was 38.3 years and the treatment cost with lanreotide was $1,578,567 and with octreotide – $1,667,052 [28].

Some studies have also estimated the total costs of treating acromegaly patients. In the publication by Knutzen R. et al., all costs for the treatment of one acromegaly patient are described. Orthopedic surgery and dental care were included. The total costs were approximately US$1 million per patient from the time of the diagnosis until 25 years later [29].

The data obtained in this study, made it possible to speak about the feasibility and effectiveness of using the drug lanreotide, a long-acting gel for the treatment of adult acromegaly patients, both in the form of preoperative therapy and in cases where remission had not been achieved after surgical treatment. Despite the significant cost of lanreotide (almost twice the cost of octreotide preparations), based on the clinical data and observational studies, a higher efficiency of lanreotide in achieving disease remissions is probable. That indicates its better clinical and economic efficiency.

The results of this work have shown that the use of Somatulin® Autogel® for the acromegaly treatment in patients over 18 years old is clinically effective and economically justified within the framework of the state drug benefit system of the Russian Federation.

CONCLUSION

The analysis of literature sources made it possible to conclude that Somatulin® Autogel® was more clinically effective and more convenient to use than long-acting octreotide preparations.

According to the Russian Register, the achievement of remission with the use of lanreotide, a long-acting s/c gel, in comparison with long-acting octreotide, is 51% vs 24% (with a comparable safety profile).

The total costs of pharmacotherapy for acromegaly patients during the first year when using long-acting octreotide was lower than when using lanreotide: 225,496.07 rubles vs 575,451.84 rubles ($3,110.29 vs $7,937.26).

According to the results of the cost-effectiveness analysis, for one achieved case of remission, the advantage of using lanreotide over long-acting octreotide was revealed: 1,251,870.56 rubles vs 1,431,005.31 rubles ($17,267.18 vs. $19,738.0). Thus, the total costs during the year per 1 patient with the achieved remission are reduced by 179,134.74 rubles ($2,470.82).

A sensitivity analysis showed that the treatment model for acromegaly patients was resistant to an increase in the price of lanreotide to +18%, a decrease in the price of octreotide to –22%, an increase in the price of transsphenoidal adenomectomy to +59%, and a decrease in the remission rate on lanreotide to –12%.

FUNDING

This study was carried out with the support of Ipsen LLC. The sponsor had no influence on the choice of materials for the publication, analysis and interpretation of the data.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Ivan S. Krysanov – concept development and study design, carrying out calculations; Ekaterina V. Makarova – carrying out calculations, text writing; Victoria Yu. Ermakova – literature search, data analysis.

REFERENCES

1. Dedov II, Molitvoslovova NN, Rozhinskaia LI, Mel'nichenko GA. Russian association of endocrinologists national practice guidelines (clinical signs, diagnosis, differential diagnosis, treatment). Acromegaly. Problems of Endocrinology. 2013;59(6):4–18. DOI: 10.14341/probl20135964-18. Russian
2. Katznelson L, Laws ER Jr, Melmed S, Molitch ME, Hassan MM, Utz A, Wass JAH. Acromegaly: An Endocrine Society Clinical Practice Guideline. The Journal of Clinical Endocrinology & Metabolism. 2014; 99(11):3933–51. DOI: 10.1210/jc.2014-2700.
3. Belaya ZE, Golounina OO, Rozhinskaya LY, Melnichenko GA, Isakov MA, Lutsenko AS, Alekseeva T, Zenkova TS, Przhiyalkovskaya EG, Panyushkina GM, Ilukhina OB, Ivanova EI, Krishtal EA, Vachygova AA, Pigarova EA, Dzeranova LK, Marova EI, Arpova SD, Mamedova EO, Grebennikova TA, Antsiferov MB, Dreval AV, Dedov II. Epidemiology, clinical manifestations and efficiency of different methods of treatment of acromegaly according to the United Russian Registry of Patients with Pituitary Tumors.Problems of Endocrinology. 2020;66(1):93–103. DOI: 10.14341/probl10333. Russian

4. Golounina OO, Dzeranova LK, Pigarova EA, Belaya ZE. Resistance to drug treatment of acromegaly and ways to overcome it. Obesity and metabolism. 2021;18(2):150–62. DOI: 10.14341/omet12710. Russian
5. Dreval AV, Pokramovich YuG, Tishenina RS. The effectiveness of analysis of octreotide-depo, a long-acting somatostatin analog, for the treatment of the patients presenting with active phase of acromegalia. Problems of Endocrinology. 2014;60(3):10–4. DOI: 10.14341/probl201460310-14. Russian
6. Giustina A, Chanson P, Bronstein MD, Klibanski A, Lamberts S, Casanueva FF, Trainer P, Ghigo E, Ho K, Melmed S; Acromegaly Consensus Group. A consensus on criteria for cure of acromegaly. J Clin Endocrinol Metab. 2010 Jul;95(7):3141–8. DOI: 10.1210/jc.2009-2670.
7. Product monograph including patient medication information. Somatuline® Autogel® lanreotide injection 60 mg, 90 mg, 120 mg lanreotide (as acetate)/unit (syringe) Antigrowth hormone, ATC Code: H01C B03. Ipsen Biopharmaceuticals Canada Inc. Last Revised March 25, 2021
8. Lesén E, Granfeldt D, Houchard A, Dinet J, Berthon A, Olsson DS, Björholt I, Johannsson G. Comorbidities, treatment patterns and cost-of-illness of acromegaly in Sweden: a register-linkage population-based study. Eur J Endocrinol. 2017 Feb;176(2):203–12. DOI: 10.1530/EJE-16-0623.
9. Orlewska E, Kos-Kudla B, Sowinski J, Sworczak K, Zgliczynski W; Lanro-Study Group. Dosage and costs of lanreotide Autogel 120 mg administered as part of routine acromegaly care in Poland – two years of data from Lanro-Study. Endokrynol Pol. 2015;66(2):142–8. DOI: 10.5603/EP.2015.0022.
10. Biermasz NR, Roelfsema F, Pereira AM, Romijn JA. Cost-effectiveness of lanreotide Autogel in treatment algorithms of acromegaly. Expert Rev Pharmacoecon Outcomes Res. 2009 Jun;9(3):223–34. DOI: 10.1586/erp.09.17.
11. Qiao N, He M, Shen M, Zhang Q, Zhang Z, Shou X, Wang Y, Zhao Y, Tritos NA. Comparative efficacy of medical treatment for acromegaly: a systematic review and network meta-analysis of integrated randomized trials and observational studies. Endocr Pract. 2020 Apr;26(4):454–62. DOI: 10.4158/EP-2019-0528.
12. Leonart LP, Ferreira VL, Tonin FS, Fernandez-Llimos F, Pontarolo R. Medical Treatments for Acromegaly: A Systematic Review and Network Meta-Analysis. Value Health. 2018 Jul;21(7):874–80. DOI: 10.1016/j.jval.2017.12.014.
13. Abu Dabrh AM, Mohammed K, Asi N, Farah WH, Wang Z, Farah MH, Prokop LJ, Katznelson L, Murad MH. Surgical interventions and medical treatments in treatment-naïve patients with acromegaly: systematic review and meta-analysis. J Clin Endocrinol Metab. 2014 Nov;99(11):4003–14. DOI: 10.1210/jc.2014-2900.
14. Marko NF, LaSota E, Hamrahian AH, Weil RJ. Comparative effectiveness review of treatment options for pituitary microadenomas in acromegaly. J Neurosurg. 2012 Sep;117(3):522–38. DOI: 10.3171/2012.4.JNS11739.
15. Bernabéu I, Fajardo C, Marazuela M, Cordido F, Venegas EM, de Pablos-Velasco P, Maroto GP, Olvera MP, de Paz IP, Carvalho D, Romero C, De la Cruz G, Escolá CÁ. Effectiveness of lanreotide autogel 120 mg at extended dosing intervals for acromegaly. Endocrine. 2020 Dec;70(3):575–83. DOI: 10.1007/s12020-020-02424-z.
16. Annamalai AK, Webb A, Kandasamy N, Elkhawad M, Moir S, Khan F, Maki-Petaja K, Gayton EL, Strey CH, O'Toole S, Ariyaratnam S, Halsall DJ, Chaudhry AN, Berman L, Scoffings DJ, Antoun NM, Dutka DP, Wilkinson IB, Shneerson JM, Pickard JD, Simpson HL, Gurnell M. A comprehensive study of clinical, biochemical, radiological, vascular, cardiac, and sleep parameters in an unselected cohort of patients with acromegaly undergoing presurgical somatostatin receptor ligand therapy. J Clin Endocrinol Metab. 2013 Mar;98(3):1040–50. DOI: 10.1210/jc.2012-3072.
17. Gariani K, Meyer P, Philippe J. Implications of Somatostatin Analogues in the Treatment of Acromegaly. Eur Endocrinol. 2013 Aug;9(2):132–5. DOI: 10.17925/EE.2013.09.02.132.
18. Li ZQ, Quan Z, Tian HL, Cheng M. Preoperative lanreotide treatment improves outcome in patients with acromegaly resulting from invasive pituitary macroadenoma. J Int Med Res. 2012;40(2):517–24. DOI: 10.1177/147323001204000213.
19. Carmichael JD. Lanreotide depot deep subcutaneous injection: a new method of delivery and its associated benefits. Patient Prefer Adherence. 2012;6:73–82. DOI: 10.2147/PPA.S20783.
20. Tutuncu Y, Berker D, Isik S, Ozuguz U, Akbaba G, Kucukler FK, Aydin Y, Guler S. Comparison of octreotide LAR and lanreotide autogel as post-operative medical treatment in acromegaly. Pituitary. 2012 Sep;15(3):398–404. DOI: 10.1007/s11102-011-0335-y.
21. Salvatori R, Gordon MB, Woodmansee WW, Ioachimescu AG, Carver DW, Mirakhur B, Cox D, Molitch ME. A multicenter, observational study of lanreotide depot/autogel (LAN) in patients with acromegaly in the United States: 2-year experience from the SODA registry. Pituitary. 2017 Dec;20(6):605–18. DOI: 10.1007/s11102-017-0821-y.
22. An Z, Lei T, Duan L, Hu P, Gou Z, Zhang L, Durand-Gasselin L, Wang N, Wang Y, Gu F; LANTERN study investigators. Efficacy and safety of lanreotide autogel compared with lanreotide 40 mg prolonged release in Chinese patients with active acromegaly: results from a phase 3, prospective, randomized, and open-label study (LANTERN). BMC Endocr Disord. 2020 May 4;20(1):57. DOI: 10.1186/s12902-020-0524-7.
23. Caron PJ, Bevan JS, Petersenn S, Houchard A, Sert C, Webb SM; PRIMARYS Investigators Group. Effects of lanreotide Autogel primary therapy on symptoms and quality-of-life in acromegaly: data from the PRIMARYS study. Pituitary. 2016 Apr;19(2):149–57. DOI: 10.1007/s11102-015-0693-y.
24. Antsiferov MB, Alekseeva TM, Pronin EV, Pronin VS. Prediktory klinicheskogo techeniya i effektivnosti lecheniya akromegalii (po materialam Moskovskogo registra) [Predictors of acromegaly clinical history and treatment effectiveness (based on Moscow Register Data)]. Endokrinologiya: novosti, mneniya, obuchenie [Endocrinology: News, Opinions, Training]. 2020;9(3):26–38. DOI: 10.33029/2304-9529-2020-9-3-26-38. Russian
25. Kulikov AYu, Nguyen TT, Tikhomirova AV. Metodologiya modelirovaniya v farmakoekonomike [Modeling methodology in pharmacoeconomics]. Pharmacoeconomics. 2011;4:8–16. Russian
26. Chanson P, Borson-Chazot F, Kuhn JM, Blumberg J, Maisonobe P, Delemer B; Lanreotide Acromegaly Study Group. Control of IGF-I levels with titrated dosing of lan-

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2022-10-2-164-173

reotide Autogel over 48 weeks in patients with acromegaly. Clin Endocrinol (Oxf). 2008 Aug;69(2):299–305. DOI: 10.1111/j.1365-2265.2008.03208.x.

27. Marty R, Roze S, Kurth H. Decision-tree model for health economic comparison of two long-acting somatostatin receptor ligand devices in France, Germany, and the UK. Med Devices (Auckl). 2012;5:39–44. DOI: 10.2147/MDER.S30913.

28. Neggers SJ, Pronin V, Balcere I, Lee MK, Rozhinskaya L, Bronstein MD, Gadelha MR, Maisonobe P, Sert C, van der Lely AJ; LEAD Study Group. Lanreotide Autogel 120 mg at extended dosing intervals in patients with acromegaly biochemically controlled with octreotide LAR: the LEAD study. Eur J Endocrinol. 2015 Sep;173(3):313–23. DOI: 10.1530/EJE-15-0215.

29. Knutzen R, Ezzat S. The cost of medical care for the acromegalic patient. Neuroendocrinology. 2006;83(3-4): 139–44. DOI: 10.1159/000095521.

AUTHORS

Ivan S. Krysanov – Candidate of Sciences (Pharmacy), Associate Professor, Head of the Pharmacy Course of the Medical Institute of Continuing Education of Moscow State University of Food Production; Head of the Department of Clinical-economic Analysis of the Institute of Clinical-economic Expertise and Pharmacoeconomics; Head of the Research Laboratory of "Health technology assessments and clinical-economic expertise" A.I. Yevdokimov Moscow State University of Medicine and Dentistry. ORCID ID: 0000-0002-3541-1120. E-mail: krysanov-ivan@mail.ru

Ekaterina V. Makarova – Candidate of Sciences (Medicine), Researcher of the Research Laboratory of "Health technology assessments and clinical-economic expertise" A.I. Yevdokimov Moscow State University of Medicine and Dentistry; Senior Researcher, Semashko National Scientific Research Institute of Public Health. ORCID ID: 0000-0003-3767-8475. E-mail: rue-royal@inbox.ru

Victoria Yu. Ermakova – Candidate of Sciences (Pharmacy), Executive Director of the Institute of Clinical and Economic Expertise and Pharmacoeconomics; Associate Professor of the Department of Chemistry, Sechenov First Moscow State Medical University. ORCID ID: 0000-0002-4822-7226. E-mail: ermakova.viktoriya.yurievna@mail.ru

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

GENDER CHARACTERISTICS OF ADVERSE DRUG REACTIONS DEVELOPMENT: EXPERIENCE OF REGIONAL DATABASE ANALYSIS

A.V. Matveev[1,2,3], A.E. Krasheninnikov[1], E.A. Egorova[2], E.I. Konyaeva[2], N.V. Matveeva[2]

[1] National Pharmacovigilance Research Center
Bld. 2/2, Malaya Sukharevskaya Sq., Moscow, Russia, 127051
[2] Medical Academy named after S.I. Georgievsky
of Vernadsky Crimean Federal University
Bld. 7, 5, Lenin Blvd., Simferopol, Republic of Crimea, Russia, 295007
[3] Russian Medical Academy of Continuing Professional Education (RMA CPE)
Bld. 1, 2, Barrikadnaya St., Moscow, Russia, 125993

E-mail: elena212007@rambler.ru

Received 15 Nov 2021 After peer review 30 March 2022 Accepted 20 Apr 2022

The aim of the article is the gender characteristics study of the adverse drug reactions (ADRs) development based on the data of the notification forms registered in the regional database ARCADe (Adverse Reactions in Crimea, Autonomic Database), for the period from 2009 to 2018.

Materials and methods. The objects of the study were 6903 notification forms about adverse drug reactions recorded in the regional database called ARCADe (Adverse Reactions in Crimea, Autonomic Database) for the period from 2009 to 2018. The classification of drugs for separate pharmacological groups was carried out using the codes of the Anatomical Therapeutic Chemical (ATC) classification system of the World Health Organization (WHO) medicinal products, the instructions data of the State Registers of medicinal preparations used in the Russian Federation and Ukraine (for the cases registered before the entry of the Republic of Crimea into the Russian Federation).

Results. A general analysis of the number of cases of the adverse drug reactions (ADRs) development in patients of different genders made it possible to determine that 59.9% (4132 notification forms) of ADRs cases were observed in female patients; 37.7% (2602 cases) – in male patients. In 169 cards (2.4%), information about a patient's gender was missing. The groups with the largest number of the registered cases of ADRs were antimicrobial agents for a systemic use (2864 cases, 41.5% of the total number of the ADRs registered cases), the drugs affecting the cardiovascular (811 cases, 11.7%) and nervous (734 cases, 10.6%) systems. In each of the presented groups, the incidence rate of ADRs in female patients exceeded that in men.

Conclusion. The study of the gender characteristics of the pharmacotherapy safety, carried out on the basis of the notification forms of the ADRs data registered in the Republic of Crimea, confirmed a higher likelihood of developing ADRs in female patients. This may be due to the peculiarities of the pharmacokinetics and pharmacodynamics of drugs in the female body, psychological factors, a more frequent use of drugs by this category of people. The implementation of the drug, taking into account specific features of each gender, can lead not only to better treatment outcomes, but also to increased patients' compliance.

Keywords: gender; gender characteristics; adverse reactions; drugs

Abbreviations: ABDs – antibacterial drugs; AH – arterial hypertension; ATE – angiotensine transforming enzyme; ATC-classification system – anatomical therapeutic chemical classification system; CCBAs – calcium channel-blocking agents; CI – confidence interval; MP – medicinal product; NRTI – Nucleoside Reverse Transcriptase Inhibitor; NNRTIs – Non Nucleoside Reverse Transcriptase Inhibitor; ADR – adverse drug reaction; RAAS – renin-angiotensin-aldosterone system; CVS – cardiovascular system.

For citation: A.V. Matveev, A.E. Krasheninnikov, E.A. Egorova, E.I. Konyaeva, N.V. Matveeva. Gender characteristics of adverse drug reactions development: experience of regional database analysis. *Pharmacy & Pharmacology.* 2022;10(2):174-186. **DOI:** 10.19163/2307-9266-2022-10-2-174-186

Для цитирования: А.В. Матвеев, А.Е. Крашенинников, Е.А. Егорова, Е.И. Коняева, Н.В. Матвеева. Гендерные особенности развития нежелательных реакций лекарственных препаратов: опыт анализа региональной базы данных. *Фармация и фармакология.* 2022;10(2):174-186. **DOI:** 10.19163/2307-9266-2022-10-2-174-186

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2022-10-2-174-186

ГЕНДЕРНЫЕ ОСОБЕННОСТИ РАЗВИТИЯ НЕЖЕЛАТЕЛЬНЫХ РЕАКЦИЙ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ: ОПЫТ АНАЛИЗА РЕГИОНАЛЬНОЙ БАЗЫ ДАННЫХ

А.В. Матвеев[1,2,3], А.Е. Крашенинников[1], Е.А. Егорова[2], Е.И. Коняева[2], Н.В. Матвеева[2]

[1] Автономная некоммерческая организация «Национальный научный центр фармаконадзора»
127051, Россия, г. Москва, Малая Сухаревская пл., д. 2, к. 2
[2] Институт «Медицинская академия имени С.И. Георгиевского»
Федерального государственного автономного образовательного учреждения высшего образования
«Крымский федеральный университет имени В.И. Вернадского»
295051, Россия, г. Симферополь, бул. Ленина, 5/7
[3] Федеральное государственное бюджетное образовательное учреждение
дополнительного профессионального образования
«Российская медицинская академия непрерывного профессионального образования»
125993, Россия, г. Москва, ул. Баррикадная, д. 2/1, стр. 1

E-mail: elena212007@rambler.ru

Получена 15.11.2021 После рецензирования 30.03.2022 Принята к печати 20.04.2022

Цель. Изучение гендерных особенностей развития нежелательных реакций (НР) на основании данных карт-извещений о НР лекарственных средств, зарегистрированных в региональной базе ARCADe (Adverse Reactions in Crimea, Autonomic Database) за период 2009–2018 гг.

Материалы и методы. Объектами исследования являлись 6903 карты-извещения о НР лекарственных средств, зарегистрированные в региональной базе НР ARCADe (Adverse Reactions in Crimea, Autonomic Database) за период 2009–2018 гг. Проведение классификации ЛС по отдельным фармакологическим группам осуществлялось с использованием кодов Анатомо-терапевтически-химической (АТХ) классификации лекарственных средств Всемирной организации здравоохранения (ВОЗ), данных инструкций Государственных реестров ЛС Российской Федерации и Украины (для случаев, зарегистрированных до вступления Республики Крым в состав Российской Федерации).

Результаты. Общий анализ количества случаев развития НР у пациентов различного пола позволил определить, что в 59,9% (4132 карт-извещений) случаев НР наблюдались у пациентов женского пола; в 37,7% случаев (2602 случая) – у пациентов мужского пола. В 169 картах (2,4%) информация о поле пациента отсутствовала. Группами с наибольшим количеством зарегистрированных случаев НР стали противомикробные средства для системного применения (2864 случая, 41,5% от всего количества зарегистрированных случаев НР); средства, влияющие на сердечно-сосудистую (811 случаев, 11,7%) и нервную (734 случаев, 10,6%) систему. В каждой из представленных групп частота развития НР у пациентов женского пола превышала таковую у мужчин.

Заключение. Изучение половых особенностей безопасности фармакотерапии, проведенное на основании данных карт-извещений о НР ЛС, зарегистрированных в Республике Крым, подтвердило более высокую вероятность развития нежелательных последствий применения ЛС у пациентов женского пола. Это может быть обусловлено особенностями фармакокинетики и фармакодинамики лекарственных препаратов в организме женщины, психологическими факторами, более частым применением ЛС данной категорией лиц. Осуществление выбора лекарственного препарата с учетом специфических для каждого пола особенностей может привести не только к лучшим результатам лечения, но и к повышению комплаентности пациентов.

Ключевые слова: пол; половые особенности; нежелательные реакции; лекарственные средства

Список сокращений: АБП – антибактериальные препараты; АГ – артериальная гипертензия; АПФ – ангиотензинпревращающий фермент; АТХ-классификация – анатомо-терапевтически-химическая классификация; БМКК – блокаторы медленных кальциевых каналов; ДИ – доверительный интервал; ЛС – лекарственное средство; НИОТ – нуклеозидные ингибиторы обратной транскриптазы; ННИОТ – ненуклеозидные ингибиторы обратной транскриптазы; НР – нежелательная реакция; РААС – ренин-ангиотензин-альдостероновая система; ССС – сердечно-сосудистая система.

INTRODUCTION

At the present stage of the healthcare development, when using certain groups of medicinal products (MPs), the study of gender characteristics of the occurrence and course of various diseases, as well as the variability of patients' of different genders' pharmacological response is becoming increasingly important. Over the past 30 years, such gender differences in the majority of cardiovascular diseases, broncho-obstructive diseases and pathologies of the gastrointestinal tract, have already been studied. For example, women are characterized by a smaller size of the heart and coronary vessels, but the stiffness of their vessels is often higher than in men [1]. The changes in the level of female sex hormones

during the menstrual cycle can affect the main parameters of their heart (blood pressure, heart rate, circulating blood volume) [2, 3]. The lipid level of blood plasma in women may also depend on the level of estrogens and gestagens. The development of cardiovascular diseases caused by atherosclerosis is observed in women 7–10 years later than in men [4]. The main pre-condition for the development of arterial hypertension in female patients is the onset of the postmenopausal period, which is accompanied by the development of hormone deficiency, the activation of the sympathoadrenal system, an increase in body mass index and fluid retention in the body. The features of the arterial hypertension (AH) development in women include a more frequent development of isolated systolic AH with a high risk of target organs damage [2].

Gender features of the bronchopulmonary pathology development are primarily due to the anatomical and physiological features of a female respiratory tract structure, which consist in thickening of the bronchi walls of a small caliber and a decrease in the bronchi lumen. Such changes predetermine a significantly higher incidence of chronic obstructive pulmonary diseases in this category of patients, and are, to a great extent, due to circadian changes in the patients' hormonal background of [4].

The differences due to the patients' gender can also significantly change their pharmacological response when the patients are administered with certain groups of drugs, affecting not only the pharmacokinetics, but also the pharmacodynamics of drugs [5, 6]. This is considerably due to the higher body weight of male patients, the large size of their internal organs and the prevailing indicators of blood plasma volume, which can significantly affect the rate of pharmacokinetic processes.

When prescribing drugs, it is also necessary to take into account the lower rate of the evacuation function of the stomach and small intestine, as well as the rate of biochemical processes in female patients [7]. The hormonal characteristics of women lead to the accumulation of their body fat mass, which is accompanied by a decrease in the water content. These factors play an important role in the distribution of lipophilic and hydrophilic drugs. So, lipophilic drugs are distributed in a woman's body much better than hydrophilic ones. A decrease in the synthesis of acid α1-glycoprotein (a protein that forms a bound fraction of drugs with the drugs of a neutral and basic nature), observed in female patients, leads to a significant increase in the free fraction of drugs and the risk of developing ADRs [5, 8, 9].

Sexual characteristics of the metabolism and excretion processes of drugs are primarily due to high rates of activity of the phase II enzymes of metabolism, a glomerular filtration rate and a renal blood flow in male patients. These factors predetermine high rates of the main pharmacokinetic parameters of drug excretion in this category of individuals. The studies have shown that women have a 10–25% lower glomerular filtration rate than men, even after adjusting for a body size. As a result, the clearance and elimination constants of drugs are significantly reduced, which makes this category of patients the most committed to the development of ADRs [10–13].

Thus, the widespread clinical practice of prescribing identical doses of drugs to women and men, does not take into account gender differences in patients' pharmacokinetics and body weight characteristics. This factor creates high risks of overdosing and the development of adverse reactions in female patients and increases the frequency of hospitalizations due to this [14, 15].

THE AIM of the article is a retrospective study of the gender characteristics of the adverse drug reactions development based on the data of the notification forms about ADRs.

MATERIALS AND METHODS

The data on the development of adverse drug reactions for the period from 1 January 2009 to 31 December of 2018, were obtained from a regional electronic database formed on the FileMaker platform and supporting the entry, storage, search and analysis of the data according to user-defined queries. The access to the database is limited (username/password) and was provided by Matveev A.V. and Konyaeva E.I., the developers of the database, the employees of the Department of Basic and Clinical Pharmacology of the Medical Academy named after S.I. Georgievsky of Vernadsky Crimean Federal University.

The database was formed on the basis of medical notification forms about the development of ADRs, containing the following information: a patient's gender and age, a suspected drug, a method of the drug administration, a description of an adverse reaction, an allergy history, and methods for correcting the AR.

The classification of drugs for separate pharmacological groups was carried out using the codes of the Anatomical Therapeutic Chemical (ATC) classification system of the World Health Organization[1] medicinal products. These instructions of the State Registers of Drugs of the Russian Federation[2] and Ukraine[3] (for the cases registered before the entry of the Republic of Crimea into the Russian Federation).

The event rate (share, % of the total number) was determined using the MS Excel 2016 software of the Microsoft Office package. Confidence intervals (95% CI) were calculated using the Klopper-Pearson method (a binominal distribution) or the Fitzpatrick-Scott method (a multinomial distribution) in the CoinMinD module of the R language. The calculation of the χ^2 test and Fisher's

[1] World Health Organization (WHO) Collaborating Centre for Drug Statistics Methodology. ATC/DDD Index 2021. Available from: https://www.whocc.no/atc_ddd_index/.
[2] State Register of Medicines of the Russian Federation. Available from: https://www.grls.rosminzdrav.ru.
[3] State Register of Medicines of Ukraine. Available from: http://www.drlz.com.ua/.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-174-186

exact test were performed using the Past 4.06b program (Oyvind Hammer, Norway).

The disadvantage of the study was the lack of information on the number of prescription cases of certain groups of drugs, which prevented us from obtaining the data on the frequency of adverse drug reactions in the population.

RESULTS

In order to study the gender characteristics of the ADRs development during the use of various drugs groups, 6903 notification forms registered for the corresponding period, were selected from the regional database of spontaneous reports. A general analysis of the number of ADRs development cases in patients of different genders, made it possible to determine that in 59.9% cases (95% CI: 58.7–61%; 4132 notification forms), ADRs were observed in female patients, in 37.7% cases (95% CI: 36.5–38.9%; 2602 cases) – in male patients. In 169 forms (95% CI: 1.3–3.6%; 2.4%), the information about the patients' gender was missing.

A further analysis was aimed at studying the gender characteristics of the ADRs development in the application of certain pharmacological drugs groups. In accordance with the ATC-classification system, 14 groups of drugs corresponding to the codes: "A", "B", "C", "D", "G", "H", "J", "L", "M", " N", "P", "R", "S", "V", are distinguished. The distribution of the ADRs development cases by gender in separate drug groups is presented in Table 1.

The groups with the largest number of registered ADRs cases were antimicrobial agents for systemic use (2,864 cases, 41.5% of the total number of registered ADRs cases); the drugs affecting the cardiovascular (811 cases, 11.7%) and nervous (734 cases, 10.6%) systems. In each of the presented groups, the incidence of ADRs in female patients exceeded that in men.

The analysis of differences in the indicators between the groups is presented in Table 2. A significant difference in the drugs of group G (the drugs that affect the genitourinary system and sex hormones) from all other groups, both in pairwise and overall comparisons, is noteworthy. In this group, the number of ADRs in women was 8.6 times higher than that in men.

The analysis of the classification groups represented above, considered in separate pharmacological groups and the gender characteristics of the ADRs development during their use, was of scientific interest. The incidence rate of ADRs in systemic antimicrobials was 41.5% (95% CI: 40.3–42.7%) of the total number of the reported ADRs cases, i.e. of the largest number among all the studied groups. That requires a more detailed study of the characteristics of the ADRs development in using antimicrobial drugs.

In accordance with the ATC classification, group "J" includes 6 main subgroups: antimicrobials; antifungal; antiviral agents; the drugs active against tuberculosis Mycobacteria; vaccines and sera. The incidence rate and distribution of ADRs cases into the above drugs groups by gender are presented in Table 3.

The results of the ADRs cases analysis made it possible to determine that the ADRs development was most often observed when a group of antimicrobial agents (1940 cases) and antiviral drugs (670 cases) for systemic use were administrated. The significance of differences between groups is presented in Table 4.

The distribution analysis of the ADRs cases between separate groups of drugs is of research interest. Thus, among antimicrobial agents for systemic use, the "leaders" in the incidence of the ADRs cases were beta-lactam antibiotics of the cephalosporin group (937 cases), penicillins (358 cases), as well as quinolone derivatives (280 cases) and preparations of the macrolides and lincosamides group (122 case). The distribution of the presented groups by gender characteristics is shown i n Fig. 1.

Among antiviral drugs, the cases of the ADRs development prevailed when combined antiretroviral drugs (266 cases), non-nucleoside reverse transcriptase inhibitors (NNRTIs) (194 cases) and nucleoside reverse transcriptase inhibitors (NRTIs) (91 cases), were prescribed. Rarely, adverse reactions were observed when a group of protease inhibitors (69 cases), which is also used in the treatment of infection caused by the human immunodeficiency virus, was administrated. The gender characteristics study of the ADRs development in the group of antiviral agents revealed that in all these groups, except NNRTIs, the adverse reactions predominated in female patients. In the NNRTIs group, the ratio of the ADRs development cases in male and female patients was 47.9% and 41.7%, respectively. In 20 notification forms, there was no information about the patients' gender.

The next group under study was the drugs that affect the cardiovascular system. Table 5 presents the analysis results of the ADRs reported cases by patients' gender, and Table 6 shows the significance of differences in the subgroups. In terms of the ADRs incidence, the "leading" groups were the drugs that affect the renin-angiotensin-aldosterone (RAAS) system – 37.48% of cases; the drugs for the heart disease treatment – 17.51% of cases; for calcium channel blockers – 12.45%; for peripheral vasodilators – 10.97% of cases.

In almost each of these groups, the number of the ADRs cases registered in female patients prevailed over the number of the ADRs cases observed in men. Among the drugs that affect the RAAS system, the majority of the ADRs cases were due to the use of angiotensin-converting enzyme (ATE) inhibitors – 199 cases. It is worth noting that the ratio of females and males with the reported ADRs cases was 67.3% in this group (134 reports; 95% CI: 60.4–74.3%) and 32.7% (65 notification forms; 95% CI: 25.7–39.6%), respectively, which indicates pronounced features of the ADRs development when this group of drugs is used in patients of different genders.

Table 1 – Distribution of ADRs development cases by gender characteristics in accordance with ATC-classification system of drugs

Group name of drugs in accordance with ATC classification system	Males, absolute number of cases	Females, absolute number of cases	Information about patients' gender is missing	Total number of ADRs development cases	Percentage of ADRs development cases in using certain drug groups
A – Digestive tract and metabolism	171	435	16	622	9.01
B – Hematopoiesis and blood	157	280	11	448	6.49
C – Cardiovascular system	265	542	4	811	11.75
D – Dermatology	47	78	1	126	1.83
G – Genitourinary system and sex hormones	11	95	1	107	1.55
H – Hormones for systemic use (excluding sex hormones and insulins)	11	28	1	40	0.58
J – Antimicrobials for systemic use	1162	1582	120	2864	41.49
L – Anticancer drugs and immunomodulators	53	69	5	127	1.84
M – Musculoskeletal system	180	310	2	492	7.13
N – Nervous system	328	399	7	734	10.63
P – Antiparasitic drugs, insecticides and repellents	15	19	0	34	0.49
R – Respiratory system	112	181	1	294	4.26
S – Preparations for the treatment of sensory processing disorders	34	37	0	71	1,03
V – Other drugs	56	77	0	133	1.93
Total	2,602	4,132	169	6,903	100

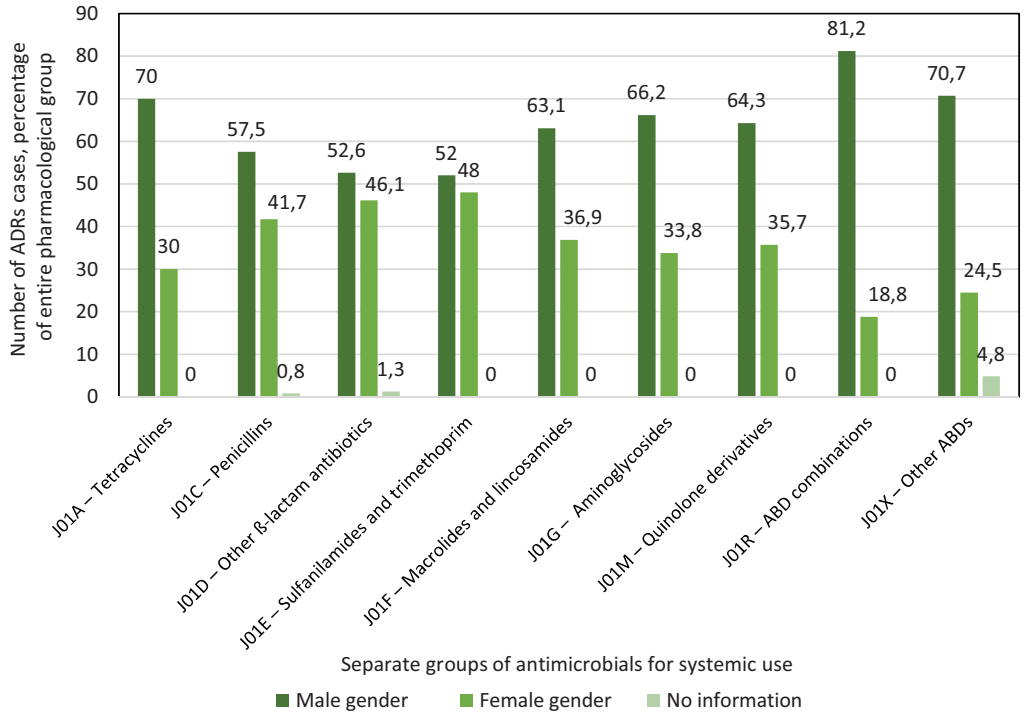

Figure 1 – Distribution of ADRs development cases by gender characteristics in antimicrobial agents group for systemic use (group "J01")

Note: ABDs – Antimicrobial drugs

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-174-186

Table 2 – Intergroup differences in main ATC groups by gender characteristics

		A	B	C	D	G	H	J	L	M	N	P	R	S	V	All other groups
		X^2 and significance level of criterion														
A – Digestive tract and metabolism			7.015; p=0.03	14.51; p<0.001	5.903; p=0.052	16.345; p<0.001	0.001; p=0.999	45.374; p<0.001	11.669; p=0.003	17.156; p<0.001	45.532; p<0.001	4.937; p=0.085	14.705; p<0.001	13.76; p=0.001	13.546; p<0.001	30.516; p<0.001
B – Hematopoiesis and blood		p=0.029		10.638; p<0.001	1.445; p=0.485	27.254; p<0.001	0.935; p=0.627	9.5541; p=0.008	3.080; p=0.214	7.2823; p=0.026	13.632; p=0.001	1.787; p=0.409	5.391; p=0.067	5.573; p=0.062	5.0142; p=0.081506	1.451; p=0.484
C – Cardiovascular system		p<0.001	p=0.006		1.285; p=0.526	22.673; p<0.001	2.985; p=0.225	50.447; p<0.001	18.863; p<0.001	2.105; p=0.349	25.468; p<0.001	2.046; p=0.359	2.884; p=0.236	6.961; p=0.031	5.0353; p=0.080648	27.95; p<0.001
D – Dermatology		p=0.06	p=0.614	p=0.344		22.616; p<0.001	1.880; p=0.391	4.726; p=0.094146	3.574; p=0.167	0.343; p=0.842	2.478; p=0.29	0.751; p=0.687	0.397; p=0.82	2.547; p=0.28	1.605; p=0.448	1.53; p=0.465
G – Genitourinary system and sex hormones		p<0.001	p<0.001	p<0.001	p<0.001		7.521; p=0.023	47.223; p<0.001	32.882; p<0.001	28.198; p<0.001	46.267; p<0.001	19.793; p<0.001	28.788; p<0.001	32.28; p<0.001	30.651; p<0.001	37.879; p<0.001
H – Hormones for systemic use (excluding sex hormones and insulins)	Fisher's exact test (significance level)	p=1	p=0.54405	p=0.181	p=0.287	p=0.016		3.49; p=0.175	3.069; p=0.216	4.01; p=0.135	5.118; p=0.077	2.871; p=0.238	4.217; p=0.121	5.796; p=0.055	5.761; p=0.056	1.8; p=0.406
J – Antimicrobials for systemic use		p<0.001	p=0.0081787	p<0.001	p=0.081	p<0.001	p=0.186		0.077; p=0.96	22.699; p<0.001	19.806; p<0.001	1.5311; p=0.465	12.631; p=0.002	4.0072; p=0.135	5.808; p=0.055	88.874; p<0.001
L – Anticancer drugs and immunomodulators		p=0.003	p=0.18022	p<0.001	p=0.169	p<0.001	p=0.207	p=0.973		13.078; p=0.001	7.127; p=0.028	1.386; p=0.5	9.132; p=0.01	3.2298; p=0.199	5.385; p=0.0677	2.392; p=0.302
M –Musculoskeletal system		p<0.001	p=0.024	p=0.35	p=0.669	p<0.001	p=0.105	p<0.001	p=0.003		9.677; p=0.008	0.883; p=0.643	0.196; p=0.907	3.569; p=0.168	1.828; p=0.401	10.071; p=0.007
N – Nervous system		p<0.001	p<0.001	p<0.001	p=0.25	p<0.001	p=0.045	p<0.001	p=0.044	p<0.001		0.34; p=0.843	5.078; p=0.079	0.885; p=0.642	1.694; p=0.429	22.297; p<0.001
P – Antiparasitic drugs. insecticides and repellents		p=0.11	p=0.47	p=0.316	p=0.648	p<0.001	p=0.222	p=0.679	p=0.763	p=0.531	p=1		0.562; p=0.755	0.131; p=0.717	0.045; p=0.832	1.301; p=0.522
R – Respiratory system		p<0.001	p=0.06	p=0.22	p=0.796	p<0.001	p=0.117	p<0.001	p=0.013	p=0.869	p=0.076	p=0.622		2.468; p=0.291	1.031; p=0.598	5.7366; p=0.057
S – Preparations for the treatment of sensory processing disorders		p=0.002	p=0.08	p=0.039	p=0.245	p<0.001	p=0.034	p=0.125	p=0.231	p=0.178	p=0.848	p=0.835	p=0.308		0.628; p=0.428	4.453; p=0.108
V – Other drugs		p=0.001	p=0.08	p=0.09	p=0.485	p<0.001	p=0.051	p=0.021	p=0.076	p=0.467	p=0.606	p=0.848	p=0.625	p=0.461		4.108; p=0.128
All other groups		p<0.001	p=0.48	p<0.001	p=0.563	p<0.001	p=0.309	p<0.001	p=0.267	p<0.001	p<0.001	p=0.724	p=0.028	p=0.126	p=0.102	

Table 3 – Distribution of cases of adverse reactions development by gender characteristics in group "J" (antimicrobials for systemic use)

ATC group	Male gender		Female gender		Information about patients' gender is missing		Total number of ADRs development cases	Percentage of total number of ADRs development cases for drugs of group "J" (95% CI)
	Absolute number of cases	% (95% CI)	Absolute number of cases	% (95% CI)	Absolute number of cases	% (95% CI)		
J01 – Antimicrobials for systemic use	800	41,24 (39,0–43,5)	1120	57,73 (55,5–60,0)	20	1,03 (0,0–3,3)	1940	67,74 (65,9–69,6)
J02 – Antifungal drugs for systemic use	11	45,83 (25,8–65,8)	13	54,17 (34,2–74,2)	0	0,0 (0,0–20)	24	0,84 (0–2,7)
J04 – Drugs active against Mycobacteria	85	57,43 (49,4–65,5)	62	41,89 (33,8–49,9)	1	0,68 (0–8,7)	148	5,17 (3,3–7,0)
J05 – Antiviral agents for systemic use	221	32,99 (29,2–36,8)	355	52,99 (49,2–56,8)	94	14,03 (10,2–17,8)	670	23,39 (21,6–25,2)
J06 – Immune sera and immunoglobulins	15	46,88 (29,6–64,2)	17	53,12 (35,8–70,4)	0	0,0 (0,0–17,3)	32	1,12 (0,0–2,9)
J07 – Vaccines	30	60,0 (46,1–73,9)	15	30,0 (16,1–43,9)	5	10,0 (0,0–23,9)	50	1,75 (0,0–3,6)
TOTAL:	1162	40,57 (38,7–42,4)	1582	55,24 (53,4–57,1)	120	4,19 (2,4–6,0)	2864	100

Table 4 – Intergroup differences in ATC subgroups of group "J" by gender characteristics

		J01	J02	J04	J05	J06	J07	All other groups
		X^2 and significance level of criterion						
J01 – Antimicrobials for systemic use	Fisher's exact test (significance level)		0.421; p=0.81	14.777; p<0.001	203.31; p<0.001	0.688; p=0.709	41.899; p<0.001	150.65; p<0.001
J02 – Antifungal drugs for systemic use		p=0.752		1.374; p=0.503	4.517; p=0.104	0.006; p=0.938	5.49; p=0.064	1.1842; p=0.553
J04 – Drugs active against Mycobacteria		p<0.001	p=0.379		40.908; p<0.001	1.5; p=0.472	12.124; p=0.002	20.566; p<0.001
J05 – Antiviral agents for systemic use		p<0.001	p=0.072	p<0.001		6.242; p=0.044	15.073; p<0.001	215.32; p<0.001
J06 – Immune sera and immunoglobulins		p=0.705	p=1	p=0.446	p=0.023		6.486; p=0.039	1.699; p=0.428
J07 – Vaccines		p<0.001	p=0.08	p=0.004	p<0.001	p=0.04		14.702; p<0.001
All other groups		p<0.001	p=0.818	p<0.001	p<0.001	p=0.568	p<0.001	

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-174-186

Table 5 – Distribution of ADRs development cases by gender characteristics in group "C" (cardiovascular system)

ATC group	Male gender		Female gender		Information about patients' gender is missing		Total number of ADRs development cases	Percentage of total number of ADRs development cases for drugs used in separate groups (95% CI)
	Absolute number of cases	% (95% CI)	Absolute number of cases	% (95% CI)	Absolute number of cases	% (95% CI)		
C01 – Drugs for heart diseases treatment	67	47.18 (39.0–55.4)	71	50.00 (41.8–58.2)	4	2.82 (0.0–11.0)	142	17.51 (14.1–21.0)
C02 – Antihypertensive drugs	0	0.00 (0.0–97.9)	1	100.00 (2.0–197.9)	0	0.00 (0.0–97.9)	1	0.12 (0.0–3.6)
C03 – Diuretics	4	25.00 (0.5–49.5)	12	75.00 (50.5–99.5)	0	0.00 (0.0–24.5)	16	1.97 (0.0–5.4)
C04 – Peripheral vasodilators	29	32.58 (22.2–43.0)	60	67.42 (57.0–77.8)	0	0.00 (0.0–10.4)	89	10.97 (7.5–14.4)
C05 – Angioprotectors	25	36.76 (24.9–48.6)	43	63.24 (51.4–75.1)	0	0.00 (0.0–11.9)	68	8.38 (4.9–11.8)
C07 – Beta–blockers	17	31.48 (18.1–44.8)	37	68.52 (55.2–81.9)	0	0.00 (0.0–13.3)	54	6.66 (3.2–10.1)
C08 – Calcium channel blockers	22	21.78 (12.0–31.5)	79	78.22 (68.5–88.0)	0	0.00 (0.0–9.8)	101	12.45 (9.0–15.9)
C09 – Drugs affecting the RAAS system	91	29.93 (24.3–35.6)	213	70.07 (64.4–75.7)	0	0.00 (0.0–5.6)	304	37.48 (34.0–40.9)
C10 – Antilipedimics	10	27.78 (11.4–44.1)	26	72.22 (55.9–88.6)	0	0.00 (0.0–16.3)	36	4.44 (1.0–7.9)
TOTAL:	265	32.68 (29.2–36.1)	542	66.83 (63.4–70.3)	4	0.49 (0.0–3.9)	811	100

Note: CI – confidence interval; RAAS – renin-angiotensin-aldosterone system.

Table 6 – Intergroup differences in subgroups of group "C" (cardiovascular system) by gender characteristics

		C01	C02	C03	C04	C05	C07	C08	C09	C10	All other groups
		X^2 and significance level of criterion									
C01 – Drugs for heart diseases treatment	Fisher's exact test (significance level)		0.993; p=0.609	3.736; p=0.154	8.239; p=0.016	4.538; p=0.103	6.206; p=0.045	20.855; P<0.001	22.812; P<0.001	6.1166; p=0.047	37.229; p<0.001
C02 – Antihypertensive drugs		p=1		0.327; p=0.567	0.481; p=0.488	0.577; p=0.448	0.456; p=0.5	0.278; p=0.598	0.427; p=0.514	0.381; p=0.537	0.497; p=0.78
C03 – Diuretics		p=0.175	p=1		0.362; p=0.547	0.793; p=0.373	0.247; p=0.619	0.083; p=0.774	0.177; p=0.674	0.043; p=0.835	0.538; p=0.764
C04 – Peripheral vasodilators		p=0.013	p=1	p=0.771		0.299; p=0.585	0.019; p=0.891	2.811; p=0.094	0.228; p=0.633	0.276; p=0.599	0.498; p=0.779
C05 – Angioprotectors		p=0.106	p=1	p=0.56	p=0.614		0.372; p=0.542	4.544; p=0.033	1.21; p=0.272	0.851; p=0.356	0.889; p=0.641
C07 – Beta-blockers		p=0.044	p=1	p=0.761	p=1	p=0.57		1.758; p=0.185	0.052; p=0.819	0.141; p=0.707	0.335; p=0.846
C08 – Calcium channel blockers		P<0.001	p=1	p=0.752	p=0.103	p=0.037	p=0.244		2.5; p=0.114	0.532; p=0.465	6.997; p=0.03
C09 – Drugs affecting the RAAS system		P<0.001	p=1	p=0.785	p=0.695	p=0.311	p=0.872	p=0.125		0.072; p=0.789	4.278; p=0.118
C10 – Antilipedimics		p=0.063	p=1	p=1	p=0.674	p=0.391	p=0.816	p=0.495	p=0.85		0.626; p=0.731
All other groups		P<0.001	p=1	p=0.63	p=1	p=0.648	p=0.91	p=0.027	p=0.12	p=0.657	

Note: RAAS – renin-angiotensin-aldosterone system.

Table 7 – Distribution of cases of adverse reactions development by gender characteristics in group "N" (nervous system)

ATC group	Male gender		Female gender		Information about patients' gender is missing		Total number of ADRs development cases	Percentage of total number of ADRs cases for drugs used in group "N" (95% CI)
	Absolute number of cases	% (95% CI)	Absolute number of cases	% (95% CI)	Absolute number of cases	% (95% CI)		
N01 – Anesthetics	57	40.71 (32.4–49.0)	80	57.14 (48.9–65.4)	3	2.14 (0.0–10.4)	140	19.07 (15.5–22.7)
N02 – Analgesics	99	51.30 (44.2–58.3)	94	48.70 (41.7–55.8)	0	0.00 (0.0–7.1)	193	26.29 (22.7–29.9)
N03 – Antiepileptic drugs	14	31.82 (17.0–46.6)	29	65.91 (51.1–80.7)	1	2.27 (0.0–17.0)	44	5.99 (2.4–9.6)
N04 – Antiparkinsonian drugs	2	50.00 (1.0–98.9)	2	50.00 (1.0–98.9)	0	0.00 (0.0–49.0)	4	0.54 (3.1–4.2)
N05 – Psycholeptics	71	44.65 (36.9–52.4)	88	55.35 (47.6–63.1)	0	0.00 (0.0–7.8)	159	21.66 (18–25.3)
N06 – Psychoanaleptics	53	44.92 (35.9–53.9)	64	54.24 (45.2–63.3)	1	0.85 (0.0–9.9)	118	16.08 (12.5–19.7)
N07 – Other drugs for treatment of nervous system diseases	32	42.11 (30.9–53.3)	42	55.26 (44.0–66.5)	2	2.63 (0.0–13.9)	76	10.35 (6.7–14.0)
TOTAL:	328	44.69 (41.1–48.3)	399	54.36 (50.7–58.0)	7	0.95 (0.0–4.6)	734	100

Note: CI – confidence interval.

Table 8 – Intergroup differences in separate subgroups of group "N" by gender characteristics

		N01	N02	N03	N04	N05	N06	N07	All other groups
		X^2 and significance level of criterion							
N01 – Anesthetics			7.18; p=0.028	1.123; p=0.57	0.202; p=0.904	3.72; p=0.156	1.055; p=0.59	0.105; p=0.949	3.422; p=0.181
N02 – Analgesics	Fisher's exact test (significance level)	0.02		9.281; p=0.01	0.003; p=0.959	1.54; p=0.215	2.69; p=0.261	6.489; p=0.039	6.6; p=0.037
N03 – Antiepileptic drugs		0.559	0.009		0.598; p=0.741	5.637; p=0.06	2.617; p=0.27	1.317; p=0.518	3.737; p=0.154
N04 – Antiparkinsonian drugs		1	1	0.63		0.045; p=0.832	0.069; p=0.967	0.18; p=0.914	0.078; p=0.962
N05 – Psycholeptics		0.183	0.239	0.066	1		1.364; p=0.506	4.26; p=0.119	1.972; p=0.373
N06 – Psychoanaleptics		0.653	0.233	0.212	1	0.651		1.044; p=0.593	1.57; p=0.456
N07 – Other drugs for treatment of nervous system diseases		0.96	0.045	0.545	1	0.174	0.691		2.642; p=0.267
All other groups		0.148	0.042	0.121	1	0.521	0.681	0.239	

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-174-186

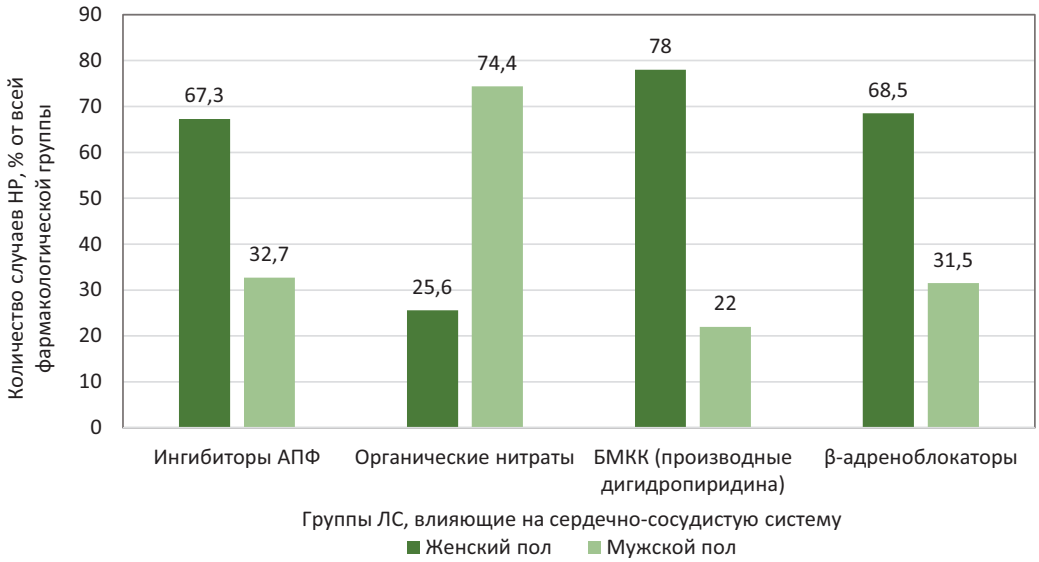

Figure 2 – Distribution of adverse reactions development cases by gender characteristics in the group of drugs that affect cardiovascular system (group "J01")

Note: ADRs – adverse drug reactions; ATE – angiotensine transforming enzyme; CCBAs – calcium channel-blocking agents.

A comparative analysis of differences in the ADRs incidence rate in the administration of combined drugs containing the ATE inhibitors and thiazide diuretics, made it possible to determine that the ratio of the ADRs cases in female and male patients was 75% (95% CI: 62.3–87.7%) (45 cases) and 25% (95% CI: 12.3–37.7%) (15 cases), respectively. Among the drugs for the treatment of heart diseases, the ADRs development cases in the group of organic nitrates prevailed (43 cases). In this pharmacological group, the ADRs cases were more often observed in male patients (32 cases) than in females (11 cases), i.e. in 74.4% (95% CI: 59.5–89.4%) and 25.6% (95% CI: 10.6–40.1%), respectively.

The study of the ADRs caused by the administration of a group of calcium channel-blocking agents (CCBAs), revealed the absolute predominance of the ADRs development cases to dihydropyridine derivatives (100 cases, 99% of the total number of the ADRs to calcium channel-blocking agents, most of which were observed in female patients (78 cases).

In accordance with gender, the ratio of the ADRs development cases in the administration of group "C07" drugs (beta-blockers), was 68.5% (37 cases; 95% CI: 62.9–91.2%) and 31.5% (11 cases; 95% CI: 8.8–37.1%) for female and male patients, respectively.

The distribution of notification forms about ADRs (by gender) of the main pharmacological drugs groups that affect the CVS is shown in Fig. 2.

In terms of the incidence rate of ADRs among the notification forms registered in the ARCADe database, the third place was occupied by the drugs that affect the nervous system functions. A greater number of ADRs in this case was due to the drugs administration of the "N02" group – analgesics (193 cases). It is noteworthy that for the entire group of analgesics, the predominant number of ADRs cases was recorded in men (99 vs 94 cases in women), which distinguishes this group from the rest analyzed.

In 159 cases, the ADRs were associated with the use of the "N05" group – psycholeptics, among which the ADRs development cases in female patients predominated (88 cases). The administration of local anesthetics (group N01) was associated with adverse reactions, 80 of which out of 140 cases, were observed in female patients. The distribution of all undesirable consequences caused by the use of agents that affect the nervous system functions, by gender characteristics, is presented in Table 7.

Among analgesics, the largest number of ADRs was caused by non-narcotic analgesics of the anilide group (93 cases). The distribution of ADRs cases by gender characteristics in the presented group was as follows: in 51 cases, ADRs were observed in male patients, and in 42 cases - in female patients, which amounted to 54.8% (95% CI: 44.7–65, 0%) and 45.2% (95% CI: 35.0–55.3%) of cases, respectively (Table 8).

In the N05 (psycholeptics) group, the majority of ADRs were associated with the use of antipsychotics (126 cases, 79.2% (95% CI: 71.5–87.0%) of the entire N05 group). Butyrophenone derivatives (28 cases) and phenothiazine derivatives with a piperazine structure (23 cases) were the leaders in the incidence rate of ADRs in the group under study. It is important to notify that in the administration of these groups, ADRs were more often observed in the male patients, which was also typical for the entire group of antipsychotic drugs (men – 52.3%, women – 47.7%). For all other groups of drugs that affect the nervous system functions, gender-specific differences were due to the predominance of the ADRs developing risk when they were used in female patients.

DISCUSSION

The results of the present study indicate a higher incidence rate of ADRs in female patients in all groups of drugs, distributed in accordance with the ATC classification system. Taking into account the peculiarities of the pharmacokinetics and pharmacodynamics of drugs in female patients, the data obtained confirm a higher risk of developing the ADRs in this category of individuals [16, 17].

The study of the group of drugs that affect the cardiovascular system functions, revealed a high incidence rate of adverse reactions to the drugs of the ACE inhibitors group in the form of monodrugs and combinations with thiazide diuretics. The data obtained in the study, are comparable with the data presented by Rydberg D.M. et al. [17], in which the ratio of female and male patients with registered ADRs cases to the drugs of the ACE inhibitor group, was 51.6% and 48.4%, respectively, and for the drugs combined with thiazide diuretics – 56.4% and 43.6%, respectively [17]. The data of the literature review studying the features of the ADRs development in patients of different genders administrated with drugs from the ACE inhibitor group, made it possible to identify possible causes of a high incidence rate of ADRs in women. Among them, a more pronounced effect of genetic polymorphisms of bradykinin receptors and ABO genes associated with the level of ATE and the risk of developing cough against the background of the ATE inhibitors use, as well as the effect of changing sex hormones levels during the menstrual cycle on the RAAS system [18]. In experimental models, androgens stimulate the RAAS system, while estrogens and progesterone decrease the plasma renin activity, the ATE activity, and aldosterone levels [19]. There is also evidence of a higher risk of dry cough developing in female patients when using ATE inhibitors, which may be due to the genetic polymorphism of bradykinin receptors and ABO genes associated with plasma ATE levels [20].

The study of gender characteristics of the ADRs development in the administration of

the calcium channel-blocking agents (CCBAs) group (dihydropyridine derivatives), conducted by Rydberg D.M. et al. [17], confirmed the results gained on the predominance of the ADRs development cases in female patients (59% of cases). These data are comparable with the results of studying the safety of amlodipine in clinical practice, which revealed a high risk of developing peripheral edema and a significant effect of this group on the level of blood pressure in females during the use of amlodipine [21].

Similar results on the predominance of the incidence rate of adverse reactions in female patients during the administration of the drugs that affect the cardiovascular system were obtained in the study conducted by the National Pharmacovigilance Center of the Netherlands and the study by Yu Y. et al. [22]. In these studies, the ratio of the ADRs frequency o in female and male patients using antihypertensive drugs was 53.1% and 46.9%, respectively.

Clinical studies have also revealed higher risks of developing ADRs in female patients when using the drugs that affect the central nervous system function (selective serotonin reuptake inhibitors, antidepressants) [23, 24].

The data gained by De Vries et al., which indicate a higher frequency of the ADRs development with the use of tetracycline, penicillin, beta-lactam antibiotics, except for penicillins, macrolides in females [25].

The results of this study concerning the gender characteristics of the ADRs development in the administration of antimicrobials, were comparable with De Vries et al.'s data, which indicate a higher incidence rate of the ADRs development when using tetracycline, beta-lactam antibiotics groups, except penicillins, macrolides in females [25].

CONCLUSION

The study of the gender characteristics of the pharmacotherapy safety, carried out on the basis of the data of the ADRs notification forms registered in the Republic of Crimea, confirmed a higher likelihood of developing undesirable consequences of the drugs in female patients. This may be due to the peculiarities of the pharmacokinetics and pharmacodynamics of drugs in the female body, psychological factors, a more frequent use of drugs by this category of people. However, despite the obvious physical and physiological gender differences, the efficacy and safety features of drugs are very rarely taken into account when conducting pharmacotherapy in females. The implementation of the drug choice, taking into account the specific gender characteristics, can lead not only to better treatment outcomes, but also to the increased patient compliance.

FUNDING

This study did not receive any financial support from outside organizations.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Alexander V. Matveev – working out the concept and design of the study, processing the results;
Anatoly E. Krasheninnikov – work out the concept of the study, analysis and interpretation of the results;
Elena A. Egorova – statistical processing of the results, writing the text of the article;
Elena I. Konyaeva – writing the text of the article; Natalia V. Matveeva – analysis of the study results.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-174-186

REFERENCES

1. Liventseva MM. Gender features of cardiovascular diseases. International Reviews: clinical practice and health. 2013;1: 15–8. Russian
2. Ibragimova KI, Mammaev SN, Omarova JA. Gender-specific regulation of blood pressure and antihypertensive treatment. Arterial Hypertension. 2018;24(3):303–8. DOI: 10.18705/1607-419X-2018-24-3-303308. Russian
3. Abdel-Rahman AA. Influence of sex on cardiovascular drug responses: role of estrogen. Curr Opin Pharmacol. 2017 Apr;33:1–5. DOI: 10.1016/j.coph.2017.02.002.
4. Di Pilla M, Bruno RM, Taddei S, Virdis A. Gender differences in the relationships between psychosocial factors and hypertension. Maturitas. 2016 Nov;93:58–64. DOI: 10.1016/j.maturitas.2016.06.003.
5. Holm L, Ekman E, Jorsäter Blomgren K. Influence of age, sex and seriousness on reporting of adverse drug reactions in Sweden. Pharmacoepidemiol Drug Saf. 2017 Mar;26(3):335–343. DOI: 10.1002/pds.4155.
6. Watson S, Caster O, Rochon PA, den Ruijter H. Reported adverse drug reactions in women and men: Aggregated evidence from globally collected individual case reports during half a century. EClinicalMedicine. 2019 Oct 25;17:100188. DOI: 10.1016/j.eclinm.2019.10.001.
7. Freire AC, Basit AW, Choudhary R, Piong CW, Merchant HA. Does sex matter? The influence of gender on gastrointestinal physiology and drug delivery. Int J Pharm. 2011 Aug 30;415(1–2):15–28. DOI: 10.1016/j.ijpharm.2011.04.069.
8. Franconi F, Campesi I. Sex Impact on Biomarkers, Pharmacokinetics and Pharmacodynamics. Curr Med Chem. 2017;24(24):2561–75. DOI: 10.2174/0929867323666161003124616.
9. Mauvais-Jarvis F, Berthold HK, Campesi I, Carrero JJ, Dakal S, Franconi F, Gouni-Berthold I, Heiman ML, Kautzky-Willer A, Klein SL, Murphy A, Regitz-Zagrosek V, Reue K, Rubin JB. Sex- and Gender-Based Pharmacological Response to Drugs. Pharmacol Rev. 2021 Apr;73(2):730–62. DOI: 10.1124/pharmrev.120.000206.
10. Hu R, McDonough AA, Layton AT. Functional implications of the sex differences in transporter abundance along the rat nephron: modeling and analysis. Am J Physiol Renal Physiol. 2019 Dec 1;317(6):F1462–F1474. DOI: 10.1152/ajprenal.00352.2019.
11. Islam MM, Iqbal U, Walther BA, Nguyen PA, Li YJ, Dubey NK, Poly TN, Masud JHB, Atique S, Syed-Abdul S. Gender-based personalized pharmacotherapy: a systematic review. Arch Gynecol Obstet. 2017 Jun;295(6):1305–17. DOI: 10.1007/s00404-017-4363-3.
12. Franconi F, Campesi I. Pharmacogenomics, pharmacokinetics and pharmacodynamics: interaction with biological differences between men and women. Br J Pharmacol. 2014 Feb;171(3):580–94. DOI: 10.1111/bph.12362.
13. Filipescu D, Ştefan M. Sex and gender differences in anesthesia: Relevant also for perioperative safety? Best Pract Res Clin Anaesthesiol. 2021 May;35(1):141–153. DOI: 10.1016/j.bpa.2020.12.006.
14. Zucker I, Prendergast BJ. Sex differences in pharmacokinetics predict adverse drug reactions in women. Biol Sex Differ. 2020 Jun 5;11(1):32. DOI: 10.1186/s13293-020-00308-5.
15. Li Q, McDonough AA, Layton HE, Layton AT. Functional implications of sexual dimorphism of transporter patterns along the rat proximal tubule: modeling and analysis. Am J Physiol Renal Physiol. 2018 Sep 1;315(3):F692–F700. DOI: 10.1152/ajprenal.00171.2018.
16. Oertelt-Prigione S, Regitz-Zagrosek V. Gender aspects in cardiovascular pharmacology. J Cardiovasc Transl Res. 2009 Sep;2(3):258–66. DOI: 10.1007/s12265-009-9114-9.
17. Tamargo J, Rosano G, Walther T, Duarte J, Niessner A, Kaski JC, Ceconi C, Drexel H, Kjeldsen K, Savarese G, Torp-Pedersen C, Atar D, Lewis BS, Agewall S. Gender differences in the effects of cardiovascular drugs. Eur Heart J Cardiovasc Pharmacother. 2017 Jul 1;3(3):163–182. DOI: 10.1093/ehjcvp/pvw042.
18. Rydberg DM, Mejyr S, Loikas D, Schenck-Gustafsson K, von Euler M, Malmström RE. Sex differences in spontaneous reports on adverse drug events for common antihypertensive drugs. Eur J Clin Pharmacol. 2018 Sep;74(9):1165–73. DOI: 10.1007/s00228-018-2480-y.
19. Sato A, Fukuda S. A prospective study of frequency and characteristics of cough during ACE inhibitor treatment. Clin Exp Hypertens. 2015;37(7):563–8. DOI: 10.3109/10641963.2015.1026040.
20. Komukai K, Mochizuki S, Yoshimura M. Gender and the renin-angiotensin-aldosterone system. Fundam Clin Pharmacol. 2010 Dec;24(6):687–98. DOI: 10.1111/j.1472-8206.2010.00854.x.
21. Mas S, Gassò P, Alvarez S, Ortiz J, Sotoca JM, Francino A, Carne X, Lafuente A. Pharmacogenetic predictors of angiotensin-converting enzyme inhibitor-induced cough: the role of ACE, ABO, and BDKRB2 genes. Pharmacogenet Genomics. 2011 Sep;21(9):531–8. DOI: 10.1097/FPC.0b013e328348c6db.
22. Kalibala J, Pechère-Bertschi A, Desmeules J. Gender Differences in Cardiovascular Pharmacotherapy-the Example of Hypertension: A Mini Review. Front Pharmacol. 2020 May 6;11:564. DOI: 10.3389/fphar.2020.00564.
23. Yu Y, Chen J, Li D, Wang L, Wang W, Hongfang L. Systematic Analysis of Adverse Event Reports for Sex Differences in Adverse Drug Events. Sci Rep. 2016; 6: 24955. DOI: 10.1038/srep24955.
24. Bigos KL, Pollock BG, Stankevich BA, Bies RR. Sex differences in the pharmacokinetics and pharmacodynamics of antidepressants: an updated review. Gend Med. 2009 Dec;6(4):522–43. DOI: 10.1016/j.genm.2009.12.004.
25. Ekhart C, van Hunsel F, Scholl J, de Vries S, van Puijenbroek E. Sex Differences in Reported Adverse Drug Reactions of Selective Serotonin Reuptake Inhibitors. Drug Saf. 2018 Jul;41(7):677–83. DOI: 10.1007/s40264-018-0646-2.
26. de Vries ST, Denig P, Ekhart C, Burgers JS, Kleefstra N, Mol PGM, van Puijenbroek EP. Sex differences in adverse drug reactions reported to the National Pharmacovigilance Centre in the Netherlands: An explorative observational study. Br J Clin Pharmacol. 2019 Jul;85(7):1507–15. DOI: 10.1111/bcp.13923.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

AUTHORS

Alexander V. Matveev – Candidate of Sciences (Medicine), Associate Professor of the Department of Basic and Clinical Pharmacology, Medical Academy named after S.I. Georgievsky of Vernadsky CFU; Executive Director of National Pharmacovigilance Research Center; Associate Professor of the Department of Clinical Pharmacology and Therapy, Head of the Training and Educational Center for Fundamental and Translational Medicine of Russian Medical Academy of Continuing Professional Education (RMA CPE). ORCID ID: 0000-0002-6636-3950. E-mail: avmcsmu@gmail.com

Anatoly E. Krasheninnikov – Doctor of Sciences (Pharmacy), General Director of National Pharmacovigilance Research Center. ORCID ID: 0000-0002-7791-6071. E-mail: anatoly.krasheninnikov@drugsafety.ru

Elena A. Egorova – Candidate of Sciences (Pharmacy), Associate Professor of the Department of Basic and Clinical Pharmacology, Medical Academy named after S.I. Georgievsky of Vernadsky CFU. ORCID ID: 0000-0003-4012-2523. E-mail: elena212007@rambler.ru

Elena I. Konyaeva – Candidate of Sciences (Medicine), Associate Professor of the Department of Basic and Clinical Pharmacology, Medical Academy named after S.I. Georgievsky of Vernadsky CFU. ORCID ID: 0000-0001-5301-8608. E-mail: konyaeva.simferopol@gmail.com

Natalia V. Matveeva – Candidate of Sciences (Medicine), Associate Professor of the Department of Physical Therapy and Sports Medicine, Physiotherapy with the Course of Physical Education, Medical Academy named after S.I. Georgievsky of Vernadsky CFU. ORCID ID: 0000-0002-0119-8106. E-mail: matveevanatasha@gmail.com

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2022-10-2-187-197

ECONOMIC DAMAGE OF PNEUMOCOCCAL VACCINATION ABSENCE AS A RISK FACTOR FOR COMPLICATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

E.A. Orlova[1], I.P. Dorfman[1], A.R. Umerova[1,2], B.I. Kantemirova[1], M.A. Orlov[1], M.A. Abdullaev[1]

[1] Astrakhan State Medical University
121, Str. Bakinskaya, Astrakhan, Russia, 414000
[2] Territorial body of Federal State Surveillance Service in Healthcare for Astrakhan Region
27, Kommunisticheskaya Str., Astrakhan, Russia, 414040

E-mail: eorlova56@mail.ru

Received 11 Dec 2021 After peer review 16 March 2022 Accepted 04 Apr 2022

The aim of the article is the evaluation of the economic damage (ED) because of the absence of pneumococcal vaccination as a leading risk factor for the development of community-acquired pneumonia (CAP) and acute exacerbations of a chronic obstructive pulmonary disease (COPD).

Materials and methods. The method of attributive statistics was used for the first time to assess the ED of the vaccination absence as an independent risk factor contributing to the development of CAP and COPD exacerbations in the Astrakhan region for the period of 2015–2019. To do this, at the beginning of the study based on the literature data, a relative risk of COPD complications associated with the absence of pneumococcal vaccination was determined. Using it as a risk factor, prevalence rates (a proportion of non-vaccinated patients with COPD), the population attributable risk (PAR) was calculated. Further, the annual economic damage (ED) from the development of CAP and COPD exacerbations was determined. To assess the cost-effectiveness of the COPD complications prevention, vaccination costs of newly registered patients were calculated and the ratio of these costs to the average annual ED was determined.

Results. A decrease in the non-vaccinated patients' proportion corresponds to the decrease in the total ED from COPD complications: from 13.16 million rubles to 6.06 million rubles during the observation period. The calculations showed that due to the increase in the vaccinated patients' proportion over a five-year observation period, the ED from the CAP development decreased by 2.1 times, from exacerbations of COPD – by 2.3 times. The vaccination costs of newly diagnosed COPD cases amounted to 0.63 million rubles. Thus, to prevent the annual ED of 3.24 million rubles, the sum for the state to spend, should be 5.2 times as small.

Conclusion. A study on the evaluation of the ED due to the risk factor (RF), the pneumococcal vaccination absence, showed that its elimination reduces the risk of acute COPD exacerbations and the development of CAP, as well the ED, as associated with them. Reducing the economic costs of the health care system with significantly lower vaccination costs, makes this preventive measure economically viable.

Keywords: chronic obstructive pulmonary disease; community-acquired pneumonia; relative risk; population attributable risk; economic damage; pneumococcal vaccination

Abbreviations: COPD – chronic obstructive pulmonary disease; CAP – community-acquired pneumonia; PI – pneumococcal infection; PV – pneumococcal vaccination; RF – risk factor; RR – relative risk; PAR population attributable risk; PPRF – proportion of individuals in population exposed to RF; ED – economic damage; DCs – direct costs; AR – Astrakhan region; RCTs – randomized clinical trials; CMI – compulsory medical insurance.

For citation: E.A. Orlova, I.P. Dorfman, A.R. Umerova, B.I. Kantemirova, M.A. Orlov, M.A. Abdullaev. Economic damage of pneumococcal vaccination absence as a risk factor for complications of chronic obstructive pulmonary disease. *Pharmacy & Pharmacology.* 2022;10(2):187-197. **DOI:** 10.19163/2307-9266-2022-10-2-187-197

Для цитирования: Е.А. Орлова, И.П. Дорфман, А.Р. Умерова, Б.И. Кантемирова, М.А. Орлов, М.А. Абдуллаев. Экономический ущерб отсутствия пневмококковой вакцинации как фактора риска осложнений хронической обструктивной болезни легких. *Фармация и фармакология.* 2022;10(2):187-197. **DOI:** 10.19163/2307-9266-2022-10-2-187-197

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

ЭКОНОМИЧЕСКИЙ УЩЕРБ ОТСУТСТВИЯ ПНЕВМОКОККОВОЙ ВАКЦИНАЦИИ КАК ФАКТОРА РИСКА ОСЛОЖНЕНИЙ ХРОНИЧЕСКОЙ ОБСТРУКТИВНОЙ БОЛЕЗНИ ЛЕГКИХ

Е.А. Орлова[1], И.П. Дорфман[1], А.Р. Умерова[1,2], Б.И. Кантемирова[1], М.А. Орлов[1], М.А. Абдуллаев[1]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Астраханский государственный медицинский университет» Министерства здравоохранения Российской федерации
414000, Россия, г. Астрахань, ул. Бакинская, д. 121
[2] Территориальный орган Федеральной службы по надзору в сфере здравоохранения по Астраханской области
414040, Россия, г. Астрахань, ул. Коммунистическая, 27

E-mail: eorlova56@mail.ru

Получена 11.12.2021 После рецензирования 16.03.2022 Принята к печати 04.04.2022

Цель. Оценка экономического ущерба (ЭУ) от отсутствия пневмококковой вакцинации, как ведущего фактора риска развития внебольничной пневмонии (ВП) и обострений хронической обструктивной болезни легких (ХОБЛ).
Материалы и методы. Методом атрибутивной статистики впервые проведена оценка ЭУ отсутствия вакцинации в качестве самостоятельного фактора риска, способствующего развитию ВП и обострений ХОБЛ в Астраханской области за период 2015–2019 гг. Для этого в начале исследования на основе данных литературных источников был определен относительный риск осложнений ХОБЛ, ассоциированных с отсутствием пневмококковой вакцинации. С помощью него и показателей распространенности фактора риска (доли не вакцинированных пациентов с ХОБЛ) рассчитан популяционный атрибутивный риск. Далее определялся ежегодный ЭУ от развития ВП и обострений ХОБЛ. Для оценки рентабельности профилактики осложнений ХОБЛ рассчитаны затраты на вакцинацию вновь зарегистрированных пациентов и определено соотношение этих затрат к среднему ежегодному ЭУ.
Результаты. Снижение доли не вакцинированных пациентов соответствует снижению суммарного ЭУ от осложнений ХОБЛ: с 13,16 млн. рублей до 6,06 млн. рублей за период наблюдения. Расчеты показали, что в связи с увеличением доли вакцинированных пациентов за пятилетний период наблюдения ЭУ, от развития ВП снизился в 2,1 раза, от обострений ХОБЛ в 2,3 раза. Затраты на вакцинацию вновь выявленных случаев ХОБЛ составили 0,63 млн. рублей. Таким образом, для предотвращения ежегодного ЭУ в 3,24 млн рублей государство должно затратить сумму в 5,2 раза меньше.
Заключение. Исследование оценки ЭУ, обусловленного фактором риска – отсутствием пневмококковой вакцинации, показало, что его устранение снижает риск обострений ХОБЛ и развития ВП, а также связанного с ними ЭУ. Сокращение экономических затрат системы здравоохранения при существенно меньших затратах на проведение вакцинации обеспечивает экономическую целесообразность этой профилактической меры.
Ключевые слова: хроническая обструктивная болезнь легких; внебольничная пневмония; относительный риск; популяционный атрибутивный риск; экономический ущерб; пневмококковая вакцинация
Список сокращений: ХОБЛ – хроническая обструктивная болезнь легких; ВП – внебольничная пневмония; ПИ – пневмококковая инфекция; ПВ – пневмококковая вакцинация; ФР – фактор риска; ОР – относительный риск; ПАР – популяционный атрибутивный риск; П – доля лиц в популяции, которые подвергаются воздействию ФР; ЭУ – экономический ущерб; ПЗ – прямые затраты; АО – Астраханская область; РКИ – рандомизированные клинические исследования; ОМС – обязательное медицинское страхование.

INTRODUCTION

The diseases that form the mortality structure comprise cardiovascular diseases, type 2 diabetes mellitus, malignant neoplasms, and COPD [1]. According to the WHO, COPD is the third leading cause of death worldwide[1]. Among the diseases spread throughout the world, COPD occupies a leading position in terms of morbidity and mortality, and are accompanied by a growing socio-economic burden on the healthcare system[2] [2]. Acute exacerbations are an integral part of this disease and cause a rapid progression of the disease against the background of a decrease in the quality of life, which leads to significant economic costs[3] [3–6]. Patients with

[1] World Health Organization (WHO). The top 10 causes of death. Available from: https://www.who.int/ru/news-room/fact-sheets/detail/the-top-10-causes-of-death.

[2] Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease. 2020, Report. Available from: https://goldcopd.org/wp-content/uploads/2019/12/GOLD-2020-FINAL-ver1.2-03Dec19_WMV.pdf.

[3] Russian Respiratory Society. Federal clinical guidelines. Chronic obstructive pulmonary disease. Available from: – Режим доступа: http://spulmo.ru/obrazovatelnye-resursy/federalnye-klinicheskie-rekomendatsii/.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-187-197

a severe COPD are at high risk for community-acquired pneumonia (CAP) [7]. The critical role of COPD in increasing rates of pneumococcal pneumonia has been supported by various independent studies. For example, in Europe, the incidence of this pneumonia was 20 times higher in the people with COPD [8, 9].

The official statistics[4] shows that 3.37 million cases of pneumonia are registered every year, one third of them requires hospitalization [10]. Acute exacerbations of COPD and the development of CAP are the main reasons for the patients to seek medical care. *Streptococcus pneumoniae* often acts as an etiological factor in the acute COPD exacerbation. Each subsequent exacerbation is especially severe, accompanied by hospitalization of the patient, associated with a severe prognosis in terms of survival, so the prevention of infectious COPD exacerbations seems critically important [11, 12]. Approximately 80% of the most severe cases of pneumococcal infection (PI) are due to 20 pneumococcal serotypes, most of which are included in modern vaccines for the prevention of PI[5] [13, 14]. In the course of various studies, it has been confirmed that pneumococcal vaccination (PV) of COPD patients, reduces the number of their hospitalizations associated with respiratory viral infections. The results of the studies showed that after vaccination with a pneumococcal conjugated 13-valent vaccine (PCV-13), the ability to eliminate pneumococcus from sputum was 65.6%, and after a year it decreased to 6.3% [13, 15, 16].

Thus, vaccination against Streptococcus pneumoniae is one of the most effective measures to prevent pneumonia and reduce the frequency of COPD exacerbations [17]. In the future, vaccination of COPD patients can significantly reduce the budget economic costs for inpatient and outpatient medical care, sick leave payments [18, 19].

The prevention of exacerbations and CAP in COPD patients is an important strategy in this pathology management. To do this, it is necessary to determine the risk factors (RFs) that can be influenced to reduce the development and frequency of exacerbations. Currently, the main risk factors contributing to the development of severe COPD exacerbations, are traditionally identified as smoking, occupational hazards, the air pollution, an excessive alcohol consumption, respiratory infections, as well as their combinations [20, 21]. An important trend is the evaluation of the economic damage caused by the impact of various risk factors. Thus, McKensey & Company experts showed that in the world, the economic damage from smoking and obesity is comparable to the damage from all wars, terrorism and armed conflicts [22].

The analysis of ED of various COPD RFs is widely reported in the world [1, 18, 23, 24]. A detailed monitoring of foreign and domestic studies of this problem showed that the absence of vaccination as an independent risk factor contributing to the development of CAP and COPD exacerbations, had had not been previously considered.

THE AIM of the article is the evaluation of the economic damage (ED) from the risk factors (RFs) represented by the absence of pneumococcal vaccination in COPD patients.

MATERIALS AND METHODS

Calculation of population attributable risk (PAR)

According to the literature database, at the first stage of the study, the relative risk (RR) of developing COPD exacerbations and the incidence of CAP associated with the absence of PV as a risk factor was determined. To do this, the available databases (Cochrane Library, PubMed, Russian scientific electronic library eLIBRARY) were searched for the studies on the PV effectiveness in preventing the development of exacerbations and CAP in COPD patients for the period from 2016 to 2021. The search queries were performed in Russian and English and included “pneumococcal vaccination in COPD patients”, “effectiveness of pneumococcal vaccination in patients with COPD”.

Initially, 100 literature sources were identified. Then they were selected according to the type and content of publications (Fig. 1). The identical publications found in different databases, the works that are not original studies, were excluded. The publications that did not evaluate the effectiveness of vaccination in COPD patients or were devoted to the study of the effectiveness of pneumococcal polysaccharide 23-valent vaccine (PPV-23) alone, were not subjected to the review, either. In addition, the studies evaluating the effects of vaccination in COPD patients with comorbid pathology were excluded.

As a result, 10 studies were selected [16, 17, 25–32], from which in 2017, the Cochrane systematic review by Walters J.A. et al. [25] was chosen out as the gold standard for high-quality, reliable information in evidence-based medicine and healthcare. This review included the results of 12 randomized controlled trials (RCTs) and 2,171 COPD patients. The average age of the participants was 66 years, 67% of them were men. All participants had a COPD diagnosis from moderate to heavy severity level. Compared to the controls, the vaccinated group had a lower likelihood of developing CAP (the relative risk (RR) 0.62, 95% confidence interval (CI) from 0.43 to 0.89; six studies, n = 1372; the level of evidence – medium). The number of patients requiring treatment to obtain an additional favorable outcome (NNTB) (avoiding one episode of CAP) was 19 (95% CI from 13 to 52). Vaccination significantly

[4] European Respiratory Society. European Lung White Book. Lausanne, Switzerland: ERS / European Respiratory Society. Available from: https://www.erswhitebook.org/files/public/Chapters/13_COPD.pdf

[5] Zverev V.V., Kostinov M.P., Magarshak O.O., et al. Rukovodstvo po klinicheskoj immunologii v respiratornoj medicine [Guidelines for Clinical Immunology in Respiratory Medicine]. Suppl. 2nd ed. Moscow: MDB, 2018. – 304 p. Russian

reduced the chance of a COPD exacerbation (OR 0.60, 95% CI from 0.39 to 0.93; four studies, n = 446; the level of evidence: moderate). The NNTB for preventing an acute exacerbation in the patient was 8 (95% CI 5 to 58).

Further, in accordance with the data obtained, RR was calculated separately for the development of acute exacerbations and CAPs in non-vaccinated (1) and vaccinated (2) patients according to the formula:

$$RR = \frac{\left[\frac{a}{(a+b)}\right]}{\left[\frac{c}{(c+d)}\right]}, \quad (1)$$

$$RR = \frac{\left[\frac{c}{(c+d)}\right]}{\left[\frac{a}{(a+b)}\right]}, \quad (2)$$

where: RR is a relative risk; a is the number of exacerbations (CAPs) in non-vaccinated patients; b denotes the absence of exacerbations (CAPs) in non-vaccinated patients; c is the number of exacerbations (CAPs) in vaccinated patients; d denotes the absence of exacerbations (CAPs) in vaccinated patients.

By calculating the RR, it is possible to show the association strength between the influencing risk factor and the outcome, e. i., how many times the probability of exacerbations and the development of CAPs caused by the lack of vaccination in COPD patients, increases. If the development of exacerbations of COPD and CAPs is higher in the non-vaccinated group, then the RR will be higher than 1, if lower, then the RR will be lower than 1; if the probability in the two groups is the same, then their ratio will be equal to 1[6].

The PAR population attributable risk (PAR) was calculated based on the FR and RR prevalence rates.

This indicator reflects the additional incidence in the population associated with risk factors. In addition, with the help of PAR, it is possible to determine the proportion of morbidity in the population associetd with this risk factor, i.e., the additional proportion of a population risk. The PAR depends on the degree the risk factors are widespread in the given population:

$$PAR = \frac{P_{nv}(RR - 1)}{P_{nv}(RR - 1) + 1},$$

where: P_{nv} is the proportion of non-vaccinated individuals in the population (exposed to risk factors); RR is the relative risk of developing a disease under the influence of the FR under consideration [33, 34].

To determine the proportion of the people exposed to the FR is the lack of vaccination (UV), the number of the non-vaccinated patients was calculated relative to the total number of the people suffering from the COPD registered in the Astrakhan region (AR), for the period from 2015 to 2019:

$$P_{nv} = \frac{N_1}{N},$$

where: N_1 is the number of non-vaccinated patients with COPD; H is the total number of patients with COPD.

The total number of COPD patients was taken from the state statistical observation form No.12 "Information on the number of diseases registered in patients living in the medical organization service area" in the Astrakhan region (AR) for the period from 2015 to 2019. The number of vaccinated patients was determined by adding the number of pneumococcal vaccinations (PVs) for each year with the indicator of the previous year, based on the data provided upon the request by medical institutions of the city and region. The number of non-vaccinated patients per year was determined by diminution the sum of those vaccinated from the total number of the COPD patients during the same period.

Calculation of government economic damage in disease management of patients with exacerbations of COPD and CAPs, due to RF

At the second stage of the study, the government economic damage was calculated in the management of patients with exacerbations of COPD and CAPs, due to RFs. The calculation was carried out according to the formula:

$$ED_{RF} = PAR_{RF} \times DCs,$$

where: ED_{RF} is the economic damage from the risk factor; PAR_{RF} denotes the population attributable risk for each analyzed disease associated with FR; DCs are direct costs of treating COPD.

Direct costs were calculated in the earlier study "Evaluating Socioeconomic Burden of COPD over a Five-Year Period – Regional Aspect" [35].

Subsequently, the ED of COPD and CAPs exacerbations were summed up.

Then, to determine PAR_{RF} for new non-vaccinated COPD patients for the period of 2015-2019, the average number of newly diagnosed patients were calculated.

Using the data obtained, the ED for CAPs was calculated for the first detected cases of the COPD exposed to RF. The calculation was carried out according to the formula:

$$ED_{rf} = PAR_{rf} \times DCs_{av},$$

where: ED_{rf} is an economic damage from the risk factor; PAR_{rf} is the population attributable risk for each analyzed disease associated with RF; $DCs_{av.}$ denotes the average direct costs of COPD treatment from 2015 to 2019.

[6] Zhukova OV. Application of attributive statistics methods in pharmaceutical and biomedical research (contingency tables). Privolzhsky Research Medical University. – Kazan: Buk Limited Liability Company, 2020. – 76 p. Russian

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-187-197

Table 1 – Contingency table for RR calculating of COPD exacerbations development

Risk factor	One exacerbation episode within a period of 6 months to 1 year	Absence of exacerbations
Absence of vaccination	608(a)	392(b)
Vaccination	482(c)	518(d)

Note: a – the number of CAPs in non-vaccinated patients, b – the absence of CAPs in non-vaccinated patients, c – the number of CAPs in vaccinated patients; d – the absence of CAPs in vaccinated patients.

Table 2 – Contingency table for RR calculating of CAP development

Risk factor	One CAP episode within a period of 6 to 36 months	Absence of CAP
Absence of vaccination	148(a)	852(b)
Vaccination	93(c)	907(d)

Note: a – the number of CAPs in non-vaccinated patients, b – the absence of CAPs in non-vaccinated patients, c – the number of CAPs in vaccinated patients; d – the absence of CAPs in vaccinated patients.

Table 3 – Information necessary for PAR CAPs calculation, COPD exacerbations and their results

Indicators	Period					
	2015	2016	2017	2018	2019	On average
Number of COPD persons	2 869	2788	2721	2913	3.180	2.894
COPD incidence (CAP first detected cases)	445	226	266	310	496	369
Annually vaccinated	333	138	559	617	940	–
Total number of vaccinated	333	471	1030	1.647	2.587	–
Non-vaccinated	2 536	2317	1691	1.266	593	369
Proportion of non-vaccinated (P_{nv})	0.88	0.83	0.62	0.43	0.19	0.13
PAR CAP	0.34	0.33	0.27	0.20	0.10	0.07
PAR exacerbations of COPD	0.19	0.18	0.14	0.10	0.05	0.03

Table 4 – Economic damage to regional healthcare system due to RF – absence of vaccination

Indicators	Period				
	2015	2016	2017	2018	2019
Direct COPD costs, mln rubles	24.83	28.14	29.6	32.94	40.39
ED due to CAPs, mln rubles	8.44	9.29	7.99	6.59	4.04
ED exacerbations of COPD, mln rubles	4.72	5.07	4.14	3.29	2.02
ED due to CAP + COPD exacerbations, mln rubles	13.16	14.35	12.14	9.88	6.06

Table 5 – Sensitivity analysis of the obtained results

Changes in initial parameters	Indicators										
	25%	20 %	15 %	10 %	5 %	Basic variant	5 %	10 %	15%	20 %	25 %
Change in vaccine price, rubles	1275	1360	1445	1530	1615	1700	1.785	1.870	1,955	2040	2125
Change in the number of people	277	295	314	332	351	369	387	406	424	443	461
Change in vaccination costs, mln rubles	0.47	0.50	0.53	0.56	0.60	0.63	0.66	0.69	0.70	0.75	0.78
Change in economic damage, mln rubles	2,43	2.59	2,75	2.92	3,08	3.24	3.40	3.56	3,73	3.89	4.05

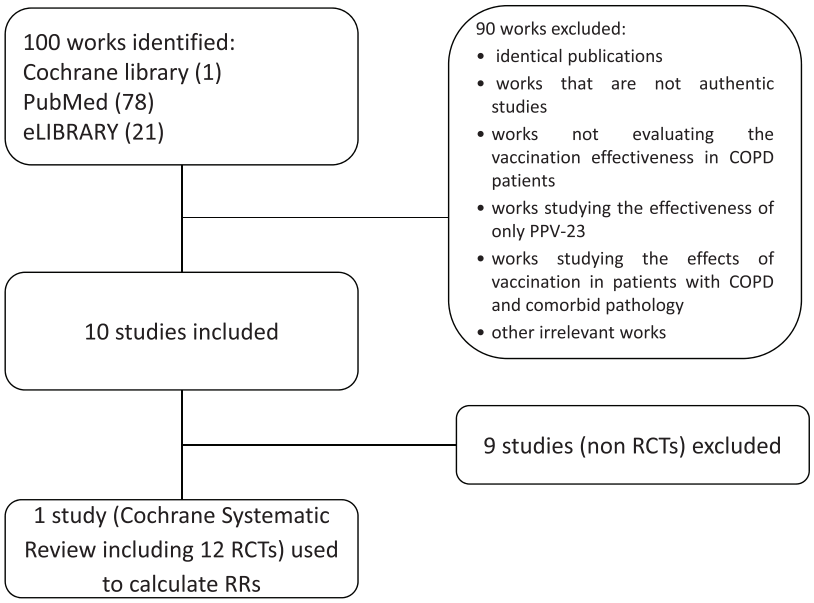

Figure 1 – Research selection methodology

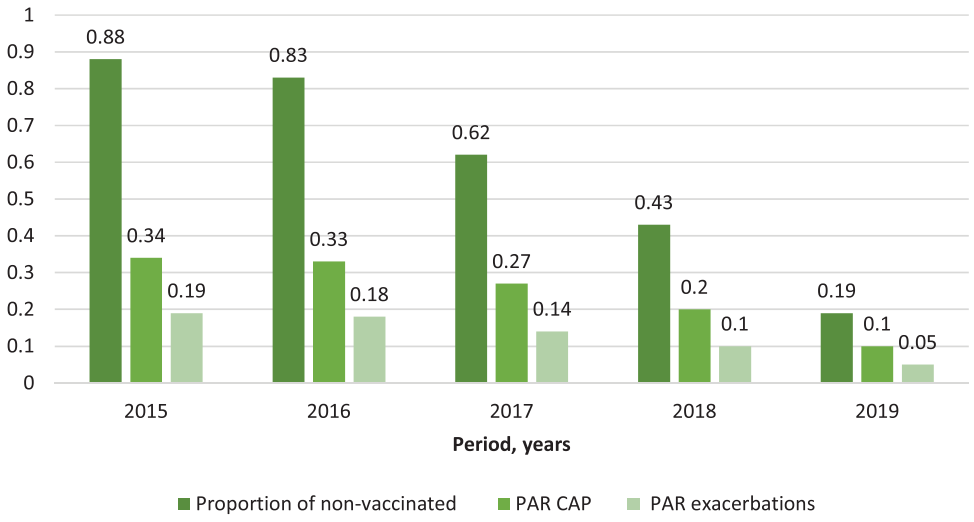

Figure 2 – Proportion ratio of non-vaccinated COPD patients related to PAR CAPs and COPD exacerbations

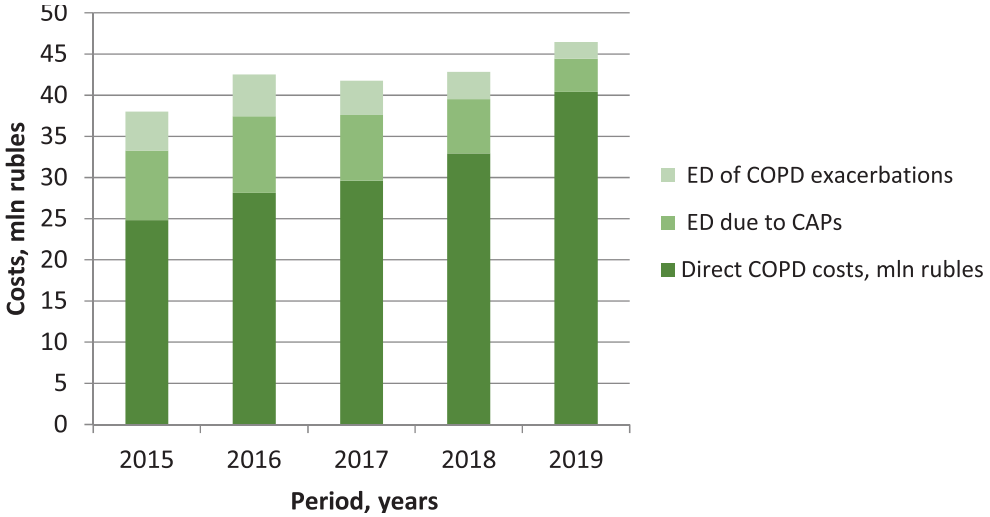

Figure 3 – Correlation of direct COPD and ED costs from exacerbations and CAPs

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-187-197

Determining economic benefit of pneumococcal vaccination of newly diagnosed COPD cases

At the third stage of the study, the calculation of the pneumococcal vaccine prophylaxis cost was carried out for the CAPs patients with the first diagnosed COPD. The calculation was carried out according to the formula [36]:

$$Cost_{vp} = Cost_{vac.} \times N,$$

where: is the cost of vaccine prophylaxis; $Cost_{vac.}$ is the cost of a medicinal product (vaccine); N is the number of patients to be vaccinated.

The cost of the vaccine was taken into account based on the results of competitive tendering for the purchase of pneumococcal vaccines held by the regional Ministry of Health. The cost of PKV-13 "Prevenar 13" remained unchanged for the entire study period and corresponded to 1700 rubles[7].

Next, the ratio of pneumococcal vaccination cost for patients with CAPs of the first detected COPD and the average possible annual ED from the occurrence of COPD and CAPs exacerbations were calculated.

Sensitivity analysis of the study results

The study result was a sensitivity analysis of the results obtained in order to determine their resistance to changes in the initial parameters. To do this, a one-way sensitivity analysis was performed with a variation in the price of pneumococcal vaccine and the number of patients by sequentially increasing them by 25% with a 5% step.

The work with the data and their statistical processing was carried out using MS Excel 10.0 (Microsoft Office 2010, USA). For these data processing, the method of population attributive statistics was used; RR, PAR.

RESULTS

Based on the systematic review data, contingency tables were compiled to calculate the OR (Tables 1, 2).

In accordance with the study program, the RRs for the development of COPD exacerbations in vaccinated and non-vaccinated PV patients were calculated and analyzed.

RRs of non-vaccinated =
[608/(608+392)]/[482/(482+518)]=1.26
RRs of vaccinated =
[482/(482+518)]/[608/(608+392)]=0.79

The RR for COPD exacerbations in non-vaccinated COPD patients based on the results of a systematic review was 1.26 versus 0.79 in the vaccinated patients.

The relative risk is the ratio of the exacerbation of COPD risk in the individuals exposed to the risk factor (of the non-vaccinated) relative to a comparison group (the vaccinated).

The next step was to calculate the RR for the CAP development in vaccinated and non-vaccinated PV patients with COPD.

RRs of non-vaccinated =
[148/(148+852)]/[93/(93+907)]=1.59
RRs of vaccinated =
[93/(93+907)]/[148/(148+852)]=0.62

Accordingly, the RRs for developing CAPs in the non-vaccinated COPD patients was 1.59 versus 0.62 in the vaccinated patients.

The obtained values may change when calculations are carried out on a different sample, for this it is necessary to determine how significant these changes can be. To confirm the results of RRs for both groups of patients, the confidence interval (CI) was calculated. In COPD exacerbations (95% CI), the CI of RRs was 1.163–1.368. For the RR of the CAP development, (95% CI) is 1.247–2.031, which confirms the statistical significance of the results.

Thus, the RR calculation performed shows that non-vaccinated COPD patients are 1.26 and 1.59 times more likely to develop exacerbations or CAPs, respectively.

The results of the RRs obtained from the data on the COPD patients taken in the systematic review, allowed us to create a computational model with which we can evaluate the RFs in the regional population.

In order to evaluate risks in the population, it is necessary to know how often the members of the population under consideration are exposed to risk factors. This study was based on the data of the COPD incidence registered in patients living in the service area of the Astrakhan region medical organizations. We

PAR CAPs and COPD exacerbations were calculated for the period of 2015–2019: The results are presented in Table 3.

Thus, over the study period, with a decrease in the proportion of non-vaccinated PV COPD patients, the population risk of developing CAPs and exacerbating COPD decreases, which is reflected in Fig. 2.

Having PAR indicators for the study period, the costs of the regional healthcare system for the treatment of CAPs and COPD exacerbations in the form of ED were estimated.

As for the compulsory medical insurance (CMI) of the AR healthcare system for the period of 2015-2019, its structure of direct costs included direct medical costs for hospitalization, outpatient treatment, calls for ambulances and emergency medical care. For the research purposes, direct cost estimates (Table 4) from a previous study assessing the economic burden of COPD in the AO, were extrapolated [35].

Having received all the necessary data, the ED from the RF were calculated in accordance with the study program. The results are presented in Table 4.

The calculations showed that, due to a decrease in the proportion of non-vaccinated patients over a five-year follow-up period, the ED from the RF due to CAPs

[7] Procurement contract card of the Ministry of Health JSC No. 2301506815919000378. Available from: https://zakupki.gov.ru/epz/contract/contractCard/document-info.html?reestrNumber=2301506815919000378.

decreased by 2.1 times, from COPD exacerbations by 2.3 times (Fig. 3).

Considering that new cases of COPD are detected every year, these patients can be considered as a risk group for exacerbations and CAPs due to the absence of PV. The mean value of the non-vaccinated newly diagnosed patients with PV makes it possible to calculate the mean annual ED from the occurrence of COPD and CAP exacerbations. In this regard, a number of CAPs of new COPD cases during the study period were analyzed. For the period of 2015-2019, the average number of newly diagnosed patients was 369 cases ± 124 (from 245 to 493). The median number of COPD patients over the same period was 2894. Then the percentage of non-vaccinated patients was calculated relative to the median of the total number of COPD patients during the study period, which made 0.13 or 13%. PAR CAP and exacerbations of COPD were determined as 0.07 (7%) and 0.03 (3%), respectively. The average DCs for COPD was calculated as the average value for 5 years under study, which amounted to 31.18 ± 4.4 million rubles. Based on the results obtained, the ED was determined equal to 2.22 million rubles from CAPs and 1.02 million rubles from COPD exacerbations. In total, this damage amounted to 3.24 million rubles. Next, we calculated the vaccination cost of newly diagnosed COPD cases, which amounted to 627,300 rubles (from 416,500 to 838,100 rubles). Thus, in order to prevent an annual economic loss of 3.24 million rubles, the government must spend the sum 5.2 times less.

The sensitivity analysis demonstrated the stability of the obtained results to changes in the initial parameters. If the vaccination cost is increased by 25% (both due to an increase in the price of the vaccine and due to the number of the vaccinated), then the indicators will not go beyond the indicators of the economic damage caused by the absence of vaccination (Table 5).

DISCUSSION

The COPD incidence among the adult population of the Russian Federation shows a steady increase, thus reflecting global trends. The increase in the incidence rate for 2007–2017 amounted to 16.5%[8]. As the results of the SUPPORT[9] study showed, in Russia, there are more than 50% of patients with frequent exacerbations. A high risk of exacerbations and the CAP development in COPD patients determine the importance of preventing complications of this disease. Risk factors make a significant contribution to the COPD progression and severity, so the study of risk factors plays an important role in the effective allocation of resources for the prevention and treatment of COPD by health authorities. In COPD studies, a significant attention is paid to risk factors such as smoking, an excessive alcohol consumption, a low physical activity, etc. [1]. Thus, the greatest contribution to the morbidity and mortality from COPD is made by smoking (9.6%), arterial hypertension (24%), an excessive alcohol consumption (9%), a low physical activity (16%) [1]. These works are carried out both in domestic and foreign studies [37, 38]. So, in the studies by Zhukova O.V. et al., the effect of smoking on the COPD exacerbation is shown [37]. In another paper by this author, an attempt to link the risk of developing CAP in patients with COPD with the use of inhaled glucocorticosteroids has been made. Against the background of cardiovascular diseases, a trend towards an increase in the development of CAP in COPD patients has also been shown [23]. The likelihood of COPD occurring against the background of the exposure to occupational hazards such as gas fumes and dust, was demonstrated in the meta-analysis conducted by scientists from the Russian Federation, Kazakhstan and Azerbaijan [20]. In the work by Khalid F. et al. [39], in Latin Americans under the age of 50 years (7,565 people), the risk factors for early COPD were determined as asthma (PAR – 26.5%), smoking (21.4%), chronic sinusitis (6.7%). The aim of the study by Shulmin A.V. et al. was the costs research related with tobacco-associated diseases, including COPD [24].

According to the data on the PV effectiveness of the Chelyabinsk "City Pulmonological Center", obtained for 2012–2015, vaccination provided a sevenfold decrease in the number of hospitalizations keeping up to the results and 3 years afterwards of follow-up in comparison with the control group [31]. Pneumococci often act as an etiological factor of exacerbations and development of pneumonia in the patients suffering from COPD, which is of interest in studying the absence of PV as an independent RF. When conducting an information search in the available databases (Cochrane Library, PubMed, e-LIBRARY), it was notified that there were no studies in which the absence of PV was considered as RF complications of COPD.

In accordance with the developed research program, based on the data of a systematic review using the method of attributive statistics, the RR of COPD complications was calculated to determine the proportion of individuals in the regional population associated with the studied RF for the period of 2015–2019. During the five-year follow-up period, there was a decrease in PAR: with CAP from 34 to 10%, and with COPD exacerbations from 19 to 5%. In the process of calculating PAR, there was a positive tendency to reduce the population risk of COPD complications against the background of an increase in the number of vaccinated patients. The decrease in the proportion of non-vaccinated patients corresponds to a decrease in the total economic damage from COPD

[8] Chronic obstructive pulmonary disease as a socially significant disease. XXVIII National Congress on Respiratory Diseases; Moscow, October 17, 2018. – P. 54–60. Available from: https://umedp.ru/upload/iblock/73b/GSK.pdf. Russian

[9] The 28th National Congress on Respiratory Diseases: a time for innovations. Pulmonologiya. 2018;28(5):632–634. Available from: https://umedp.ru/upload/iblock/73b/GSK.pdf. Russian

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-187-197

complications: from 13.16 million rubles to 6.06 million rubles during the follow-up period. To assess the cost-effectiveness of COPD complications prevention, the ratio of the average number of non-vaccinated newly diagnosed patients to the average annual economic damage was calculated. The calculation of the average PV costs showed that the costs necessary to prevent the RF action will be 5.2 times less than the possible annual ED. The results of the study indicate that pneumococcal vaccination is an effective method in reducing healthcare costs associated with the treatment of COPD complications.

Study limitations

When analyzing the contribution of risk factors to the development of COPD complications, the literature data were used to determine the RR for CAP and COPD exacerbations, which could affect the accuracy of the PAR calculation.

CONCLUSION

CAP was the first study to assess ED using an attributive statistics methodology, in which the absence of PV acted as RF. The results obtained suggest that pneumococcal vaccination significantly reduces the risk of COPD exacerbations and the CAP development. Reducing the risks of COPD complications leads to a marked reduction in the economic costs of the health care system, with significantly lower costs for vaccination, which, in turn, makes this preventive measure economically viable. The determination of the population risk makes it possible to provide people with important information about the potential impact of prevention programs and interventions on the health system and will be extremely useful to health decision makers. In addition to traditional financial aspects, investments in population-based preventive measures have an important social value, since a decrease in the burden of various diseases leads to an increase in well-being, motivation and harmonization of patients' private lives.

FUNDING

This study did not receive financial support from third parties.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Ekaterina A. Orlova – material collection, text writing and editing; Inna P. Dorfman – study design, data collection and editing; Adela R. Umerova – text writing and editing; Bela I. Kantemirova – data search, editing; Mikhail A. Orlov – text writing and editing; Musalitdin A. Abdullaev – compiling drawings and bibliographic lists. All the authors made a significant contribution to the search and analytical work and preparation of the article, read and approved the final version before the publication.

REFERENCES

1. Kontsevaya AV, Mukaneeva DK, Myrzamatova AO, Balanova YuA, Khudyakov MB, Drapkina OM. Economic damage of risk factors associated with morbidity and mortality from major chronic non-communicable diseases in Russia in 2016. Cardiovascular Therapy and Prevention. 2020;19(1):48–55. DOI: 10.15829/1728-8800-2020-1-2396.
2. Iheanacho I, Zhang S, King D, Rizzo M, Ismaila AS. Economic Burden of Chronic Obstructive Pulmonary Disease (COPD): A Systematic Literature Review. Int J Chron Obstruct Pulmon Dis. 2020 Feb 26;15:439–60. DOI: 10.2147/COPD.S234942.
3. Lee J, Jung HM, Kim SK, Yoo KH, Jung KS, Lee SH, Rhee CK. Factors associated with chronic obstructive pulmonary disease exacerbation, based on big data analysis. Sci Rep. 2019 Apr 30;9(1):6679. DOI: 10.1038/s41598-019-43167-w.
4. Alqahtani JS, Njoku CM, Bereznicki B, Wimmer BC, Peterson GM, Kinsman L, Aldabayan YS, Alrajeh AM, Aldhahir AM, Mandal S, Hurst JR. Risk factors for all-cause hospital readmission following exacerbation of COPD: a systematic review and meta-analysis. Eur Respir Rev. 2020 Jun 3;29(156):190166. DOI: 10.1183/16000617.0166-2019.
5. Avdeev SN. Exacerbation of chronic obstructive pulmonary disease: a choice of antibacterial treatment. Pulmonologiya. 2014;(6):65–72. DOI: 10.18093/0869-0189-2014-0-6-65-72. Russian
6. Zhukova OV, Kononova SV, Konyshkina TM. Statistical relationship between smoking and frequent exacerbations of chronic obstructive pulmonary disease. Profilaktich-eskaya Meditsina. 2019;22(1):79–83. DOI: 10.17116/profmed20192201179. Russian
7. Postnikova LB, Klimkin PF, Boldina MV, Gudim AL, Kubysheva NI. Fatal severe community-acquired pneumonia: risk factors, clinical characteristics and medical errors of hospital patients. Terapevticheskii arkhiv. 2020; 92(3):42–9. DOI: 10.26442/00403660.2020.03.000538. Russian
8. Myint PK, Lowe D, Stone RA, Buckingham RJ, Roberts CM. U.K. National COPD Resources and Outcomes Project 2008: patients with chronic obstructive pulmonary disease exacerbations who present with radiological pneumonia have worse outcome compared to those with non-pneumonic chronic obstructive pulmonary disease exacerbations. Respiration. 2011;82(4):320–7. DOI: 10.1159/000327203.
9. Matanock A, Lee G, Gierke R, Kobayashi M, Leidner A, Pilishvili T. Use of 13-Valent Pneumococcal Conjugate Vaccine and 23-Valent Pneumococcal Polysaccharide Vaccine Among Adults Aged ≥ 65 Years: Updated Recommendations of the Advisory Committee on Immunization Practices. MMWR Morb Mortal Wkly Rep. 2019;68:1069–75. DOI: 10.15585/mmwr.mm6846a5.
10. Reinert R, Taysi B. Effectiveness of the 13-valent pneumococcal conjugate vaccine: emerging data from invasive

pneumococcal disease, pneumonia, acute otitis media and nasopharyngeal carriage. Pediatric pharmacology. 2012;9(3):8–11. DOI: 10.15690/pf.v9i3.315.

11. Kostinov MP, Ryzhov AA, Magarshak OO, Zhirova SN, Protasov AD, Erofeev IuV, Migunova OV, Tolokonnikova IN, Liverko EV. The clinical aspects of efficiency of the prevention of pneumococcal infection with vaccines in chronic obstructive pulmonary disease patients living in the West Siberian Region. Ter Arkh. 2014;86(3):28–33. Russian
12. Halpin DM, Miravitlles M, Metzdorf N, Celli B. Impact and prevention of severe exacerbations of COPD: a review of the evidence. Int J Chron Obstruct Pulmon Dis. 2017 Oct 5;12:2891–908. DOI: 10.2147/COPD.S139470.
13. Kostinov AM, Kostinov MP, Mashilov CV. Antagonism between pneumococcal vaccines and COVID-19. Meditsinskiy sovet = Medical Council. 2020;(17):66–73. DOI: 10.21518/2079-701X-2020-17-66-73. Russian
14. Zaripova AZ, Bayazitova LT, Tyupkina OF, Chazova TA, Tyurin YA, Isaeva GSh, Pokrovskaya EM. Phenotypic and genotypic properties of Streptococcus pneumoniae in case of bacteria carrying. Practical Medicine. 2018; 16(9): 106–12. Russian
15. Protasov A, Kostinov M, Zhestkov A. Microbiological effect of anti-pneumococcal vaccination in COPD patients. 10th Intern Symposium On Pneumococci and Pneumococcal Diseases. 2016: 26–30.
16. Protasov AD, Kostinov MP, Zhestkov AV, Shteiner ML, Magarshak OO, Kostinova TA, Ryzhov AA, Pakhomov DV, Blagovidov DA, Panina MI. [Choice of optimal vaccination tactics against pneumococcal infection from immunological and clinical standpoints in patients with chronic obstructive pulmonary disease]. Ter Arkh. 2016;88(5):62–9. Russian. DOI: 10.17116/terarkh201688562-69.
17. Ignatova GL, Avdeev SN, Antonov VN. Comparative effectiveness of pneumococcal vaccination with PPV23 and PCV13 in COPD patients over a 5-year follow-up cohort study. Sci Rep. 2021;11: 15948. DOI: 10.1038/s41598-021-95129-w.
18. Artyukhov IP, Shulmin AV, Dobretsova EA, Arshukova IL. Ocenka mediko-demograficheskih poter' i ekonomicheskih zatrat, obuslovlennyh hronicheskoj obstruktivnoj bolezn'yu legkih (na primere Krasnoyarskogo kraya) [Assessment of medical and demographic losses and economic costs caused by chronic obstructive pulmonary disease (on the example of the Krasnoyarsk Territory)]. Healthcare of the Russian Federation. 2015;59(5):32–7. Russian
19. Rycroft CE, Heyes A, Lanza L, Becker K. Epidemiology of chronic obstructive pulmonary disease: a literature review. Int J Chron Obstruct Pulmon Dis. 2012;7:457–94. DOI: 10.2147/COPD.S32330.
20. Vinnikov D, Rybina T, Strizhakov L, Babanov S, Mukatova I. Occupational Burden of Chronic Obstructive Pulmonary Disease in the Commonwealth of Independent States: Systematic Review and Meta-Analysis. Front Med (Lausanne). 2021 Jan 18;7:614827. DOI: 10.3389/fmed.2020.614827.
21. Smirnova MI, Antipushina DN, Kurekhyan AS. Chronic obstructive pulmonary disease and COVID-19: data by summer 2020, approaches to health care and prevention. Profilakticheskaya Meditsina. 2020;23(8):37–44. DOI: 10.17116/profmed20202308137. Russian
22. Dobbs R, Sawers C, Thompson F, Manyika J, Woetzel JR, Child P, McKenna S, Spatharou A. Overcoming obesity: An initial economic analysis. Executive summary. Discussion paper, 2014. Available from: http://www.mckinsey.com/mgi.
23. Zhukova OV, Khokhlov AL, Zhukova OV, Khokhlov AL. IGKS-terapiya kak faktor riska razvitiya vnebol'nichnoj pnevmonii u pacientov s hronicheskoj obstruktivnoj bolezn'yu legkih [Inhaled corticosteroid therapy as a risk factor for the development of community-acquired pnumonia in patients with chronic obstructive pulmonary disease]. Medicinal Bulletin. 2021; 15(3(83):28–35. Russian
24. Shulmin AV, Kozlov VV, Kutumova OY, Dobretsova EA, Arshukova IL. Assessment of the costs of the health care system at the krasnoyarsk territory, due to diseases associated with tobacco smoking. Siberian Medical Review. 2013;4:74–7. Russian
25. Walters JA, Tang JN, Poole P, Wood-Baker R. Pneumococcal vaccines for preventing pneumonia in chronic obstructive pulmonary disease. Cochrane Database Syst Rev. 2017 Jan 24;1(1):CD001390. DOI: 10.1002/14651858.CD001390.pub4.
26. Figueira-Gonçalves JM, Bethencourt-Martín N, Pérez-Méndez LI, Díaz-Pérez D, Guzmán-Sáenz C, Viña-Manrique P, Pedrero-García AJ. Impact of 13-valent pneumococcal conjugate polysaccharide vaccination in exacerbations rate of COPD patients with moderate to severe obstruction. Rev Esp Quimioter. 2017 Aug;30(4):269–75. English, Spanish.
27. Jgnatova GL, Antonov VN. Analysis of compliance dynamics in patients with chronic obstructive pulmonary disease on the background of vaccination against pneumococcal infection. Terapevticheskii arkhiv. 2018; 90(3): 47–52. DOI: 10.26442/terarkh201890347-52. Russian
28. Fekete M, Pako J, Nemeth AN, Tarantini S, Varga JT. Prevalence of influenza and pneumococcal vaccination in chronic obstructive pulmonary disease patients in association with the occurrence of acute exacerbations. J Thorac Dis. 2020 Aug;12(8):4233–42. DOI: 10.21037/jtd-20-814.
29. Protasov AD, Zhestkov AV, Kostinov MP, Shteiner ML, Tezikov IuV, Lipatov IS, Yastrebova NE, Kostinova AM, Ryzhov AA, Polishchuk VB. Analysis of the effectiveness and long-term results of formation of adaptive immunity in the use of various medications and vaccination schemes against pneumococcal infection in patients with chronic obstructive pulmonary disease. Terapevticheskii Arkhiv. 2017;89(12):165–74. DOI: 10.17116/terarkh20178912165-174. Russian
30. Ignatova GL, Antonov VN. Efficacy analysis of five-year experience of vaccination with conjugate pneumococcal vaccine in patients with chronic obstructive pulmonary disease. Pulmonologiya. 2018;28(2):185–92. DOI: 10.18093/0869-0189-2018-28-2-185-192. Russian
31. Ignatova GL, Antonov VN, Rodionova OV. The effectiveness of using the conjugated pneumococcal vaccine in patients with chronic obstructive pulmonary disease for 3 years. Consilium Medicum. 2016; 3: 42–6. Russian
32. Antonov VN. Vaccinal effect on early and late results in patients with chronic obstructive pulmonary disease. Modern problems of science and education. 2016;4:45. Russian
33. Krysanova VS, Zhuravleva MV, Dralova OV, Rogacheva OA, Kameneva TR. The problem of obesity and overweight in the russian federation and its pharmacoeconomic assessment. Almanac of Clinical Medicine. 2015; 1(1): 36–41. DOI: 10.18786/2072-0505-2015-1-36-41.
34. Askari M, Namayandeh SM. The Difference between

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-187-197

the Population Attributable Risk (PAR) and the Potentioal Impact Fraction (PIF). Iran J Public Health. 2020 Oct;49(10):2018-2019. DOI: 10.18502/ijph.v49i10.4713.

35. Orlova EA, Umerova AR, Dorfman IP, Orlov MA, Abdullaev MA. Estimation of socio-economic burden of chronic obstructive pulmonary disease for a 5-year period: a regional aspect. Pharmacy & Pharmacology. 2021;9(2):130–8. DOI: 10.19163/2307-9266-2021-9-2-130-138.
36. Orlova EA, Dorfman IP, Orlov MA, Abdullaev MA. Pharmacoeconomic evaluation of anti-pneumococcal vaccination in risk groups for the prevention of community-acquired pneumonia among adults in the Astrakhan region. Pharmacy & Pharmacology. 2020;8(6):436–45. DOI: 10.19163/2307-9266-2020-8-6-436-445.
37. Zhukova OV, Konyshkina TM, Kononova SV. The concept of risk factors in assessing the impact of smoking on an exacerbation of chronic obstructive pulmonary disease. Terapevticheskii arkhiv. 2015;87(3):23–6. Russian
38. Brown KF, Rumgay H, Dunlop C, Ryan M, Quartly F, Cox A, Deas A, Elliss-Brookes L, Gavin A, Hounsome L, Huws D, Ormiston-Smith N, Shelton J, White C, Parkin DM. The fraction of cancer attributable to modifiable risk factors in England, Wales, Scotland, Northern Ireland, and the United Kingdom in 2015. Br J Cancer. 2018 Apr;118(8):1130–41. DOI: 10.1038/s41416-018-0029-6.
39. Khalid F, Wang W, Mannino D, Diaz AA. Prevalence and Population Attributable Risk for Early Chronic Obstructive Pulmonary Disease in U.S. Hispanic/Latino Individuals. Ann Am Thorac Soc. 2022 Mar;19(3):363–71. DOI: 10.1513/AnnalsATS.202103-253OC.

AUTHORS

Ekaterina A. Orlova – Candidate of Sciences (Medicine), Associate Professor, Head of the Department of Pharmacology, Astrakhan State Medical University. ORCID ID: 0000-0001-7169-3586, E-mail: eorlova56@mail.ru

Inna P. Dorfman – Candidate of Sciences (Medicine), Associate Professor, Associate Professor of the Department of Clinical Pharmacology, Astrakhan State Medical University. ORCID ID: 0000-0003-1561-0592. E-mail: inna1977@inbox.ru

Adela R. Umerova – Doctor of Sciences (Medicine), Associate Professor, Head of the Department of Clinical Pharmacology, Astrakhan State Medical University; Head of the Territorial body of Federal State Surveillance Service in Healthcare for Astrakhan Region. ORCID ID: 0000-0002-3129-2443. E-mail: adelya_umerova@mail.ru

Bela I. Kantemirova – Doctor of Sciences (Medicine), Associate Professor, Professor of the Department of Pharmacology, Astrakhan State Medical University. ORCID ID:0000-0003-3278-2556. E-mail: belakantemirova@rambler.ru

Mikhail A. Orlov – Doctor of Sciences (Medicine), Professor, Head of the Department of Medical Rehabilitation of Astrakhan State Medical University. ORCID ID: 0000-0002-8995-6572. E-mail: orlovdoc56@gmail.com

Musalitdin A. Abdullaev – post-graduate student of the Department of Pharmacology, Astrakhan State Medical University. ORCID ID: 0000-0001-7374-2660. E-mail: abdullaev-musalitdin@mail.ru

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

CLINICAL AND ECONOMIC ANALYSIS OF GENETICALLY ENGINEERED BIOLOGICS CONSUMPTION BY PATIENTS WITH COVID-19

V.I. Petrov, A.Yu. Ryazanova, A.V. Ponomareva, O.V. Shatalova, Ya.V. Levina

Volgograd State Medical University
1, Pavshikh Bortsov Sq., Volgograd, Russia, 400131

E-mail: nastasyakus@mail.ru

Received 21 Feb 2022 After peer review 15 March 2022 Accepted 20 Apr 2022

The aim of the article is a comparative clinical and economic assessment of genetically engineered monoclonal antibodies against interleukins in infectious diseases facilities in Volgograd region, reassigned to treat COVID-19 patients.

Materials and methods. ABC analysis of the drug consumption in infectious disease facilities in Volgograd region in 2020 and 2021, cost-minimization analysis, and volume of consumption (standard dose per 1000 patients) for genetically engineered monoclonal antibodies against interleukins, were performed on the basis of pharmacies dispensing drug reports on infectious diseases facilities, Russian State Register of maximum selling prices, and Russian guidelines for COVID-19 treatment.

Results. Only a small proportion of COVID-19 patients (43.6 standard doses per 1000 patients in 2020 and 137.8 per 1000 patients in 2021) received genetically engineered biologics in infectious disease facilities in Volgograd Region. Nevertheless, in the studied facilities, medical drug expenses on them exceeded from 20% in 2020 to 40% of the total inventory value in 2021. In mild COVID-19 patients with a high comorbidity index, netaquimab was the least expensive drug therapy and levilimab was the most expensive one. For moderate COVID-19, a standart recommended dose of sarilumab was the least expensive among the drugs used in the studied facilities, and anakinra was the least expensive drug among all the recommended GEBs. In severe and extremely severe COVID-19 courses, tocilizumab and sarilumab were less the least expensive among the GEBs used in the infectious disease facilities, and anakinra was the least expensive among all the recommended GEBs.

Conclusion. Accepting a possible equal effectiveness based on the currently available data, sarilumab is the least expensive for moderate COVID-19 and tocilizumab is the least expensive for severe and extremely severe COVID-19.

Keywords: genetically engineered biologics; interleukin antagonists; COVID-19; ABC-analysis; cost-minimization analysis

Abbreviations: GEB(s) – genetically engineered biologics; IL(s) – interleukins; IgG – immunoglobulin G; MA(s) – monoclonal antibodies; INN – international nonproprietary name; VED – vital and essential medicines; RF – Russian Federation; SD – standard dose; CI – confidence interval; RR – relative risk.

КЛИНИКО-ЭКОНОМИЧЕСКИЙ АНАЛИЗ ПОТРЕБЛЕНИЯ ГЕННО-ИНЖЕНЕРНЫХ БИОЛОГИЧЕСКИХ ПРЕПАРАТОВ ПАЦИЕНТАМИ С COVID-19

В.И. Петров, А.Ю. Рязанова, А.В. Пономарева, О.В. Шаталова, Я.В. Левина

Федеральное государственное бюджетное образовательное учреждение высшего образования
«Волгоградский государственный медицинский университет»
Министерства здравоохранения Российской Федерации
400131, Россия, г. Волгоград, пл. Павших Борцов, 1

E-mail: nastasyakus@mail.ru

Получена 21.02.2022 После рецензирования 15.03.2022 Принята к печати 20.04.2022

For citation: V.I. Petrov, A.Yu. Ryazanova, A.V. Ponomareva, O.V. Shatalova, Ya.V. Levina. Clinical and economic analysis of genetically engineered biologics consumption by patients with COVID-19. *Pharmacy & Pharmacology.* 2022;10(2):198-206. **DOI:** 10.19163/2307-9266-2022-10-2-198-206

Для цитирования: В.И. Петров, А.Ю. Рязанова, А.В. Пономарева, О.В. Шаталова, Я.В. Левина. Клинико-экономический анализ потребления генно-инженерных биологических препаратов пациентами с COVID-19. *Фармация и фармакология.* 2022;10(2):198-206. **DOI:** 10.19163/2307-9266-2022-10-2-198-206

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2022-10-2-198-206

Цель. Сравнительный клинико-экономический анализ применения генно-инженерных биологических препаратов антагонистов интерлейкинов в инфекционных отделениях Волгоградской области, перепрофилированных для лечения пациентов с COVID-19.

Материалы и методы. На основании отчётно-учётной документации по выдаче лекарственных средств в отделениях, Государственного реестра, предельных отпускных цен и временных методических рекомендаций по лечению COVID-19, проведён АВС-анализ расходования лекарственных средств в инфекционных отделениях Волгоградской области в 2020 и 2021 гг., выполнен анализ минимизации затрат и рассчитан объём потребления генно-инженерных биологических препаратов, антагонистов интерлейкинов (стандартная доза на 1000 пациентов).

Результаты. В инфекционных отделениях Волгоградской области только небольшая доля пациентов с COVID-19 (43,6 стандартных доз на 1000 больных 2020 г. и 137,8 на 1000 больных в 2021 г.) получала генно-инженерные биологические препараты. Тем не менее, расходы на них в изучаемых отделениях в 2020 году превысили 20%, а в 2021 г. – 40% от всех расходов на лекарственные средства. При лёгком течении COVID-19 у пациентов с высоким индексом коморбидности наименьшей стоимостью на курс терапии обладал нетакимаб, а наибольшей стоимостью – левилимаб. При среднетяжёлом течении COVID-19 среди препаратов, закупаемых отделениями, наименьшей стоимостью 1 введения обладал сарилумаб, а среди всех рекомендованных препаратов – анакинра. При тяжёлом и крайне тяжёлом течении, наименьшей стоимостью 1 введения обладали тоцилизумаб и сарилумаб, а среди всех рекомендованных препаратов – анакинра.

Заключение. При равной эффективности на основании имеющихся в настоящий момент данных при среднетяжёлом течении COVID-19 экономически наиболее оправдано применение сарилумаба, а при тяжёлом и крайне тяжёлом течении – тоцилизумаба.

Ключевые слова: генно-инженерные биологические препараты; антагонисты интерлейкинов; COVID-19; АВС-анализ; анализ минимизации затрат

Список сокращений: ГИБП – генно-инженерные биологические препараты; ИЛ – интерлейкины; IgG – иммуноглобулин G; МА – моноклональные антитела; ЛС – лекарственные средства; МНН – международное непатентованное название; ЖНВЛС – жизненно необходимые и важнейшие лекарственные средства; РФ – Российская Федерация; СД – стандартная доза; ДИ – доверительный интервал; ОР – относительный риск.

INTRODUCTION

In the winter and spring of 2020, a wave of a rapidly progressing respiratory failure and deaths from a COVID-19 infection spread in many countries. In February 2022, the COVID-19 mortality in the Russian Federation was 2.4%. In the Volgograd region, it achieved 3.7%[1]. The resources of most countries in the world are now oriented to the novel infection`s treatment. Medical costs for COVID-19 therapy, as well as its effectiveness, are also of interest to the healthcare system.

In the first versions of Russian guidelines on the management of COVID-19 patients[2], the main emphasis was made on antiviral and symptomatic therapy. The discovery of the role of the hyperimmune response or cytokine storm as the basis for the pathogenesis of acute respiratory distress syndrome and multiorgan dysfunction in COVID-19 prompted the initiation of tocilizumab as preventive anti-inflammatory therapy. Anti-inflammatory drugs widely used in rheumatology, such as corticosteroids, Janus kinase inhibitors, tocilizumab and other genetically engineered biologics (GEBs), became the basis of pathogenetic therapy of the novel infection in hospitals in many countries in the world and the Russian Federation, as well. In the 14th version of Russian guidelines on the management of COVID-19 patients[3], janus kinase inhibitors and GEBs in combination with corticosteroids are indicated in patients hospitalized both with mild COVID-19 with risk factors of a severe disease, and with a moderate or severe disease. In the latter cases, the prescription of GEBs is preferred.

In the rheumatology practice, the first GEB infliximab, a monoclonal antibody to the tumor necrosis factor-alpha, was approved for a clinical use in 1998. In the 2000s, when infliximab was not effective, monoclonal antibodies (MAs) that block the action of interleukins (IL), such as IL-6, IL-1 and IL-17, began to be used [1, 2].

IL-6 is a multifunctional cytokine produced by various cell types, and it is involved in the paracrine regulation, systemic physiological and pathological processes such as stimulation of immunoglobulin secretion, activation of T cells, and stimulation of the acute phase inflammatory proteins production in the liver and stimulation of hematopoiesis. IL-6 is involved in the pathogenesis of various diseases, playing an important role in the development of the “cytokine storm” in the novel coronavirus infection COVID-19. IL-1β induces gene expression and production of inflammatory mediators such as IL-6 and cyclooxygenase-2. IL-17A, a proinflammatory cytokine, stimulates T-cell immunity and increased the production of inflammatory mediators: IL-1, IL-6, the tumor necrosis factor alpha, and other [3, 4].

The first reports on the successful usage of tocilizumab (MA to IL-6 receptor) in patients with severe COVID-19, were published by Chinese researchers just after the start of the COVID-19 pandemic caused by the SARS-CoV-2 virus [5, 6]. Subsequently, the efficiency of the drug was demonstrated in numerous observational studies conducted in different countries including the Russian Federation [7–9].

[1] Operational data: Coronavirus COVID-19. Official information about coronavirus in Russia at stopcoronavirus.rf. Available from: https://xn--80aesfpebagmfblc0a.xn--p1ai/information/.

[2] Prevention, diagnosis and treatment of novel coronavirus infection (COVID-19). Interim guidelines. Version 4 (27 March 2020). Ministry of Health of the Russian Federation; 2020.

[3] Prevention, diagnosis and treatment of novel coronavirus infection (COVID-19). Interim guidelines. Version 14 (27.12.2021). Ministry of Health of the Russian Federation; 2021.

Currently, in addition to tocilizumab in the 14th version of Russian guidelines on the management patients with COVID-19[4], there were also 6 other anti-inflammatory GEBs including three Russian biosimilars (Table 1), two of which were registered at the outbreak of the COVID-19 pandemic. On February 22, 2022, a new version of Russian guidelines was issued, there the treatment regimens for COVID-19 patients did not include netakimab[5].

THE AIM of the study was to perform a comparative clinical and economic assessment of genetically engineered monoclonal antibodies against interleukins in infectious diseases facilities in Volgograd region, reassigned to treat patients with COVID-19 in 2020–2021.

MATERIALS AND METHODS

For ABC analysis of the drug consumption in infectious disease facilities in Volgograd region, pharmacies dispensing drug reports in 2020 and 2021, were used. According to international nonproprietary names (INNs), all items were ranged in compliance with the expired costs from the highest to the lowest; the costs were calculated for each INN as a percentage of the total inventory value and the cumulative percentage.

Groups of the drugs that account for 80% of the total inventory (segment A), 15% of the total inventory (segment B), and 5% of the total inventory (segment C), were identified and the percentage of consumption within each group was determined.

The recommended doses of GEBs for different COVID-19 severity were calculated according to the 14th version of Russian guidelines on the management patients with COVID-19[6]. The costs of 1 GEB injection or 1 therapy course were calculated according to the pharmacies dispensing drug reports on infectious disease facilities in 2020 and 2021, as well as on the basis of the Russian Register of maximum selling prices[7]. Due to the fact that in the treatment of COVID-19, the studied drugs in most cases are prescribed as a single injection (not daily), and according to the low frequency of these drugs usage in real clinical practice, in order to estimate the volume of consumption, the indicator Standard Dose per 1000 treated patients was calculated. The Standard Dose of 1 administration or course of therapy SD was determined according to the recommended single or course dose for mild and moderate COVID-19. For tocilizumab, 1 SD was 320 mg (16 ml of 20 mg/ml concentrate), olokizumab – 64 mg (1 vial, 160 mg/ml – 0.4 ml), levilimab – 324 mg (2 syringes of 180 mg/ml – 0.9 ml each), sarilumab – 400 mg (2 syringes of 175 mg/ml – 1.14 ml each) and secukinumab – 300 mg (2 syringes of 150 mg/ml – 1 ml each). In terms of US dollars (USD), the exchange rate of 1 ruble = 0.012 USD on February 25, 2022 was used.

RESULTS

In 2020, about 30 million rubles (USD 360 000) were spent on 117 INN drugs in 5 infectious disease facilities, including over 8 million rubles (USD 96 000) of anti-inflammatory therapy (corticosteroids, janus kinase inhibitors and GEBs) (27.0% of the total inventory value). About 7 million rubles (USD 84 000) out of this sum was spent on GEBs (23.4% of the total inventory value). In 2021, about 80 mln rubles (USD 960 000) was spent in 4 infectious disease facilities on 129 INN drugs. Out of this sum, a little over 36 mln rubles (USD 432 000) (45.9% of the total inventory value) was spent for anti-inflammatory therapy with 5 GEBs (32.6 mln rubles; USD 391 200 – 41.5% of the total inventory value).

In 2020, 51.8% of the Segment A value was represented by antibacterials (RUB 12 494 680/ USD 149 936); 26.5% – by antimicrobials (RUB 6 395 410/ USD 76 745); 14.5% – by anticoagulants (RUB 3 494 986/ USD 41 940). In 2021, 49.2% of the Segment A value was represented by antiplatelet agents (RUR 31 325 961/ USD 375 912); 37.2% – by anticoagulants (RUR 23 641 908/ USD 283 703); 13.6% – by antibiotics (RUR 8 653 352/ USD 103 840) (Table 2).

In 2020, according to Russian guidelines, 3 GIBPs out of 5 (possible at the end of 2020) were purchased in the facilities. 77 vials of olokizumab (64 mg, 160 mg/ml – 0.4 ml), 64 concentrates of tocilizumab (400 mg, 20 mg/ml – 20 ml) and 10 packages of levilimab (2 syringes 162 mg, 180 mg/ml – 0.9 ml) were used. In 2021, 4 out of 7 GEBs presented in Russian guidelines, were procured. 413 vials of olokizumab (64 mg, 160 mg/ml – 0.4 ml), 14 concentrates of tocilizumab (400 mg, 20 mg/ml – 20 ml) and 20 (80 mg, 20 mg/ml – 4 ml), 197 packages of levilimab (2 syringes 162 mg, 180 mg/ml – 0.9 ml), 35 packages of sarilumab (2 syringes 200 mg, 175 mg/ml – 1.14 ml), and 10 syringes of secukinumab (150 mg/ml – 1 ml) were used (Fig. 1).

According to Russian guidelines[8], the choice of GEBs and dosing regimen depend on the severity of COVID-19. A cost minimization analysis in mild COVID-19 patients with a high comorbidity index revealed that baricitinib, a Janus kinase inhibitor, an alternative to GEB in this particular group of patients, had the lowest cost per

[4] Ibid.

[5] Prevention, diagnosis and treatment of novel coronavirus infection (COVID-19). Interim guidelines. Version 15 (22.02.2022). Ministry of Health of the Russian Federation.; 2022.

[6] Prevention, diagnosis and treatment of novel coronavirus infection (COVID-19). Interim guidelines. Version 14 (27.12.2021). Ministry of Health of the Russian Federation; 2021.

[7] State Register of Medicines. Available from: https://grls.rosminzdrav.ru/Default.aspx.

[8] Prevention, diagnosis and treatment of novel coronavirus infection (COVID-19). Interim guidelines. Version 14 (27.12.2021). Ministry of Health of the Russian Federation; 2021.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-198-206

course of therapy. Among GEBs in mild COVID-19, the lowest cost per administration was that of netakimab, which was not used in the studied infectious disease facilities. Russian drug levilimab had the highest cost in mild COVID-19. Administration of one standard dose of levilimab in the infectious disease facilities in Volgograd region at the procurement prices, cost more than 57 thousand rubles (684 USD), and in case of ineffectiveness, a patient could need a repeated administration of the drug, which doubled the GEBs cost in this category of patients (Table 3).

For moderate COVID-19, among the drugs procured by the infectious disease facilities, sarilumab had the lowest cost per administration; and among all the recommended GEBs, anakinra had the lowest cost per administration. For severe and extremely severe COVID-19, tocilizumab (the most studied drug for treating COVID-19) and sarilumab had the lowest possible cost per administration among the drugs procured by the infectious disease facilities. Among all the recommended GEBs, anakinra had also the lowest possible cost. According to the maximum prices in the State Register of Medicines (the Register of Vital and Essential Medicines of 2022), the cost of 1 canakinumab injection for severe and extremely severe COVID-19 exceeds 1 million rubles (12 000 USD). The fact makes it impossible to include this medicine in the infectious disease facilities procurement plan because of its extremely high cost.

Table 1 – Anti-inflammatory GEBs recommended for the treatment of COVID-19

INN	Worlds first registration[9] / registration in Russia	Type of monoclonal antibodies	Target	Russian guidelines. Prevention, diagnosis and treatment of novel coronavirus infection (COVID-19)										
				Version 5 08.04.20	Version 6 28.04.20	Version 7 03.06.20	Version 8 03.09.20	Version 9 26.10.20	Version 10 08.02.21	Version 11 07.05.21	Version 12 21.09.21	Version 13 13.10.21	Version 14 27.12.21	Version 15 22.02.22
Tocilizumab	2003/2009	Humanized IgG1	IL-6 soluble and membrane receptors	+	+	+	+	+	+	+	+	+	+	+
Sarylumab	2017/2018	Human IgG1	IL-6 soluble and membrane receptors		+	+	+	+	+	+	+	+	+	+
Olokizumab	No/2020	Humanized IgG4/kappa	Circulating IL-6			+	+	+	+	+	+	+	+	+
Kanakinumab	2009/2012	Human IgG1/kappa	IL-1β (induces IL-6 production)			+	+	+	+	+	+	+	+	+
Levilimab	No/2020	Human IgG1	IL-6 soluble and membrane receptors					+	+	+	+	+	+	+
Netakimab	No/2019	Humanized	IL-17A						+	+	+	+	+	
Anakinra	2001/2021	Recombinant version of the antagonist protein	IL-1α and IL-1β receptors								+	+	+	+
Secukinumab	2015/2016	Human IgG1	IL-17A	Absent Russian guidelines										

[9] DrugBank database. Available from: https://go.drugbank.com/.

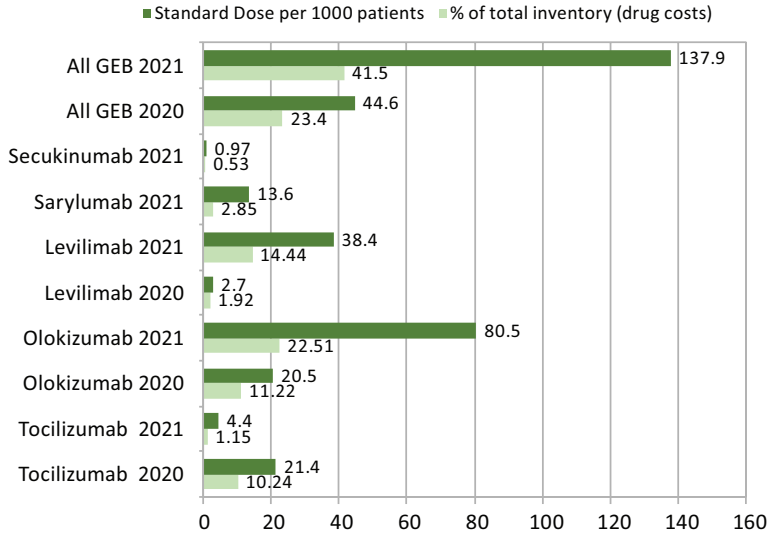

Figure 1 – GEBs consumption in infectious disease facilities in Volgograd region, reassigned to treat COVID-19 patients (2020–2021)

Table 2 – Drug consumption structure in infectious diseases facilities in Volgograd region in 2020-2021

2020 5 infectious diseases facilities 3 750 patients (45 315 days)				2021 4 infectious diseases facilities 5 130 patients (58 439 days)			
INN	Rubles	USD	% per category	INN	Rubles	USD	% per category
Category A 24 089 776 rubles/289 077 USD				**Category A** 63 621 220.88 rubles/763 455 USD			
Meropenem	4 059 700	48 716	16.85%	**Olokizumab**	17 717 700	212612	27.85%
Olokizumab	3 343 260	40 119	13.88%	**Levilimab**	11 364 764	136377	17.86%
Tocilizumab	3 052 150	36 626	12.67%	Sodium Heparin	9 955 524	119 466	15.65%
Cefoperazone Sulbactam	3 049 501	36 594	12.66%	Calcium Nadroparin	6 949 819	83 398	10.92%
Levofloxacin	2 540 382	30 485	10.55%	Enoxaparin	6 736 565	80 839	10.59%
Heparin Sodium	2 538 824	30 466	10.54%	Meropenem	3 600 000	43 200	5.66%
Linezolid	1 592 352	19 108	6.61%	Levofloxacin	3 433 352	41 200	5.40%
Ceftriaxone	1 252 745	15 033	5.20%	**Sarilumab**	2 243 497	26 922	3.53%
Sodium Chloride	1 010 270	12 123	4.19%				
Enoxaparin	956 162	11 474	3.97%	Cefoperazone Sulbactam	1 620 000	19 440	2.55%
Dexamethasone	694 431	8 333	2.88%				
Category B 4 327 479.67 rubles/USD 51 930				**Category B** 11 199 427.98 rubles/USD 134 393			
Interferon Beta-1b	618 984	7 428	14.30%	Dexamethasone	1 542 966	18 516	13.78%
Favipiravir	581 350	6 976	13.43%	Sodium Chloride	1 318 901	15 827	11.78%
Levilimab	571 664	6 860	13.21%	Baricitinib	1 122 851	13 474	10.03%
Azithromycin	527 163	6 326	12.18%	Ceftriaxone	1 121 652	13 460	10.02%
Lopinavir Ritonavir	345 789	4 149	7.99%	**Tocilizumab**	901 725	10 821	8.05%
Propofol	292 905	3 515	6.77%	Remdesivir	895 400	10 745	8.00%
Baricitinib	243 296	2 920	5.62%	Surfactant	696 160	8 354	6.22%
Amoxicillin Clavulanate	177 126	2 126	4.09%	Favipiravir	672 546	8 071	6.01%
Omeprazole	163 793	1 966	3.78%	Omeprazole	669 779	8 037	5.98%
Clopidogrel	163 269	1 959	3.77%	Azithromycin	500 324	6 004	4.47%
Umifenovir	139 440	1 673	3.22%	Propofol	492 577	5 911	4.40%
Methylprednisolone	133 102	1 597	3.08%	Prednisolone	434 442	5 213	3.88%
Vancomycin	129 092	1 549	2.98%	**Secukinumab**	419 400	5 033	3.74%
Insulin	128 818	1 546	2.98%	Methylprednisolone	410 706	4 928	3.67%
Ambroxol	111 689	1 340	2.58%				
Category C 1 383 459.64 rubles/USD16 602 91 INNs				**Category C** 3 893 959.03 rubles/USD 46 728 106 INNs			

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-198-206

Table 3 – GEBs cost minimization analysis according to COVID-19 severity

INN	Drug formulation and Strengths	Recommended dosage	Repeated dose in case of insufficient effect	Costs per administration or per course (7 or 14 days)			
				Procurement in infectious diseases facilities		List of Vital and Essential Drugs[10] 2022	
				Rubles	USD	Rubles	USD
Mild COVID-19 (patients with high comorbidity index)							
Netakimab*	Syringe 60 mg/1 ml	2 syringes				18 181.82	218
Levilimab	Syringe 162 mg (180 mg/ml – 0.9 ml)	2 syringes	Every 24 hours	57 166.38 (2020) 57 689.16 (2021)	686 692	47 531.10	570
Olokizumab	Vial 64 mg (160 mg/ml, 0.4 ml)	1 vial	Every 24 hours	43 418.96 (2020) 42 900 (2021)	521 515	39 000.00	468
Baricitinib	Tablets 4 mg (packages of 14, 28, 56 tablets)	4 mg/day for 7–14 days		12 164.8–24 329.6 (2020) 12 204.9 – 24 409.8 (2021)	146–292 146–293	10 064.6 – 20 129.20	121–242
Tofacitinib	Tablets 5 and 10 mg (package of 56 tablets)	10 mg twice a day for 7–14 days				20 129.46 – 40 258.92	242–483
Moderate COVID-19							
Levilimab	Syringe 162 mg (180 mg/ml – 0.9 ml)	2 syringes	Every 24 hours	57 166.38 (2020) 57 689.16 (2021)	686 692	47 531.10	570
Olokizumab	Vial 64 mg (160 mg/ml, 0.4 ml)	1–2 vials	Every 12 hours	43 418.96–86 937.92 (2020) 42 900–85 800 (2021)	521–1,043 515–1,030	39 000.00–78 000.00	468–936
Tocilizumab	Syringe 162 mg/0.9 ml (4 pc.)	4 mg/kg 320 mg for 80 kg 2 syringes or 16 ml				26 526.75	318
	Concentrate 20 mg/ml, 2 ml			38 151.88 (2020) 39 298.56 (2021)	458	28 139.984	338
	Concentrate 20 mg/ml, 4 ml			42 800 (2021)	472–514	29 291.08	351
	Concentrate 20 mg/ml, 10 ml					29 291.088	351
Sarylumab	Syringe 200 mg (175 mg/ml, 1.14 ml)	1 syringe	Every 12 hours	32 049.96 (2021)	385	26 493.16	318
Anakinra	Syringe 100 mg (150 mg/ml, 0.67 ml)	100 mg/day subcutaneously for 7 days				14 867.42	178
Severe and extremely severe COVID-19 (pneumonia with respiratory failure, ARDS)							
Tocilizumab	Syringe 162 mg/0.9 ml (4 pc.)	4–8 mg/kg 320–640 mg at a weight of 80 kg 2–4 syringes or 16–32 ml	Every 12 hours			26 526.75–53 053.5	318–637
	Concentrate 20 mg/ml, 2 ml			38 151.88–76 303.75 (2020) 39 298.56–78 597.12 (2021)	458–916 472–943	28 139.984–56 279 968	338–675
	Concentrate 20 mg/ml, 4 ml			42 800–85 600 (2021)	514–1,027	29 291.08–58 582 16	351–703
	Concentrate 20 mg/ml, 10 ml					29 291.088–58 582 176	351–703
Sarylumab	Syringe 200 mg (175 mg/ml, 1.14 ml)	2 syringes	Every 12 hours	64 099.92 (2021)	769	52 986.32	636
Kanakinumab	Vial 150 mg/ml, 1 ml	4–8 mg/kg 2–4 vials at a weight of about 80 kg				1 061 845.34 – 2 123 690.68	12 742–25 484
Anakinra	Syringe 100 mg (150 mg/ml, 0.67 ml)	200 to 400 mg/day subcutaneously For 7 days				29 734.84–59 469.68	357–714
Levilimab	Syringe 162 mg (180 mg/ml – 0.9 ml)	4 syringes	Every 12 hours	114 332.76 (2020) 115 378.32 (2021)	1,372 1,385	95 062.2	1 141
Olokizumab	Vial 64 mg (160 mg/ml, 0.4 ml)	4 syringes		173 875.84 (2020) 171 600 (2021)	2,087 2,059	156 000	1 872
Beyond Guidelines							
Secukinumab	Syringe 150 mg (150 mg/ml, 1 ml)	2 syringes**		83 880 (2021)	1,007	69 097.32	829

Note: * – not present in version 15 of Russian guidelines; ** – the dose used in severe COVID-19 in the study by Hasan M.J. et al. [10].

[10] State register of maximum selling prices. Available from: https://grls.rosminzdrav.ru/PriceLims.aspx.

DISCUSSION

GEBs are now quite widely used to treat various diseases associated with immune inflammation, both in rheumatology, and in gastroenterology, dermatology, pulmonology. GEB therapy of rheumatological patients, patients with severe forms of psoriasis, Crohn's disease, nonspecific ulcerative colitis, bronchial asthma favorably affects the disease prognosis, leads to improved quality of life and achievement of persistent remission [11–16]. More often, GEBs are used as second-line drugs in case of ineffectiveness or poor tolerability of standard baseline anti-inflammatory drugs. The main obstacle to the prescription of GEBs is their high cost, which leads to increased treatment costs and an economic burden on the healthcare system. Nevertheless, in the Russian Federation, a model of clinical and statistical groups has been developed at the federal level, which allows providing the patients requiring GEBs prescription, at the expense of the OMI system [17].

The use of anti-inflammatory GEBs in COVID-19 patients under current conditions of the novel coronavirus infection and the limited evidence base for the treatment of COVID-19 is «off-label» and is based on the international guidelines[9] and consensual expert opinions based on the assessment of the benefit and risk degree in the "off-label" use[10]. In the actual clinical practice, only a small proportion of COVID-19 patients fewer than 4.2% in 2020 and fewer than 13.8% in 2021) in the studied infectious disease facilities received GEBs. Nevertheless, the consumption of these drugs in 2020 exceeded 20%, and in 2021 – 40% of the-total inventory value of the drugs in the OMI system in the studied facilities.

In most cases, the reason for the use of GEBs in COVID-19 patients was fever that did not resolve with the use of systemic corticosteroids (if the drug was available in the facilities), appropriate changes in the inflammatory markers and the absence of contraindications. For two years of the pandemic, more and more publications have become available to evaluate the role of different drugs, including GEBs.

At the beginning of the pandemic, observational studies revealed an association between elevated levels of IL-6 in the severe COVID-19 and the patient mortality [18–20]. A number of the subsequent uncontrolled clinical trials have found a reduction in the disease severity and inflammatory markers after the administration of tocilizumab (MA to IL-6 receptor) [6, 21, 22]. Two major studies, RECOVERY and REMAP-CAP, have demonstrated a significant reduction in mortality after the administration of MA to IL-6 receptor [8, 23].

The RECOVERY study [8] included hospitalized COVID-19 patients who required oxygen support and had C-reactive protein levels ≥ 75 mg/l. The 28-day mortality rate was 31% (621/2022) in the tocilizumab group and 35% (729/2 094) in the standard therapy group (OR 0.85; 95% CI 0.76–0.94; p=0.028). However, this trend was observed only in patients receiving tocilizumab in combination with dexamethasone. The REMAP-CAP study [23] enrolled 2 274 COVID-19 patients requiring a respiratory support (high-flow oxygen therapy, noninvasive or invasive pulmonary ventilation) at the time of inclusion in the study. 972 of these received 1–2 doses of tocilizumab (MA to IL-6 receptor), 485 received sarilumab (MA to IL-6 receptor), 378 received anakinra (MA to IL-1 receptor), and other 418 were included in the control group. In-hospital, survival rates were 66.4% for tocilizumab; 67.3% for sarilumab; 60.3% for anakinra; and 63.1% for controls. Compared with the controls, the mean adjusted odds ratios for the in-hospital survival were 1.42 (95% CI 1.05–1.93) for tocilizumab, 1.51 (95% CI 1.06, 2.20) for sarilumab, and 0.97 (95% CI 0.66, 1.40) for anakinra. Thus, tocilizumab and sarilumab showed a comparable effect in reducing COVID-19 patients' mortality requiring a respiratory support, whereas anakinra showed no positive effect on the severity of COVID-19.

A single-center observational retrospective comparative study of two Russian drugs and tocilizumab in patients with severe COVID-19 was conducted in the Russian Federation [24]. The study included 200 patients with a single administration of tocilizumab (MA to IL-6 receptor), 100 patients who received levilimab (MA to IL-6 receptor) and 100 patients who received olokizumab (MA to IL-6). A comparative analysis of clinical outcomes between the groups revealed a statistically insignificant increase in the risk of sepsis and death in the levilimab group compared to the tocilizumab and olokizumab groups.

The study by Hasan M.J. et al. [10] compared the effectiveness of 300 mg secukinumab (MA to IL 17A) added to baricitinib in the severe course of COVID-19 in 17 patients, compared to the control group that received only baricitinib. The secukinumab+baricitinib group showed a statistically significant reduction in the need for invasive pulmonary ventilation, a shorter ICU stay, and a lower 30-day mortality rate with a greater risk of secondary infections compared to the baricitinib group.

Pavlov R.E. et al. [25] described the experience of using netakimab with corticosteroids in outpatient settings. Netakimab (MA to IL 17A), a Russian drug, a biosimilar of secukinumab, is used for treatment of severe psoriasis forms. The authors conducted a retrospective analysis of treatment of 12 patients with severe COVID-19 who received therapy with netakimab (the first injection of 60–120 mg subcutaneously and, if indicated, the second injection of 60 mg) plus betamethasone dipropionate/betamezon phosphate at the dose of 2 ml (an officinal solution) intramuscularly. The treatment started on the 7th day from the onset of the disease. A repeated administration of netakimab was performed in older patients

[11] COVID-19: clinical guidelines. Available from: https://www.ersnet.org/covid-19/covid-19-guidelines-and-recommendations-directory/.

[12] Prevention, diagnosis and treatment of novel coronavirus infection (COVID-19). Interim guidelines. Version 14 (27.12.2021). Ministry of Health of the Russian Federation; 2021.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-198-206

due to the insufficient effect in controlling hyperthermia and/or hypoxemia. A simultaneous administration of netakimab and corticosteroids resulted in reducing hyperthermia and/or increased oxygen saturation 2.5 days after the first injection, decreased levels of inflammatory markers, positive dynamics according to the lung computed tomography data. An increased respiratory support (transfer to the artificial lung ventilation) or a change of antibiotic therapy, as well as hospitalization were not required in any case.

Thus, based on the available data, we can conclude that tocilizumab, sarilumab, and probably olokizumab have a comparable efficacy in COVID-19 patients requiring a respiratory support. The extremely high cost of canakinumab raises the question whether it is reasonable to study the effectiveness of this drug in COVID-19 patients. The data obtained on the use of anakinra suggest a lack of efficacy. The efficacy of netakinumab and secukinumab, as well as levilimab, requires a further study.

CONCLUSION

Under conditions of possible equal effectiveness based on the currently available data, the use of sarilumab is the least expensive in moderate COVID-19, and tocilizumab – in severe and extremely severe COVID-19. Among Russian GEBs, the use of olokizumab, compared to levilimab, is the least expensive with possibly higher efficacy and safety. However, the efficacy and safety of anti-inflammatory GEBs in patients with COVID-19, mild COVID-19, requires a further study.

FUNDING

This study had no financial support from outside organizations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Vladimir I. Petrov – development of research design, article editing and its final approval;
Anastasiya Yu. Ryazanova – material collection, data processing, article writing and its final approval;
Angelika V. Ponomareva – article editing, planning and development of research design and its final approval;
Olga V. Shatalova – article editing, planning and development of research design and its final approval;
Ya.V. Levina – article editing, data processing and article final approval.

REFERENCES

1. Melsheimer R, Geldhof A, Apaolaza I, Schaible T. Remicade® (infliximab): 20 years of contributions to science and medicine. Biologics. 2019 Jul 30;13:139–78. DOI: 10.2147/BTT.S207246.
2. Choy EH, Isenberg DA, Garrood T, Farrow S, Ioannou Y, Bird H, Cheung N, Williams B, Hazleman B, Price R, Yoshizaki K, Nishimoto N, Kishimoto T, Panayi GS. Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis: a randomized, double-blind, placebo-controlled, dose-escalation trial. Arthritis Rheum. 2002 Dec;46(12):3143–50. DOI: 10.1002/art.10623.
3. Nasonov EL, Eliseev MS The role of interleukin 1 in the development of human diseases. Scientific-Practical Rheumatology. 2016;54(1): 60–77. DOI: 10.14412/1995-4484-2016-60-77. Russian
4. Mihara M, Hashizume M, Yoshida H, Suzuki M, Shiina M. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. Clin Sci (Lond). 2012 Feb;122(4):143–59. DOI: 10.1042/CS20110340.
5. Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. Tocilizumab treatment in COVID-19: a single center experience. J Med Virol. 2020;92(7):814–8. DOI: 10.1002/jmv.25801.
6. Xu X, Han M, Li T, Sun W, Wang D, Fu B, Zhou Y, Zheng X, Yang Y, Li X, Zhang X, Pan A, Wei H. Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci U S A. 2020 May 19;117(20):10970–5. DOI: 10.1073/pnas.2005615117.
7. Fomina DS, Lysenko MA, Beloglazova IP, Mutovana ZYu, Poteshkina NG, Samsonova IV, Kruglova TS, Chernov AA, Karaulov AV, Lederman MM. Temporal clinical and laboratory response to interleukin-6 receptor blockade with tocilizumab in 89 hospitalized patients with COVID-19 pneumonia. Pathog Immun. 2020;5(1):327–41. DOI: 10.20411/pai.v5i1.392
8. RECOVERY Collaborative Group. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet. 2021;397(10285):1637–45. DOI: 10.1016/s0140-6736(21)00676-0.
9. Moiseev SV, Avdeev EA Tao EA, Brovko MA, Yavorovsky AG, Umbetova KT, Bulanov NM, Zykova AS, Akulkina LA, Smirnova IG, Fomin VV, Efficiency of tocilizumab in patients with COVID-19 hospitalized in the ORIT: a retrospective cohort study. Clinical Pharmacology and Therapy. 2020.29(4):17–25. DOI: 10.32756/0869-5490-2020-4-17-25. Russian
10. Hasan MJ, Rabbani R, Anam AM, Huq SMR. Secukinumab in severe COVID-19 pneumonia: Does it have a clinical impact? J Infect. 2021 Jul;83(1):e11–e13. DOI: 10.1016/j.jinf.2021.05.011.
11. Rubbert-Roth A, Enejosa J, Pangan AL, Haraoui B, Rischmueller M, Khan N, Zhang Y, Martin N, Xavier RM. Trial of Upadacitinib or Abatacept in Rheumatoid Arthritis. N Engl J Med. 2020 Oct 15;383(16):1511-21. DOI: 10.1056/NEJMoa2008250.
12. Scott LJ. Tocilizumab: A Review in Rheumatoid Arthritis. Drugs. 2017 Nov;77(17):1865-1879. DOI: 10.1007/s40265-017-0829-7.
13. Van den Bosch F, Coates L. Clinical management of psoriatic arthritis. Lancet. 2018 Jun 2;391(10136):2285–94. DOI: 10.1016/S0140-6736(18)30949-8.
14. D'Amico F, Parigi TL, Bonovas S, Peyrin-Biroulet L, Danese S. Long-term safety of approved biologics for ulcerative colitis. Expert Opin Drug Saf. 2020 Jul;19(7):807–16. DOI: 10.1080/14740338.2020.1773430.
15. Honap S, Cunningham G, Tamilarasan AG, Irving PM.

Positioning biologics and new therapies in the management of inflammatory bowel disease. Curr Opin Gastroenterol. 2019 Jul;35(4):296–301. DOI: 10.1097/MOG.0000000000000546.

16. McGregor MC, Krings JG, Nair P, Castro M. Role of Biologics in Asthma. Am J Respir Crit Care Med. 2019 Feb 15;199(4):433–45. DOI: 10.1164/rccm.201810-1944CI.
17. Sura MV, Ignatova TN, Rudneva NS, Sukhanova YN, Ulyanova NG, Khudyaev AS, Shelyakin VA, Shcherbakov DV. Regional adaptation of the federal model of payment for medical care by clinical and statistical groups on the example of hospitalization cases of patients requiring prescription of genetically engineered biological drugs. Pharmacoeconomics. Modern Pharmacoecon & Pharmacoepidem. 2018;11(2):44–52. DOI: 10.17749/2070-4909.2018.11.2.044-052. Russian
18. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, Lavin Y, Swartz TH, Madduri D, Stock A, Marron TU, Xie H, Patel M, Tuballes K, Van Oekelen O, Rahman A, Kovatch P, Aberg JA, Schadt E, Jagannath S, Mazumdar M, Charney AW, Firpo-Betancourt A, Mendu DR, Jhang J, Reich D, Sigel K, Cordon-Cardo C, Feldmann M, Parekh S, Merad M, Gnjatic S. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med. 2020 Oct;26(10):1636–43. DOI: 10.1038/s41591-020-1051-9.
19. McElvaney OJ, McEvoy NL, McElvaney OF, Carroll TP, Murphy MP, Dunlea DM, Ní Choileáin O, Clarke J, O'Connor E, Hogan G, Ryan D, Sulaiman I, Gunaratnam C, Branagan P, O'Brien ME, Morgan RK, Costello RW, Hurley K, Walsh S, de Barra E, McNally C, McConkey S, Boland F, Galvin S, Kiernan F, O'Rourke J, Dwyer R, Power M, Geoghegan P, Larkin C, O'Leary RA, Freeman J, Gaffney A, Marsh B, Curley GF, McElvaney NG. Characterization of the Inflammatory Response to Severe COVID-19 Illness. Am J Respir Crit Care Med. 2020 Sep 15;202(6):812–21. DOI: 10.1164/rccm.202005-1583OC.
20. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, Wang T, Zhang X, Chen H, Yu H, Zhang X, Zhang M, Wu S, Song J, Chen T, Han M, Li S, Luo X, Zhao J, Ning Q. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest. 2020 May 1;130(5):2620–9. DOI: 10.1172/JCI137244.
21. Toniati P, Piva S, Cattalini M, Garrafa E, Regola F, Castelli F, Franceschini F, Airò P, Bazzani C, Beindorf EA, Berlendis M, Bezzi M, Bossini N, Castellano M, Cattaneo S, Cavazzana I, Contessi GB, Crippa M, Delbarba A, De Peri E, Faletti A, Filippini M, Filippini M, Frassi M, Gaggiotti M, Gorla R, Lanspa M, Lorenzotti S, Marino R, Maroldi R, Metra M, Matteelli A, Modina D, Moioli G, Montani G, Muiesan ML, Odolini S, Peli E, Pesenti S, Pezzoli MC, Pirola I, Pozzi A, Proto A, Rasulo FA, Renisi G, Ricci C, Rizzoni D, Romanelli G, Rossi M, Salvetti M, Scolari F, Signorini L, Taglietti M, Tomasoni G, Tomasoni LR, Turla F, Valsecchi A, Zani D, Zuccalà F, Zunica F, Focà E, Andreoli L, Latronico N. Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: A single center study of 100 patients in Brescia, Italy. Autoimmun Rev. 2020 Jul;19(7):102568. DOI: 10.1016/j.autrev.2020.102568.
22. Ramiro S, Mostard RLM, Magro-Checa C, van Dongen CMP, Dormans T, Buijs J, Gronenschild M, de Kruif MD, van Haren EHJ, van Kraaij T, Leers MPG, Peeters R, Wong DR, Landewé RBM. Historically controlled comparison of glucocorticoids with or without tocilizumab versus supportive care only in patients with COVID-19-associated cytokine storm syndrome: results of the CHIC study. Ann Rheum Dis. 2020 Sep;79(9):1143–51. DOI: 10.1136/annrheumdis-2020-218479.
23. Angus DC, Berry S, Lewis RJ, Al-Beidh F, Arabi Y, van Bentum-Puijk W, Bhimani Z, Bonten M, Broglio K, Brunkhorst F, Cheng AC, Chiche JD, De Jong M, Detry M, Goossens H, Gordon A, Green C, Higgins AM, Hullegie SJ, Kruger P, Lamontagne F, Litton E, Marshall J, McGlothlin A, McGuinness S, Mouncey P, Murthy S, Nichol A, O'Neill GK, Parke R, Parker J, Rohde G, Rowan K, Turner A, Young P, Derde L, McArthur C, Webb SA. The REMAP-CAP (Randomized Embedded Multifactorial Adaptive Platform for Community-acquired Pneumonia) Study. Rationale and Design. Ann Am Thorac Soc. 2020 Jul;17(7):879–91. DOI: 10.1513/AnnalsATS.202003-192SD.
24. Bobkova SS, Zhukov AA, Protsenko DN, Samoilenko VV, Tyurin IN. Comparative efficacy and safety of monoclonal antibody preparations to IL-6 in patients with new severe COVID-19 coronavirus infection. A retrospective cohort study. Annals of Critical Care. 2021;1:69–76. DOI: 10.21320/1818-474X-2021-1-69-76. Russian
25. Pavlov R.E., Tsarenko S.V., Sekinaeva A.V., Rybin PN, Nagibina MV, Soshnikova EV, Klimova MS. Experience of using glucocorticoids and netakimab in the treatment of patients with COVID-19 in outpatient. Clinical Medicine. 2020. 98(6): 449–55. DOI:10.30629/0023-2149-2020-98-6-449-455. Russian

AUTHORS

Vladimir I. Petrov – Doctor of Sciences (Medicine), Professor, Academician of RAS, Head of the Department of Clinical Pharmacology and Intensive Care of Volgograd State Medical University, chief freelance specialist, clinical pharmacologist of the Ministry of Healthcare of the Russian Federation, Honored Scientist of the Russian Federation, Honored Doctor of the Russian Federation. ORCID ID: 0000-0002-0258-4092. E-mail: brain@sprintnet.ru

Anastasiya Yu. Ryazanova – Candidate of Sciences (Medicine), Associate Professor at the Department of Clinical Pharmacology and Intensive Care, Volgograd State Medical University. ORCID ID: 0000-0002-4778-5015. E-mail: nastasyakus@mail.ru

Angelika V. Ponomareva – Candidate of Sciences (Medicine), Professor at the Department of Clinical Pharmacology and Intensive Care, Volgograd State Medical University. ORCID ID: 0000-0002-8237-8335. E-mail: angelvr@yandex.ru

Olga V. Shatalova – Doctor of Sciences (Medicine), Professor at the Department of Clinical Pharmacology and Intensive Care, Volgograd State Medical University. ORCID ID: 0000-0002-7311-4549. E-mail: shov_med@mail.ru

Yana V. Levina – Candidate of Sciences (Medicine), Associate Professor at the Department of Clinical Pharmacology and Intensive Care, Volgograd State Medical University. ORCID ID: 0000-0003-2019-2444. E-mail: yanalevin@mail.ru

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2022-10-2-207-216

EVALUATION OF ANTIOXIDANT ACTIVITY LEVEL OF *ACTINIDIA ARGUTA* (SIEBOLD ET ZUCC.) PLANCH. EX MIQ. PLANT RAW MATERIAL, GROWN IN THE CAUCASIAN MINERAL WATERS REGION

D.I. Pozdnyakov, S.L. Adzhiakhmetova, N.N. Vdovenko-Martynova

Pyatigorsk Medical and Pharmaceutical Institute –
a branch of Volgograd State Medical University
11, Kalinin Ave., Pyatigorsk, Russia, 357532

E-mail: pozdniackow.dmitry@yandex.ru

Received 15 Oct 2021 After peer review 24 Jan 2022 Accepted 10 March 2022

The aim of the study is the identification and evaluation of a new antioxidant activity in a potentially new medicinal raw material of *Actinidia arguta folia*.

Materials and methods. The total content of antioxidants was measured on a Tsvet Yauza-01-AA liquid chromatograph using the amperometric method. In parallel, the antioxidant activity of *Actinidia arguta* extracts was studied *in vitro* in the following dilution range: 62.5 μg/ml, 125 μg/ml, 250 μg/ml, 500 μg/ml and 1000 μg/ml. Herewith, DPPH, superoxide, and hydroxyl radical inhibitory properties of the analyzed samples were evaluated.

The studies of the antioxidant activity with the determination of the activity of superoxide dismutase, glutathione peroxidase, catalase, the concentration of malondialdehyde and diene conjugates, have been conducted *in vivo*.

Results. When studying the antiradical activity (*in vitro* tests), it was found out that the highest radical-inhibiting activity comparable to the individual compound - quercetin, has the extraction from *Actinidia arguta folia*, obtained by the extraction with 40% ethyl alcohol. The IC_{50} value for the given extract in relation to DPPH; superoxide and hydroxyl radical, amounted to 537.6±23.924 μg/ml; 26.6±2.627 μg/ml and 72.6±3.264 μg/ml, respectively, which may indicate that this extract has reducing and radical scavenging properties. In parallel, the study of the total content of antioxidants in terms of quercetin and gallic acid has been carried out. It has also been found out that in the *Actinidia arguta folia* extract, obtained by the extraction with 40% ethyl alcohol, the content of the antioxidants is maximum.

Conclusion. The data obtained using the *in vitro* test were confirmed in the *in vivo* study, in which the course application of the *Actinidia arguta folia* extract, obtained by the extraction with 40% ethyl alcohol to the degree comparable to quercetin, contributed to an increase in the superoxide dismutase activity, a decrease in the lipid peroxidation products. The maximum content of antioxidants for *Actinidia arguta folia* was 0.73±0.007 and 0.47±0.005 mg/g in terms of quercetin and gallic acid, respectively. The extractant was 40% ethyl alcohol.

Keywords: *Actinidia arguta;* flavonoids; antioxidant activity; medicinal plant materials; ethnomedicine

Abbreviations: DPPH- 2,2-diphenyl-1-picrylhydrazyl; EDTA – ethylenediaminetetraacetic acid; DC – diene conjugates; TBA-AP – active products of thiobarbituric acid; ROS – reactive oxygen species; SOD – superoxide dismutase; GP – glutathione peroxidase; NADPH – reduced nicotinamide adenine dinucleotide phosphate; ERK – extracellular regulated kinase; MAPK – mitogen-activated protein kinase.

For citation: D.I. Pozdnyakov, S.L. Adzhiakhmetova, N.N. Vdovenko-Martynova. Evaluation of antioxidant activity level of *Actinidia Arguta* (Siebold et Zucc.) Planch. ex Miq. plant raw material, grown in the Caucasian Mineral Waters region. *Pharmacy & Pharmacology.* 2022;10(2):207-216. **DOI:** 10.19163/2307-9266-2022-10-2-207-216

Для цитирования: Д.И. Поздняков, С.Л. Аджиахметова, Н.Н. Вдовенко-Мартынова. Оценка уровня антиоксидантной активности растительного сырья *Actinidia Arguta* (Siebold et Zucc.) Planch. ex Miq., выращиваемой в регионе Кавказских Минеральных Вод. *Фармация и фармакология.* 2022;10(2):207-216. **DOI:** 10.19163/2307-9266-2022-10-2-207-216

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

ОЦЕНКА УРОВНЯ АНТИОКСИДАНТНОЙ АКТИВНОСТИ РАСТИТЕЛЬНОГО СЫРЬЯ ACTINIDIA ARGUTA (SIEBOLD ET ZUCC.) PLANCH. EX MIQ., ВЫРАЩИВАЕМОЙ В РЕГИОНЕ КАВКАЗСКИХ МИНЕРАЛЬНЫХ ВОД

Д.И. Поздняков, С.Л. Аджиахметова, Н.Н. Вдовенко-Мартынова

Пятигорский медико-фармацевтический институт – филиал федерального государственного бюджетного образовательного учреждения высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации
357532, Россия, г. Пятигорск, пр-кт Калинина, д. 11

E-mail: pozdniackow.dmitry@yandex.ru

Получена 15.10.2021 После рецензирования 24.01.2022 Принята к печати 10.03.2022

Цель. Выявление и оценка антиоксидантной активности нового лекарственного сырья – актинидии аргута листьев (*Actinidia arguta folia*).

Материалы и методы. Суммарное содержание антиоксидантов проводили на жидкостном хроматографе «Цвет Яуза-01-АА» амперометрическим методом. Параллельно изучали *in vitro* антиоксидантную активность извлечений *Actinidia argut*a в следующем диапазоне разведений: 62,5 мкг/мл, 125 мкг/мл, 250 мкг/мл, 500 мкг/мл и 1000 мкг/мл. При этом оценивались DPPH, супероксид и гидроксил-радикал ингибирующие свойства анализируемых образцов. Были проведены исследования *in vivo* антиоксидантной активности с определением активности супероксиддисмутазы, глутатионпероксидазы, каталазы, концентрации малонового диальдегида и диеновых конъюгатов.

Результаты. При изучении антирадикальной активности (*in vitro* тесты) установлено, что наиболее высокой радикал-ингибирующей активностью сопоставимой с индивидуальным соединением – кверцетином, обладает извлечение из актинидии аргута листьев, полученные экстракцией спиртом этиловым 40%. Величина IC_{50} для данного извлечения в отношении DPPH; супероксид и гидроксил-радикала составила 537,6±23,924 мкг/мл; 26,6±2,627 мкг/мл и 72,6±3,264 мкг/мл соответственно, что может свидетельствовать о наличии у данного извлечения восстановительных и радикал-скэвенджерных свойств. Параллельно проводилось изучение суммарного содержание антиоксидантов в пересчете на кверцетин и галловую кислоту. Также было обнаружено в извлечении актинидии аргута листьев, полученном экстракцией спиртом этиловым 40%, содержание антиоксидантов максимально.

Заключение. Данные, полученные с помощью испытания *in vitro*, были подтверждены в исследовании *in vivo*, в котором курсовое применение извлечения актинидии листьев, полученного экстракцией спиртом этиловым 40% в сопоставимой с кверцетином степени, способствовало увеличению активности супероксиддисмутазы, снижению продуктов липопероксидации. Максимальное содержание антиоксидантов для актинидии аргута листьев составило 0,73±0,007 и 0,47±0,005 мг/г в пересчете на кверцетин и галловую кислоту соответственно. Экстрагент – спирт этиловый 40%.

Ключевые слова: актинидии аргута; флавоноиды; антиоксидантная активность; лекарственное растительное сырье; этномедицина

Список сокращений: DPPH- 2,2-дифенил-1-пикрилгидразил; ЭДТА – этилендиаминтетрауксусная кислота; ДК – диеновые конъюгаты; ТБК-АП – активные продукты тиобарбитуровой кислоты; АФК – активные формы кислорода; СОД – супероксиддисмутаза; ГП – глутатионпероксидаза; НАДФН – никотинамидадениндинуклеотидфосфат восстановленный; ERK – внеклеточная регулируемая киназа; MAPK – митоген-активируемая протеинкиназа.

INTRODUCTION

The genus *Actinidia* Lindl. includes about thirty species, the places of natural growth of which are Central and East Asia, the island of Java. In Russia, these are relict plants of the Far East (species: *Actinidia giraldi* Diels; *Actinidia kolomikta* (Maxim.) Maxim; *Actinidia argut*a (Siebold et Zucc.) Planch. ex Miq.; *Actinidia polygama* (Siebold et Zucc.) Maxim. Breeding work with the Far Eastern actinidia was started in 1906 by I.V. Michurin, who created a selection fund of domestic actinidia [1].

One of the promising species is *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq., as it tolerates temperature changes well and is resistant to low temperatures (in the winter period), and it has a high yield. The following varieties were bred by Michurin I.V. from the samples of this species – “Early” and “Yeilding” [2]. At present, the growth of *Actinidia arguta* has spread far enough beyond its habitat, and every year it is more and more interesting as a promising species for horticulture and medicine [4]. Fruits are evaluated for the content of ascorbic acid

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-207-216

[3, 8], as well as due to the high content of biologically active substances (BASs), which have antioxidant, adaptogenic and immunomodulatory properties [5–7]. *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq. is a well-known Japanese plant called sarunushi. In its fruits, the content of biologically active substances such as catechins, ascorbic acid, anthocyanins, beta-carotenes and other polyphenols, which are well preserved in processed products, has been established. The studies by Japanese scientists show that *A. arguta* juice components are promising for a potential use as chemopreventive agents [8, 9]. There are studies that the genus *Actinidia* Lindl. species can be a potential source of natural antioxidants [10].

At present, *Actinidia* arguta (Siebold et Zucc.) Planch. ex Miq., successfully grown under the conditions of the Caucasian Mineral Waters, is of interest for scientific research. Over the past ten years, the specimens growing under the climatic conditions of Pyatigorsk, have been monitored. Their unpretentiousness in care, frost resistance, annual fruiting and good yields should be notified.

By a screening phytochemical analysis, the content of flavonoids and tannins, the presence of which predicts a possible antioxidant activity, has been established in the *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq. *folia* [11].

THE AIM of the study is the identification and evaluation of a new antioxidant activity in a potentially new medicinal raw material of *Actinidia arguta folia*.

MATERIALS AND METHODS

Object of study

The object of the study is *Actinidia arguta folia* harvested in the fruiting phase (autumn) of the producing plant – *Actinidia arguta* of the *Actinidiaceae* family, from the specimens grown under the climatic conditions of the city of Pyatigorsk, the Stavropol Territory, in the open field near Novopyatigorsk-Skachki, the coordinatesare are: 44°01’07” N and 43°03’12” E, 545 m above the sea level. The identification of the raw materials was carried out by Vdovenko-Martynova N.N., an Associate Professor of the Department of Pharmacognosy, Botany and Technology of Phytopreparations of the Pyatigorsk Medical and Pharmaceutical Institute – a branch of the Volgograd State Medical University. *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq. is a dioecious perennial twining vine up to 15 meters high with a stem, which eventually becomes woody. The leaves are large, up to twelve centimeters long, green, turn bright yellow in autumn and fall off in November. It bears fruits annually. The fruits are berries up to 3 cm long, juicy, sweet and sour, their taste reminds that of kiwi.

Obtaining extracts and determining the total content of antioxidants in terms of quercetin and gallic acid

The extracts for the research were prepared from the dried crushed raw materials. The total content of antioxidants was determined in terms of quercetin and gallic acid. Using a calibration plot of the output signal dependence on the quercetin and/or gallic acid concentration, the mass concentration of antioxidants was measured. The determination was carried out on a Tsvet Yauza-01-AA liquid chromatograph (OJSC “Khimavtomatika”, Russia) by the amperometric method [12–15].

The analyzed raw material was crushed to the size of particles passing through a sieve with a diameter of 0.5 mm. About 1.0 g (an accurately weighed portion) was placed in a conical flask with a thin section with a capacity of 100 ml, 30 ml of extractant (purified water, ethyl alcohol of various concentrations: 95%, 70%, 40%) was added, heated in the water bath under reflux for 30 minutes. After cooling, it was filtered through a paper filter into a 100 ml volumetric flask. The extraction was repeated twice, using 30 ml of the extractant. The extracts were combined and made up to the mark [12–14].

For each sample, five consecutive measurements of the output signal (the peak area) of the analyzed extraction were recorded. The dilutions were taken into account in the calculations.

The mass concentration X (mg/g) was calculated by the formula:

$$X = \frac{X_g * V_n * N}{m_n * 1000}, \quad (1)$$

where: X_g is the mass concentration of the antioxidants found from the calibration curve, mg/l; V_n is the volume (extraction) of the plant material, ml; m_n is a sample of the vegetable raw materials, g; N is the dilution factor of the analyzed sample.

Determination of pharmacological activity

The antioxidant activity of *Actinidia arguta* extracts was studied *in vitro* in the following dilution range: 62.5 μg/ml, 125 μg/ml, 250 μg/ml, 500 μg/ml and 1000 μg/ml. In this case, the inhibitory properties of 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide, and the hydroxyl radical of the analyzed samples were evaluated. All the tests were performed in the triplet form.

DPPH test

The ability of the studied *Actinidia arguta* extracts to inhibit the formation of the DPPH radical in the model medium was evaluated according to the method described by Flieger J. et al. [16]. The mixture consisting of 1 ml of the analyzed extract at various concentrations and 0.5 ml of a 0.4 mM DPPH solution in methanol (the analytical grade, Vekton, Russia) was incubated for 30 min. at room temperature. Further, the change in the optical density of the studied samples was recorded at λ=518 nm relative to methyl alcohol. The methanolic solution of DPPH was taken as the positive control (A_0). Quercetin (Sigma-Aldrich, USA) at similar concentrations was used as a comparison. The percentage of the inhibition was calculated using the formula [16]:

$$\text{inhibition } \% = \frac{A_x * 100}{A_0}, \quad (2)$$

where: A_x is the sample optical density of the extraction reference standard; A_0 is the optical density of the positive control sample.

Evaluation of hydroxyl radical inhibitory activity

A method based on the spectrophotometric detection of a colored condensed complex of 2-thiobarbituric acid and degradation products of 2-deoxyribose degraded by the hydroxyl radical generated in the Fenton reaction, was used. The model medium included: 0.1 ml of a 2.8 mM deoxyribose solution; 0.1 ml of a 0.1 mM ethylenediaminetetraacetic acid (EDTA) solution; 0.1 ml of a 0.1 mm ascorbate solution; 0.1 ml of a phosphate buffer (pH 7.4) and 1 ml of the *Actinidia folia* assay extracts in the estimated concentration range. The resulting mixture was incubated at 37°C for one hour. Next, 1 ml of the 2.8% trichloroacetic acid solution and 1 ml of the 1% solution 2-thiobarbituric acid were added, heated for 20 minutes in the water bath (100°C). After cooling, the extinction of the samples was measured at λ=532 nm relative to the air. Fenton's medium without the addition of the studied extracts served as a positive control. As a comparison, quercetin of similar concentrations was used. The degree of hydroxyl radical formation inhibition was calculated by the formula (2) [17].

Evaluation of superoxide radical inhibitory activity

An analysis method based on the spectrophotometric detection of riboflavin photoconversion reaction products was used. The model medium included: 0.1 ml solution of the studied *Actinidia folia* extracts in various concentrations; 0.1 ml of a 1.5 mM nitro-blue tetrazolium solution; 0.2 ml of a 0.1 M EDTA solution; 0.05 ml of a 0.12 mM riboflavin solution and 2.55 ml of phosphate buffer (pH 7.4). The mixture was incubated for 5 min at room temperature. The sample extinction was measured at λ=560 nm relative to the air. The positive control was the incubation medium without the addition of the studied extracts. As a comparison, quercetin was used at similar concentrations. The percentage of the superoxide radical formation inhibition was calculated by formula (2) [18].

During the *in vitro* testing, the optical density was measured on a spectrophotometer PE-5400V (Promecolab, Russia).

Evaluation of "acute toxicity"

The toxicity study of *Actinidia folia* extracts in an acute experiment was carried out according to the "Up and Down" testing procedure, the main provisions of which are presented in the OECD Guideline for the evaluation of oral chemical compounds toxicity No. 425.

According to the principles of "the acute toxicity" evaluation, set out in OECD No. 425, the toxicity study experiment of the test samples involves two stages. The first stage is "a limit test", in which the studied objects were administered orally at the dose of 5000 mg/kg. When 3 animals died, the main testing was performed; otherwise, the value of LD_{50} was taken as 5000 mg/kg. The animals were observed for 14 days from the moment of the introduction of the studied objects. The "acute toxicity" study of the *Actinidia folia* analyzed extracts was performed on Balb/c male mice weighing 20–25 grams, obtained from the Rappolovo laboratory animal nursery (Leningrad region, Russia), which had undergone microbiological control and a 2-week quarantine. The animals were kept under standard conditions: the air temperature was 20±2°C; the relative humidity was 60±5% with a daily cycle of 12 hours day/12 hours night and a free access to food and water. The design of the study and the conditions under which the animals were kept, were in accordance with generally accepted standards of experimental ethics[1]. The concept of the work was approved by the local ethics committee of the Pyatigorsk Medical and Pharmaceutical Institute, a branch of Volgograd State Medical University (Protocol No. 2 dated 03/20/2019).

Antioxidant activity evaluation of studied *in vivo* extracts

An *in vivo* study of the antioxidant *Actinidia folia* properties of extracts was performed on 60 male Wistar rats weighing 200–230 grams. Keeping the animals corresponded to that in the assessment of "acute toxicity". The studied extracts (95% ethyl alcohol – code A95; 70% ethyl alcohol – code A70; 40% ethyl alcohol – code A40 and the water extract - code AB) were administered to the rats without pathology per os at the dose of 1/50 of LD50 for 10 days. After that, blood was taken from the animals' abdominal aorta into a citrate-coated syringe, then the rats were decapitated under chloral hydrate anesthesia (350 mg/kg intraperitoneally). The blood was centrifuged at 1000g for 15 minutes to obtain serum, in which the change in the pro/antioxidant balance was determined. The reference substance was quercetin at the dose of 100 mg/kg, which was administered according to the scheme similar to the studied extracts [19].

Determination of diene conjugates concentration

The content of diene conjugates (DCs) in the animals' blood serum was determined by a spectrophoto-

[1] Directive 2010/63 / EU of the European Parliament and of the councilon the protection of animals used for scientific purposes, September 22, 2010.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-207-216

metric method. DC was extracted with the mixture of heptane+isopropanol (1:1). The amount of DC was calculated by the molar extinction coefficient of conjugated dienes at λ=233 nm 2,2×10^5 M^1 · см$^{-1}$ and expressed in nmol/ml. The optical density was measured on a PE-5400V spectrophotometer (Promecolab, Russia) [20].

Determination of the TBA-active products (TBA-AP) concentration

The content of TBA-AP was determined by a spectrophotometric detection at λ=532 nm of the colored reaction products of the peroxide products condensation reaction with 2-thiobarbituric acid. In this case, the color of the resulting solution is proportional to the TBA-AP concentration. The content of TBA-AP was calculated by the molar extinction coefficient of malondialdehyde (1.56×10^5 lmol^{-1} · cm^{-1}). The results obtained were expressed in nmol/ml. The optical density was measured on a PE-5400V spectrophotometer [21].

Determination of catalase activity

A catalase activity was evaluated by the spectrophotometric method in the reaction of a hydrogen peroxide destruction determined by the interaction with a 4% ammonium molybdate solution. The color intensity of the reaction product was evaluated at λ=410 nm.

The catalase activity was calculated by the difference between the extinctions of the experimental and blank samples, using the molar extinction coefficient of hydrogen peroxide equal to 22.2×10^3 mM^{-1} · cm^{-1} and expressed in nmol/min/ml. The optical density was measured on a PE-5400V spectrophotometer [22].

Determination of superoxide dismutase activity

The activity of superoxide dismutase (SOD) was evaluated by the xanthine oxidase method. The incubation medium contained: 0.05 mmol/l xanthine; 0.025 mmol/l 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride; 0.94 mmol/l EDTA, 80 U/l xanthine oxidase, 40 mmol/l CAPS buffer. The sample extinction was recorded at λ=505 nm. The SOD activity was expressed in U/l. The optical density was measured on a PE-5400V spectrophotometer [23].

Determination of glutathione peroxidase activity

The activity of glutathione peroxidase (GP) was determined in the coupled glutathione reductase reaction by the decrease in NADPH. The incubation medium included: 1 mmol/l EDTA, 50 mM K,Na-phosphate buffer, pH 7.4; 1 unit act./ml glutathioreductase; 20 mmol/l NADPH; 1 mmol/L glutathione (GSH); 30–60 μg of protein per 1 ml of medium. The sample extinction was recorded at λ=340 nm. The reaction was started by adding cumene hydroperoxide at the concentration of 1.5 mmol/l and carried out at the temperature of 25°C. The GP activity was expressed in U/l. The optical density was measured on a PE-5400V spectrophotometer [24].

Statistical analysis

The obtained results were statistically processed using the Statistica 6.0 software package (StatSoft, USA). The data were expressed as M±SEM. Statistically significant differences between the *in vivo* testing groups were determined by a one-way analysis of variance with the Newman-Keuls post-test. The IC_{50} value for *in vitro* tests was calculated by a probit analysis. The LD_{50} index was determined by the maximum likelihood method using the AOT425statpgm report software (OECD TG 425 2002, USA) [25].

RESULTS

Study of antioxidants total content

The total content of the antioxidants in terms of quercetin and gallic acid, the peak areas, the dilution ratio, are presented in Table 1.

The content of antioxidants in the studied extracts obtained by extracting *Actinidia arguta folia* with purified water and ethyl alcohol of various concentrations, was established. Analyzing the data in Table 1, it can be concluded that the maximum content of the total antioxidants in terms of quercetin and gallic acid was found out in the extract of *Actinidia arguta folia*, obtained by the extraction with 40% ethyl alcohol.

Evaluation of antioxidant activity *in vitro*

When studying the antioxidant properties of the studied extracts in *in vitro* tests, it was found out that, with respect to the DPPH radical, the analyzed extracts were characterized by an insignificant inhibitory activity, as evidenced by the IC50 value. For the objects under the codes A95, A70 and AB it made up 1150.9±52.321 μg/ml, 1660.9±45.954 μg/ml and 1918.5±85.617 μg/ml, respectively. At the same time, the IC_{50} for extracts from *Actinidia folia* obtained by the extraction with 40% ethyl alcohol and quercetin, was 537.6±23.924 μg/mL and 519.4±45.296 μg/mL, respectively (Fig. 1).

With regard to the superoxide radical, the most pronounced inhibitory activity was shown by the studied extracts under the codes A95, A40 and quercetin: the IC value was 30.7±1.238 μg/ml, 26.6±2.627 μg/ml and 11.3±1.974 μg/ml, respectively. At the same time, the extracts of A70 (IC_{50} = 204.3±9.114 μg/mL) and AB (IC_{50}=262.9±7.856 μg/mL) inhibited the generation of the superoxide radical in the model medium to a lesser extent (Fig. 2).

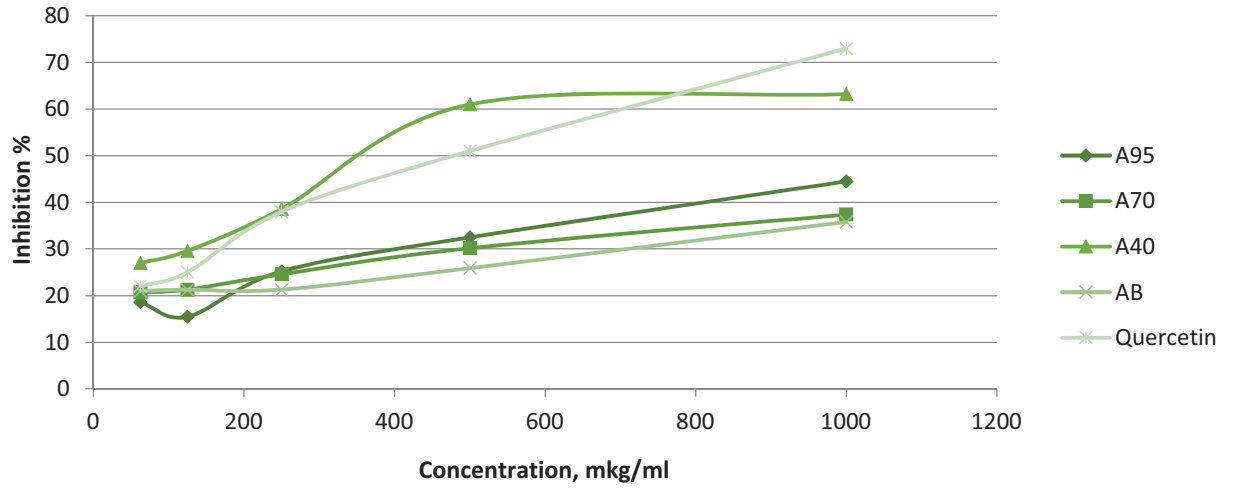

Figure 1 – Evaluation results of the DPPH-inhibitory activity of the studied extracts and quercetin

Note: A95 is the extract from *Actinidia folia*, obtained by the extraction with 95% ethyl alcohol; A70 – extract from *Actinidia folia*, obtained by the extraction with 70% ethyl alcohol; A40 is the extract from *Actinidia folia*, obtained by the extraction with 40% ethyl alcohol; AB is the extract from *Actinidia folia*, obtained by the extraction with purified water.

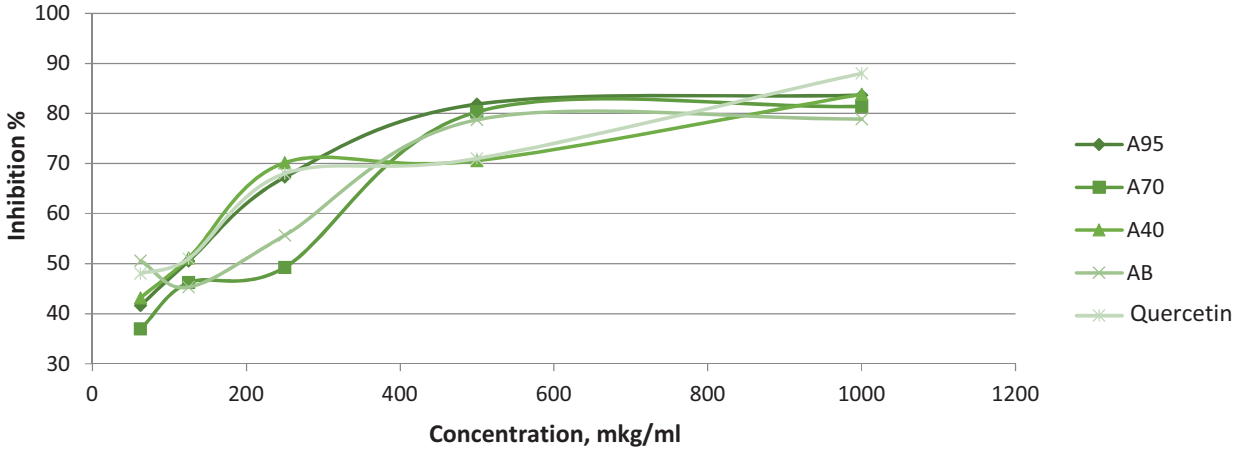

Figure 2 – Evaluation results of the superoxide-radical-inhibiting activity of the studied extracts and quercetin

Note: A95 is the extract from *Actinidia folia*, obtained by the extraction with 95% ethyl alcohol; A70 – extract from *Actinidia folia*, obtained by the extraction with 70% ethyl alcohol; A40 is the extract from *Actinidia folia*, obtained by the extraction with 40% ethyl alcohol; AB is the extract from *Actinidia folia*, obtained by the extraction with purified water.

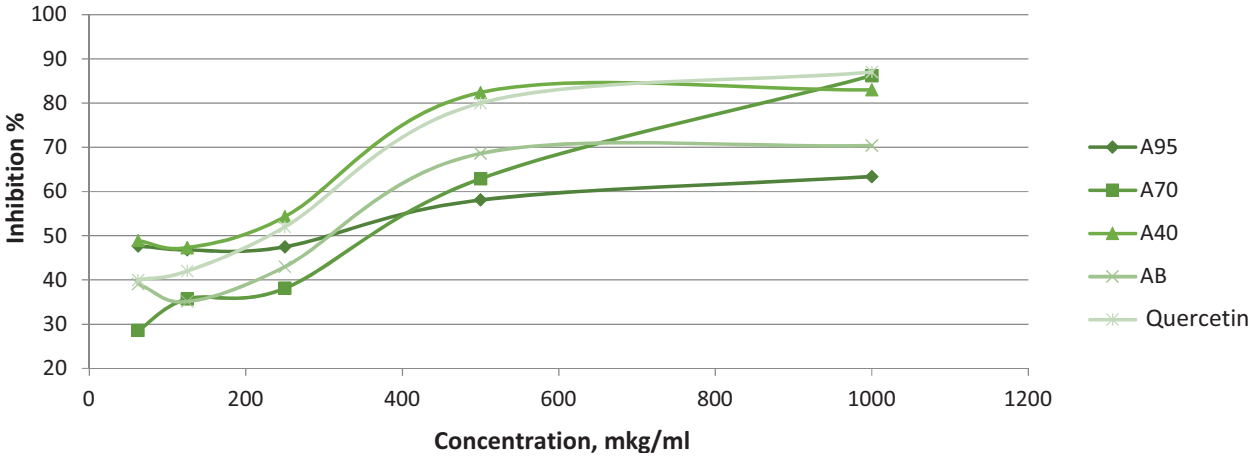

Figure 3 – Evaluation results of the hydroxyl-radical-inhibiting activity of the studied extracts and quercetin

Note: A95 is the extract from *Actinidia folia*, obtained by the extraction with 95% ethyl alcohol; A70 – extract from *Actinidia folia*, obtained by the extraction with 70% ethyl alcohol; A40 is the extract from *Actinidia folia*, obtained by the extraction with 40% ethyl alcohol; AB is the extract from *Actinidia folia*, obtained by the extraction with purified water.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-207-216

Table 1 – The content of antioxidants (in terms of quercetin and gallic acid) in extracts from *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq. *folia*

Raw materials name	Extractants used	Peak areas (pA/sec)	Dilution ratio of analyzed samples	Total content of antioxidants, mg/g (n=6) in terms of	
				quercetin	gallic acid
Leaves	95% ethyl alcohol	2963.04	–	0.268±0,005	0.172±0.003
	70% ethyl alcohol	3210.95	2	0.584±0,009	0.375±0.004
	40% ethyl alcohol	4262.38	6	0.734±0,007	0.475±0.005
	Purified water	3991.70	2	2.215±0,007	1.436±0.006

Table 2 – Results of determining "the acute toxicity" of *Actinidia folia* investigated extracts

Animal No.	Weight, g	Investigated objects				Dose
		A95	A70	A40	AB	
1	22	O (S)				5000 mg/kg, *per os*
2	21	O (S)				
3	20	O (S)				
4	23		O (S)			
5	22		O (S)			
6	20		O (S)			
7	24			O (S)		
8	25			O (S)		
9	25			O (S)		
10	24				O (S)	
11	21				O (S)	
12	22				O (S)	

Note: O – no death on the first day of observation; (S) – no death during 14 days of observation.

Table 3 – Changes in the pro / antioxidant balance against the background of the course administration of the studied extracts and quercetin to the animals without pathology

Group	Intact animals (n=10)	A95 (n=10)	A70 (n=10)	A 40 (n=10)	AB (n=10)	Quercetin (n=10)
SOD, Unit/l	300.5±7.129 µ	304.4±8.432 µ	307.1±8.391µ	371±8.377*	309.5±7.973 µ	395.47±8.439*
GP, Unit/l	602.6±5.946	601.56±6.188	603.71±6.163	664.91±9.779*	604.75±9.050	657.29±6.129
Catalase, nmol/min/ml	0.863±0.0145	0.944±0.024	0.76±0.076 µ	1.188±0.084*	0.919±0.041	1.287±0.082*
TBA-AP, nmol/ml	5.4±0.524	4.36±0.611	4.43±0.298	3.75±0.173*	4.62±0.596	3.67±0.655*
DC, nmol/ml	10.8±0.696	9.71±0.31	9.74±0.454	7.31±0.271*	9.55±0.21	7.52±0.481*

Note: * is statistically significant relative to the intact animals ($p<0.05$; Newman-Keuls test); µ is statistically significant relative to the animals treated with A40 extract ($p<0.05$; Newman-Keuls test).

The formation of a hydroxyl radical in the model mixture most significantly inhibited the addition of the studied extract from *Actinidia folia*, obtained by the extraction with 40% ethyl alcohol (IC_{50} = 72.6±3.264 µg/ml) into the medium. At the same time, the IC_{50} values for the studied objects A95; A70; AV and quercetin amounted to 245.6±10.237 µg/ml; 382.5±11.974 µg/ml; 356.0±12.987 µg/ml and 192.2±7.515 µg/ml, respectively (Fig. 3).

"Acute toxicity" evaluation of *Actinidia folia* investigated extracts

In the course of evaluating "the acute toxicity" (Table 2) of the studied extracts, it was found out that during "the limit test" (the administration of the studied objects at the dose of 5000 mg/kg, per os), neither early nor delayed death of animals was noted. At the same time, there were no significant deviations in the general condition of the animals, their behavioral activity and sensorimotor perception. Thus, based on the data obtained during the implementation of "the limit test", the main testing was not started, and the value of LD_{50} for all the studied objects was taken as 5000 mg/kg, which allows us to attribute the studied extracts of *Actinidia folia* to the 5th toxicity class according to the GSH-classification[2].

[2] Ibid

Antioxidant activity evaluation of the studied Actinidia folia extracts *in vivo*

Based on the results of determining "the acute toxicity" of the studied *Actinidia folia* extracts, when evaluating the antioxidant activity *in vivo*, the administered dose of the studied objects was 100 mg/kg (p. o.). The results of this block of the experimental work are presented in Table 2.

Against the background of a 10-day administration of quercetin to the animals without a pathological background, an increase in the activity of SOD and catalase was notified compared to the intact animals by 31.6% ($p<0.05$) and 49.1% ($p<0.05$). At the same time, the catalytic properties of HP in the rats treated with quercetin did not statistically significantly differ from those in the intact animals (Table 3). It should also be notified that when using quercetin, there was a decrease (relative to the intact rats) in the concentration of DC and TBA-AP in the blood serum of the animals by 30.3% ($p<0.05$) and 32% ($p<0.05$), respectively. As can be seen from the data obtained (Table 3), the course administration of extracts under the codes A95, A70 and AB to the animals without pathology did not have a significant effect on the change in the pro/antioxidant balance. At the same time, when using A40 extract, an increase in the activity of SOD, GP and catalase was notified in comparison with the intact rats by 23.5% ($p<0.05$); 10.3% ($p<0.05$) and 37.7% ($p<0.05$), respectively, accompanied by a decrease in the concentration of TBA-AP and DC by 30.5% ($p<0.05$) and 32.3% ($p<0.05$), respectively.

At the same time, the indicators characterizing the state of the pro/antioxidant balance in the animals treated with quercetin and the studied extracts of *Actinidia folia* obtained by the extraction with 40% ethyl alcohol, did not differ statistically significantly. It should be notified that the activity of SOD in the blood serum of the rats that had been injected with A40 extract, was 21.9% ($p<0.05$); 20.8% ($p<0.05$) and 19.8% ($p<0.05$) higher than the same indicator in the animals treated with the studied objects under codes A95, A70 and AB, respectively. A catalase activity with A40 extracts was also superior to that of the animals that received A70 extract, by 56.3% ($p<0.05$).

DISCUSSION

Reactive oxygen species (ROS) are formed in cells during metabolism and perform many physiological processes, such as a regulation of cell proliferation, a microcirculatory blood flow, apoptosis reactions, and the gene expression [26].

Within certain physiological limits, the endogenous antioxidant defense system is designed to reduce the negative effect of oxidants on the body. Endogenous antioxidant enzymes, such as catalase, superoxide dismutase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, and glutathione reductase, provide a timely inactivation of ROS, preventing their negative effect on the cells [28].

At the same time, a shift in the redox status towards prooxidants and the insufficient activity of endogenous antioxidant defense enzymes contribute to the development of the oxidative stress, which plays a significant role in the pathogenesis of a number of diseases: oncopathology, diabetes mellitus, cardiovascular diseases, alcoholic liver dystrophy, dementia, atherosclerosis, Parkinson's disease [29].

It is known that in the series of ROS, a superoxide radical and its derivatives (peroxonitrite), as well as the hydroxyl radical, have the highest cytotoxicity, which, through a direct destructive action and indirect reactions (usually involving secondary effector systems in the pathological cascade, for example, ERK and MAPK kinase) lead to the cell death [30].

An insufficient activity of the endogenous antioxidant defense and the associated increase in the amount of ROS, require the administration of exogenous antioxidants, among which the agents of the natural origin are especially prominent [31].

This research was devoted to the antioxidant activity study of the extracts obtained from *Actinidia folia.* The antioxidant activity of 95%, 70% and 40% alcohol extracts, as well as water extracts, was evaluated using *in vitro* and *in vivo* approaches. Thus, when studying the antiradical activity (*in vitro* tests), it was found out that the highest (among the studied objects) radical-inhibiting activity, comparable with the individual compound – quercetin, is the extraction from *Actinidia arguta folia,* obtained by the extraction with 40% ethyl alcohol. In relation to DPPH; superoxide and hydroxyl radical, the IC_{50} values for the given extract were 537.6±23.924 μg/mL, 26.6±2.627 μg/mL, and 72.6±3.264 μg/mL, respectively, which may indicate that this extract has reducing and radical scavenger properties [32].

In parallel, the study of the total content of the antioxidants in terms of quercetin and gallic acid, was carried out. In the *Actinidia arguta folia* extract obtained by the extraction with 40% ethyl alcohol, the maximum content of antioxidants was also found out.

It is known that the DPPH test is the most common approach for evaluating the acceptor properties of phenolic compounds. The analysis principle is based on the principle of the DPPH free radical reduction by accepting a hydrogen atom from a donor compound and converting the color from violet to yellow. Thus, this approach makes it possible to evaluate the acceptor properties of biologically active substances with a sufficiently high reliability [33].

At the same time, *in vitro* tests on superoxide and hydroxyl radicals are based on the ability of the analyzed object to suppress the formation of ROS in a model medium and, accordingly, make it possible to evaluate the scavenger activity [34, 35].

CONCLUSION

The maximum content of antioxidants in *Actinidia arguta folia* is 0.73±0.007 and 0.47±0.005 mg/g in terms

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-207-216

of quercetin and gallic acid, respectively. The extractant is 40% ethyl alcohol.

The data obtained in the *in vitro* test were confirmed in the *in vivo* study in which the course application of the *Actinidia arguta folia* extract obtained by the extraction with 40% ethyl alcohol, to a degree comparable to quercetin, contributed to an increase in the activity of SOD, GP and catalase. This extract also contributed to the reduction of lipid peroxidation products in the animals without a pathological background, which suggests that the studied A40 extract has a high antioxidant activity. All of the above, combined with a low toxicity (LD_{50}≥5000 mg/kg, *per os*), makes this extract a promising object for a further study in order to create a drug with an antioxidant effect.

ACKNOWLEDGMENT

The authors team expresses their sincere gratitude to the untimely deceased, Doctor of Sciences (Medicine), Professor A.V. Voronkov for help in article preparing.

FUNDING

This study did not receive financial support from third parties.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Dmitry I. Pozdnyakov – the study concept development, the *in vivo* experiment setting up, statistical processing of the study results, preparing the manuscript final version; Similla L. Adzhiakhmetova - the study concept development, the analyzed extracts obtaining, conducting *in vitro* studies, preparing the manuscript final version; Natalia N. Vdovenko-Martynova – the literature analysis, raw materials identification, the analyzed extracts obtaining, preparing the manuscript final version.

REFERENCES

1. Kozak NV, Temirbekova SK, Kulikov IM. Novyj sort aktinidii kolomikta pamyati Kolbasinoj [A new variety of actinidia kolomikta in memory of Kolbasina]. Fruit and berry growing in Russia.2014;38(1):194–9. Russian
2. Denisov NI. Opyt introdukcii aktinidii arguty (Actinidia arguta (Siebold&Zucc.) Planch. Ex Miq.) na yug Dal'nego Vostoka Rossii [Experience of introduction of Actinidia arguta (Siebold&Zucc.) Planch. Ex Miq.) to the south of the Russian Far East]. Bulletin of the IrGSHA. 2012;52: 34–41. Russian
3. Bryksin DM. Harakteristika sortov Actinidia kolomikta po srednej masse ploda. [Characteristics of Actinidia kolomikta varieties by average fruit weight]. In the collection of Agroecological aspects of sustainable development of the agro-industrial complex materials of the XV International. scientific conference. 2018:384–6. Russian
4. Koveshnikova EYu. Ocenka ustojchivosti sortov aktinidii k boleznyam i vreditelyam v usloviyah CCHR [Assessment of the resistance of actinidia varieties to diseases and pests in the conditions of the CCHR]. Fruit and berry growing in Russia: scientific collection. works. 2013; 36: 275–81. Russian
5. Kozak NV, Deadishcheva ME, Motyleva SM. Izuchenie obrazcov geneticheskoj kollekcii aktinidii arguta Actinidia arguta (SieboldExZucc.) Planch. ex Miq. po priznakam «obshchaya antioksidantnaya aktivnost'» i «soderzhanie askorbinovoj kisloty» v plodah [Study of samples of the genetic collection of actinidia arguta Actinidia arguta (SieboldExZucc.) Planch. ex Miq. according to the signs of "total antioxidant activity" and "ascorbic acid content" in fruits]. Current directions of scientific research: from theory to practice. 2016;1(7):178–9. Russian
6. Motyleva SM, Kozak NV, Deadishcheva ME. Antioksidantnaya aktivnost' list'ev i plodov trekh vidov Actinidia Lindl., introducirovannyh v Podmoskov'e [Antioxidant activity of leaves and fruits of three species of Actinidia Lindl. introduced in the Moscow region]. New and unconventional plants and prospects for their use. 2015;11:57–60. Russian
7. Latocha P. The Nutritional and Health Benefits of Kiwiberry (Actinidia arguta) - a Review. Plant Foods Hum Nutr. 2017;72(4):325–34. DOI: 10.1007/s11130-017-0637-y.
8. Gomel D, Ryazanova LG. Aktinidiya v usloviyah Prikubanskoj zony sadovodstva [Actinidia in the conditions of the Kuban gardening zone. In the collection]: Scientific support of the agro-industrial complex. Collection of articles based on the materials of the X All-Russian Conference of Young Scientists dedicated to the 120th anniversary of I. S. Kosenko. 2017:676–7. Russian
9. Nishimura M, Okimasu Y, Miyake N, Tada M, Hida R, Negishi T, Arimoto-Kobayashi S. Inhibitory effect of Actinidia argute on mutagenesis, inflammation and two-stage mouse skin tumorigenesis. Genes Environ. 2016; 38: 25. DOI: 10.1186/s41021-016-0053-9.
10. Stefaniak J, Łata B. Actinidia arguta Leaf as a Donor of Potentially Healthful Bioactive Compounds: Implications of Cultivar, Time of Sampling and Soil N Level. Molecules. 2021;26(13):3871. DOI: 10.3390/molecules26133871.
11. Vdovenko-Martynova NN, Adjiakhmetova SL, Bezrodnova EI, Pozdnyakov DI. Issledovaniya po vyyavleniyu pokazatelej podlinnosti rastitel'nogo syr'ya – Actinidia arguta folia i soderzhaniyu osnovnyh grupp biologicheski aktivnyh veshchestv [Studies on the identification of indicators of authenticity of plant raw materials - Actinidia arguta folia and the content of the main groups of biologically active substances]. Bulletin of the Nikitsky State Botanical Garden. 2021; 138:101–9. DOI 10.36305/0513-1634-2021-138-101-109. Russian
12. Patent 2238554 Russian Federation, MCI G01N 33/15 N27/26. Sposob opredeleniya summarnoj antioksidantnoj aktivnosti biologicheski aktivnyh veshchestv [Method for determining the total antioxidant activity of biologically active substances] Pakhomov VP [et al.] (RF). No. 2003123072/15; application 25.07. 03; publ. 20.10.04, Byul15:3. Russian
13. Yashin AYa, Yashin YaI. Pribor dlya opredeleniya antioksidantnoj aktivnosti rastitel'nyh lekarstvennyh ekstraktov i napitkov [A device for determining the antioxidant activ-

ity of herbal medicinal extracts and beverages]. Journal. international. information system on resonant technologies. 2004;34:10–4. Russian

14. Yashin AYa. Inzhekcionno-protochnaya sistema s amperometricheskim detektorom dlya selektivnogo opredeleniya antioksidantov v pishchevyh produktah i napitkah [Injection-flow system with an amperometric detector for the selective determination of antioxidants in food and beverages]. Russian Chemical. D.I. Mendeleev University. 2008;2:130–5. Russian
15. Adjiakhmetova SL, Andreeva AO, Oganesyan ET. Antioksidantnaya aktivnost' ekstraktov iz list'ev, plodov i steblej kryzhovnika otklonennogo (Grossularia reclinata (L.) Mill.) [Antioxidant activity of extracts from leaves, stems and fruits of gooseberry rejected (species G. reclinata (L.) Mill.)]. Fundamental research. 2013;10(6):1297–301 Russian
16. Flieger J, Flieger M. The [DPPH●/DPPH-H]-HPLC-DAD Method on Tracking the Antioxidant Activity of Pure Antioxidants and Goutweed (Aegopodium podagraria L.) Hydroalcoholic Extracts. Molecules. 2020 18;25(24):6005. DOI: 10.3390/molecules25246005.
17. Monga D, Ilager D, Shetti NP, Basu S, Aminabhavi TM. 2D/2d heterojunction of MoS2/g-C3N4 nanoflowers for enhanced visible-light-driven photocatalytic and electrochemical degradation of organic pollutants. J Environ Manage. 2020; 274: 111208. DOI: 10.1016/j.jenvman.2020.111208.
18. Gushiken LFS, Beserra FP, Hussni MF, Gonzaga MT, Ribeiro VP, de Souza PF, Campos JCL, Massaro TNC, Hussni CA, Takahira RK, Marcato PD, Bastos JK, Pellizzon CH. Beta-caryophyllene as an antioxidant, anti-inflammatory and re-epithelialization activities in a rat skin wound excision model. Oxid Med Cell Longev. 2022; 2022: 9004014. DOI: 10.1155/2022/9004014.
19. Sohaib M, Butt MS, Shabbir MA, Shahid M. Lipid stability, antioxidant potential and fatty acid composition of broilers breast meat as influenced by quercetin in combination with α-tocopherol enriched diets. Lipids Health Dis. 2015; 14: 61. DOI: 10.1186/s12944-015-0058-6.
20. Gavrilov VB, Mishkorudnaya MI. Spektrofotometricheskoe opredelenie soderzhaniya gidroperekisej lipidov v plazme krovi [Spectrophotometric determination of the content of lipid hydroperoxides in blood] plasma. Lab. delo.1983;6:33–5. Russian
21. Aguilar Diaz De Leon J, Borges CR. Evaluation of Oxidative Stress in Biological Samples Using the Thiobarbituric Acid Reactive Substances Assay. J Vis Exp. 2020 12;(159). DOI: 10.3791/61122.
22. Lu G, Tan W, Li G, Yang M, Wang H. Effects of carbendazim on catalase activity and related mechanism. Environ Sci Pollut Res Int. 2020;27(20):24686–91. DOI: 10.1007/s11356-019-06125-7.
23. Robbins ME, Cho HY, Hansen JM, Luchsinger JR, Locy ML, Velten M, Kleeberger SR, Rogers LK, Tipple TE. Glutathione reductase deficiency alters lung development and hyperoxic responses in neonatal mice. Redox Biol. 2021; 38: 101797. DOI: 10.1016/j.redox.2020.101797.
24. Derindağ G, Akgül HM, Kızıltunç A, Özkan Hİ, Kızıltunç Özmen H, Akgül N. Evaluation of saliva glutathione, glutathione peroxidase, and malondialdehyde levels in head-neck radiotherapy patients. Turk J Med Sci. 2021;51(2):644–9. DOI: 10.3906/sag-2006-84.
25. Blum CA, Velly L, Brochet C, Ziegler F, Tavolacci MP, Hausfater P, Lvovschi VE. Relevance of cortisol and copeptin blood concentration changes in an experimental pain model. Sci Rep. 2022;12(1):4767. DOI: 10.1038/s41598-022-08657-4.
26. Pisoschi AM, Pop A, Cimpeanu C, Predoi G. Antioxidant Capacity Determination in Plants and Plant-Derived Products: A Review. Oxid Med Cell Longev. 2016; 2016: 9130976. DOI: 10.1155/2016/9130976.
27. Lin C, Zheng X, Lin S, Zhang Y, Wu J, Li Y. Mechanotransduction Regulates the Interplays Between Alveolar Epithelial and Vascular Endothelial Cells in Lung. Front Physiol. 2022; 13: 818394. DOI: 10.3389/fphys.2022.818394.
28. Kumar P, Tanwar R, Gupta V, Upadhyay A, Kumar A, Gaikwad KK. Pineapple peel extract incorporated poly(vinyl alcohol)-corn starch film for active food packaging: Preparation, characterization and antioxidant activity. Int J Biol Macromol. 2021; 187: 223–31. DOI: 10.1016/j.ijbiomac.2021.07.136.
29. Zhang MS, Liang JH, Yang MJ, Ren YR, Cheng DH, Wu QH, He Y, Yin J. Low Serum Superoxide Dismutase Is Associated with a High Risk of Cognitive Impairment After Mild Acute Ischemic Stroke. Front Aging Neurosci. 2022; 14: 834114. DOI: 10.3389/fnagi.2022.834114.
30. Chainy GBN, Sahoo DK. Hormones and oxidative stress: an overview. Free Radic Res. 2020;54(1):1–26. DOI: 10.1080/10715762.2019.1702656.
31. Kim S, Yang HY, Lee HJ, Ju J. In Vitro Antioxidant and Anti-Colon Cancer Activities of Sesamum indicum L. Leaf Extract and Its Major Component, Pedaliin. Foods. 2021;10(6):1216. DOI: 10.3390/foods10061216.
32. Ahmad NA, Jumbri K, Ramli A, Abd Ghani N, Ahmad H, Lim JW. A Kinetic Approach of DPPH Free Radical Assay of Ferulate-Based Protic Ionic Liquids (PILs). Molecules. 2018;23(12):3201. DOI: 10.3390/molecules23123201.
33. Rajan VK, Muraleedharan K. A computational investigation on the structure, global parameters and antioxidant capacity of a polyphenol, Gallic acid. Food Chem. 2017; 220: 93-99. DOI: 10.1016/j.foodchem.2016.09.178.
34. Hung MW, Yeung HM, Lau CF, Poon AMS, Tipoe GL, Fung ML. Melatonin Attenuates Pulmonary Hypertension in Chronically Hypoxic Rats. Int J Mol Sci. 2017;18(6):1125. DOI: 10.3390/ijms18061125.
35. Szanto I. NADPH Oxidase 4 (NOX4) in Cancer: Linking Redox Signals to Oncogenic Metabolic Adaptation. Int J Mol Sci. 2022;23(5):2702. DOI: 10.3390/ijms23052702.

AUTHORS

Dmitry I. Pozdnyakov – Candidate of Sciences (Pharmacy), Associate Professor of the Department of Pharmacology with a Course of Clinical Pharmacology, Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID ID: 0000-0002-5595-8182. E-mail: pozdniackow.dmitry@yandex.ru

Similla L. Adzhiakhmetova – Candidate of Sciences (Pharmacy), Associate Professor of the Department of Organic Chemistry, Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID ID: 0000-0001-6924-8563. E-mail: simillа503@mail.ru

Natalia N. Vdovenko-Martynova – Candidate of Sciences (Pharmacy), Associate Professor of the Department of Pharmacognosy, Botany and Technology of Phytopreparations. Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID ID: 0000-0001-6425-4315. E-mail: martynovann@yandex.ru

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2022-10-2-217-228

STUDY OF LONG-TERM CLINICAL AND PATHOGENETIC EFFECTS OF FAVIPIRAVIR-BASED ANTI-VIRAL DRUG IN PATIENTS WITH METABOLIC SYNDROME IN POST-COVID PERIOD

O.A. Radaeva[1], L.A. Balykova[1], K.Ya. Zaslavskaya[1], A.V. Taganov[2], P.A. Bely[3], Yu.A. Kostina[1], E.V. Negodnova[1], S.V. Mashnina[1], D.D. Bessheinov[1], M.S. Iskandyarova[1], V.V. Eremeev[1], N.M. Chumakov[1]

[1] N.P. Ogarevs Mordovia State University
Bld. A, 26, Ulyanov St., Saransk, Republic of Mordovia, Russia, 430005
[2] Peoples Friendship University of Russia
6, Miklukho-Maklay Str., Moscow, Russia, 117198
[3] Moscow State University of Medicine and Dentistry named after A.I. Evdokimov
Bld. 1, 20, Delegatskaya Str., Moscow, Russia 127473

E-mail: radaevamed@mail.ru

Received 07 Feb 2022 After peer review 15 March 2022 Accepted 20 Apr 2022

The article presents modern scientific data on long-term clinical and pathogenetic effects of the antiviral drug Areplivir (Favipiravir) in patients with metabolic syndrome in the post-COVID period.

The aim of the article is to study long-term cytokine-mediated (IL-6/sIL6r and LIF/sLIFr) pathogenetic effects of the favipiravir (Areplivir®) based drug on the incidence of complications in patients with metabolic syndrome in the post-COVID period.

Material and methods. With the approval of the local ethics committee at the N.P. Ogarevs Mordovia State University (Protocol No. 5 dated May 17, 2020) "An open prospective comparative study of the Areplivir® (Favipiravir) drug effectiveness in reducing the risk of complications in the post-COVID period in patients with metabolic syndrome" in the Republic of Mordovia was carried out.

The study included 190 metabolic syndrome patients who received the outpatient treatment for COVID-19 at Saransk polyclinics from February 2021 to March 2021. The case of COVID-19 was diagnosed in accordance with the current Temporary Guidelines for the prevention, diagnosis and treatment of the new coronavirus infection.

Results. The analysis of the metabolic syndrome patients' follow-up within 1 year after undergoing COVID-19, revealed significant differences in the incidence of complications depending on the intake of the favipiravir based drug. The patients who were administrated with favipiravir at the early stage of infection, were characterized by lower serum levels of four members of the interleukin 6 family – IL-6 (IL-6, sIL6r and LIF, sLIFr) 10, 30 and 180 days after a clinical and laboratory recovery ($p<0.001$). The average statistical changes in the IL-6 /sIL6r system of the group administrated with favipiravir, were 90%, and they were higher than in the group not administrated with antiviral drugs. In the group of the patients administrated with favipiravir, there was a significant ($p<0.001$) positive dynamic of the sLIFr indicator, while in the comparison group, there was an increase in this indicator.

A protective effect of the early favipiravir use was characterized by a decrease in the frequency of cardiovascular complications, a 2.66-fold decrease in the risk of a stroke and the ACS in the post-COVID period.

Conclusion. The areplivir therapy in the acute period of coronavirus infection made it possible to timely reduce the viral load. It helps to correct the pro-inflammatory vector of the immune response at the post-COVID stage and, accordingly, reduces the risk of progression of atherosclerosis, transient cerebrovascular accidents with a cognitive decline, an endothelial dysfunction, and can be considered a secondary prevention of life-threatening cardiovascular complications.

Keywords: Areplivir; favipiravir; COVID-19; postcovid syndrome; metabolic syndrome

For citation: O.A. Radaeva, L.A. Balykova, K.Ya. Zaslavskaya, A.V. Taganov, P.A. Bely, Yu.A. Kostina, E.V. Negodnova, S.V. Mashnina, D.D. Bessheinov, M.S. Iskandyarova, V.V. Eremeev, N.M. Chumakov. Study of long-term clinical and pathogenetic effects of favipiravir-based anti-viral drug in patients with metabolic syndrome in post-covid period. *Pharmacy & Pharmacology.* 2022;10(2):217-228. **DOI:** 10.19163/2307-9266-2022-10-2-217-228

Для цитирования: О.А. Радаева, Л.А. Балыкова, К.Я. Заславская, А.В. Таганов, П.А. Белый, Ю.А. Костина, Е.В. Негоднова, С.В. Машнина, Д.Д. Бесшейнов, М.С. Искандярова, В.В. Еремеев, Н.М. Чумаков. Изучение отдаленных клинико-патогенетических эффектов противовирусного лекарственного препарата на основе фавипиравира в постковидном периоде у пациентов с метаболическим синдромом. *Фармация и фармакология.* 2022;10(2):217-228. **DOI:** 10.19163/2307-9266-2022-10-2-217-228

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Abbreviations: MS – metabolic syndrome; AH – arterial hypertension; DM – diabetes mellitus; ACVE – Acute Cerebrovascular Event; ACS – acute coronary syndrome; MI – Miocardial Infarction; ALT – Alanine transaminase; AST – aspartate aminotransferase; BMI – body mass index; PCR – polymerase chain reaction; ECG – electrocardiogram; VED – vital essential drugs; RNA – ribonucleic acid; ELISA – enzyme-linked immunoelectrodiffusion essay; CI – confidence interval; IL-6 – interleukin 6; sIL-6R – soluble interleukin 6 receptor; sLIFr – leukemia inhibiting factor soluble receptor; LIF – Leukemia inhibitory factor (leukemia inhibitory factor); iNOS – Nitric oxide synthase; inducible (inducible nitric oxide synthase); eNOS – endothelial nitric oxide synthase (endothelial nitric oxide synthase); ADMA – asymmetric dimethylarginine (asymmetric dimethylarginine); SDMA – symmetric dimethylarginine (symmetrical dimethylarginine); NO – nitric oxide; PWVcf – carotid to femoral artery pulse wave velocity; EchoCG – echo-cardiography; PVR – peripheral vascular resistance; GFR – glomerular filtrate rate; gp – glycoprotein; STAT3 – signaling protein and transcription activator of signal transducers and activators of transcription (STAT).

ИЗУЧЕНИЕ ОТДАЛЕННЫХ КЛИНИКО-ПАТОГЕНЕТИЧЕСКИХ ЭФФЕКТОВ ПРОТИВОВИРУСНОГО ЛЕКАРСТВЕННОГО ПРЕПАРАТА НА ОСНОВЕ ФАВИПИРАВИРА В ПОСТКОВИДНОМ ПЕРИОДЕ У ПАЦИЕНТОВ С МЕТАБОЛИЧЕСКИМ СИНДРОМОМ

О.А. Радаева[1], Л.А. Балыкова[1], К.Я. Заславская[1], А.В. Таганов[2], П.А. Белый[3], Ю.А. Костина[1], Е.В. Негоднова[1], С.В. Машнина[1], Д.Д. Бесшейнов[1], М.С. Искандярова[1], В.В. Еремеев[1], Н.М. Чумаков[1]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Национальный исследовательский Мордовский государственный университет имени Н.П. Огарёва»
430005, Россия, Республика Мордовия, г. Саранск, ул. Ульянова, д. 26а
[2] Федеральное государственное автономное образовательное учреждение высшего образования «Российский университет дружбы народов»
117198, г. Москва, ул. Миклухо-Маклая, д. 6
[3] Федеральное государственное бюджетное образовательное учреждение высшего образования «Московский государственный медико-стоматологический университет имени А.И. Евдокимова» Министерства здравоохранения Российской Федерации,
127473, Россия, г. Москва, ул. Делегатская, д. 20, стр. 1

E-mail: radaevamed@mail.ru

Получена 07.02.2022 После рецензирования 15.03.2022 Принята к печати 20.04.2022

В статье представлены современные научные данные в отношении отдаленных клинико-патогенетических эффектов противовирусного препарата Арепливир (фавипиравир) в постковидном периоде у пациентов с метаболическим синдромом.

Цель. Изучить отдаленные цитокин-опосредованные (IL-6/sIL6r и LIF/sLIFr) патогенетические эффекты применения препарата на основе фавипиравира («Арепливир®») на частоту развития осложнений у пациентов с метаболическим синдромом в постковидном периоде.

Материал и методы. С одобрения локального этического комитета при ФГБОУ ВО «МГУ им. Н.П. Огарева» (протокол № 5 от 17 мая 2020) проведено «Открытое проспективное сравнительное исследование эффективности применения препарата «Арепливир®» (фавипиравир) в отношении снижения риска развития осложнений в постковидном периоде у пациентов с метаболическим синдромом» в Республике Мордовия.

В исследование включены 190 пациентов с метаболическим синдромом, получавших амбулаторное лечение в связи с COVID-19 на базе поликлиник г. Саранска в период с февраля 2021 по март 2021. Диагноз COVID-19 был выставлен в соответствие с актуальными временными методическими рекомендациями по профилактике, диагностике и лечению новой коронавирусной инфекции.

Результаты. Анализ наблюдения пациентов с метаболическим синдромом в течение 1 года после перенесенного COVID-19 определил достоверные отличия в частоте осложнений в зависимости от приема лекарственного препарата на основе фавипиравира. Пациенты, получавшие фавипиравир на раннем этапе заражения, характеризовались более низким уровнем содержания в сыворотке крови четырех представителей семейства интерлейкина 6-IL-6 (IL-6, sIL6r и LIF, sLIFr) через 10, 30 и 180 дней после клинико-лабораторного выздоровления ($p<0,001$). Среднестатистические изменения в системе IL-6/sIL6r группы, принимающих фавипиравир, составляли 90% и были выше, чем у группы без приема противовирусных препаратов. В группе пациентов, принимавших фавипиравир, наблюдалась значимая ($p<0,001$) положительная динамика показателя sLIFr, тогда как в группе сравнения наблюдался рост данного показателя.

Протективное действие при раннем использовании фавипиравира характеризовалось уменьшением частоты сердечно-сосудистых осложнений, снижением риска развития ОНМК и ОКС в 2,66 раза в постковидном периоде.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-217-228

Заключение. Терапия арепливиром в остром периоде коронавирусной инфекции позволила своевременно снизить вирусную нагрузку, что способствует корректировке провоспалительного вектора иммунного реагирования на постковидном этапе и, соответственно, снижает риск прогрессирования атеросклероза, транзиторных нарушений мозгового кровообращения со снижением когнитивных функций, эндотелиальной дисфункции и может рассматриваться в качестве вторичной профилактики жизнеугрожающих сердечно-сосудистых осложнений.
Ключевые слова: Арепливир; фавипиравир; COVID-19; постковидный синдром; метаболический синдром
Список сокращений: МС-метаболический синдром; АГ – артериальная гипертензия; СД – сахарный диабет; ОНМК – острое нарушение мозгового кровообращение; ОКС – острый коронарный синдром; ИМ – инфаркт миокарда; АлТ – аланинаминотрансфераза; АсТ – аспартатаминотрансфераза; ИМТ – индекс массы тела; ПЦР – полимеразная цепная реакция; ЭКГ – электрокардиограмма; ЖНВЛП – жизненно необходимые важнейшие лекарственные препараты; РНК – рибонуклеиновая кислота; ИФА – иммуноферментный анализ; ДИ – доверительный интервал; IL-6 – интерлейкин 6; sIL-6R – растворимый рецептор интерлейкина 6; sLIFr – растворимый рецептор лейкемия-ингибирующего фактора; LIF – лейкемия-ингибирующий фактор; iNOS –индуцибильная синтаза оксида азота; eNOS – эндотелиальная синтаза оксида азота; ADMA – асимметричный диметиларгинин; SDMA – симметричный диметиларгинин; NO – оксид азота; СПВкф – скорости пульсовой волны на каротидно-феморальном сегменте; ЭХО-КГ – эхокардиография; ОПСС – общее периферическое сопротивление сосудов; СКФ – скорость клубочковой фильтрации; gp – гликопротеин; STAT3 – сигнальный белок и активатор транскрипции из семейства белков STAT.

INTRODUCTION

A significant proportion of people who have had COVID-19, suffer from persistent pathological symptoms that reduce the quality of life and increase the risk of disability. In a number of sources, it is referred to as post-COVID syndrome [1–3]. Herewith, the term “post-COVID syndrome” has a number of limitations that do not allow to unambiguously consider the progression of concomitant diseases after SARS-CoV-2 infection, in particular, arterial hypertension, increased glucose levels, etc., as components of post-COVID syndrome [4]. The data from the studies analyzing cytokine-mediated mechanisms of non-communicable diseases immunopathogenesis (including essential arterial hypertension (EAH) and metabolic syndrome (MS) in the post-COVID period, demonstrating the relevance of the problem, have already been published [5]. The changes in the cytokine regulation associated with complications in the post-COVID period, are studied by many international scientific groups [6]. The data on the “unexpected” increase in the levels of pro-inflammatory markers after 7–8 months in the patients who have undergone the SARS-CoV-2 infection asymptomatically, have been presented [7]. In the severity of post-infection changes, heterogeneity may be also associated with differences in therapy during the acute period of COVID-19. It is known that the earliest possible use of etiotropic therapy is the most important therapeutic tactics for the timely relief of an increasing viral load and reducing the risk of developing a complicated course of the disease. Blocking the virus vital activity in the body due to the drugs of a direct antiviral action allows, in its turn, to reduce the pathological effect of the virus and, accordingly, will help to reduce the severity of post-infection complications [8].

One of the most studied modern molecules used in the treatment of COVID-19 and able of suppressing the reproduction of RNA viruses is favipiravir (6-fluoro-3-hydroxy-2-pyrazinecarboxamide). The effectiveness of this compound has been proven against various RNA viruses (influenza, including strains H1N1, H5N1, H7N9, arenaviruses, flaviviruses, alphaviruses, etc.), which has been demonstrated in clinical and experimental studies [9–11, 13]. The active form of favipiravir selectively interacts with RdRp, is included in the emerging viral RNA chain, or binds to preserved polymerase domains, blocking the viral RNA replication, which leads to the utilization of the “defective” RNA and disappearance of the viral genome. The drug-induced “fatal” mutagenesis in widespread coronaviruses, as well as a selective inhibition of RdRp, allows us to consider favipiravir as a universal inhibitor of epidemiologically significant RNA-containing viruses – the main causative agents of seasonal ARVI [14, 15].

According to the clinical trials, in COVID-19 patients, the favipiravir-based direct antiviral drug Areplivir, registered in Russia and widely used, has manifested a high efficacy and safety in comparison with the standard therapy [16]. The use of favipiravir for the treatment of the infection caused by the SARS-CoV-2 coronavirus, reduces the clinical improvement period by an average of 4 days compared with the standard therapy, reaches, according to the computer tomography (CT) data, the lungs state improvement and eliminates the virus in more than 90% of patients. All these factors contribute to the acceleration of recovery. Timely initiation of therapy with favipiravir (Areplivir) improves the prognosis of the disease and reduces the global socio-economic burden of the current pandemic [8, 9].

The scheme of the early and effective direct etiotropic favipiravir therapy at the outpatient stage, preserved in the Interim Guidelines[1] for the Prevention, Diagnosis and Treatment of a New Coronavirus Infection, Version 15, takes priority and prevents the development of severe forms of infection.

The etiotropic therapy of acute respiratory viral infections in a pandemic, even with a negative polymerase chain reaction (PCR) test for COVID-19, is reasonable in comorbid patients in the context of preventing the pro-

[1] Interim guidelines “Prevention, diagnosis and treatment of a new coronavirus infection (COVID-19)”. Version No. 15 of 22 Feb 2022. Available from: https://static-0.minzdrav.gov.ru/system/attachments/attaches/000/059/392/original/BMP_COVID-19_V15.pdf. Russian

gression of the disease to a more severe form and the development of life-threatening conditions, minimizes the risk of post-infectious complications and improves the quality of patients' life [10, 12, 16].

It is important to study the long-term effects of favipiravir not only in terms of registering clinical differences (complication rates, etc.), but also through the analysis of immune-regulatory mechanisms, and, most significantly, in the group of patients with mild and moderate COVID-19 forms. Having pantropism and a proven importance in the COVID-19 pathogenesis, cytokines are relevant candidate molecules that determine post-COVID complications. The IL-6 family is being paid additional attention: a number of researchers argue that IL-6 is an independent predictor of severity and mortality from COVID-19 [17].

THE AIM of the article is to study long-term cytokine-mediated (IL-6/sIL6r and LIF/sLIFr) pathogenetic effects of the favipiravir (Areplivir®) based drug on the incidence of complications in patients with metabolic syndrome in the post-COVID period.

MATERIALS AND METHODS

With the approval of the local ethics committee at the National Research Ogarev Mordovia State University (Protocol No. 5 dated May 17, 2020) "An open prospective comparative study of the Areplivir (Favipiravir) drug effectiveness in reducing the risk of complications in the post-COVID period in patients with metabolic syndrome" in the Republic of Mordovia was carried out. The study included 190 metabolic syndrome patients who received the outpatient treatment for COVID-19 at Saransk polyclinics from February 2021 to March 2021. The case of COVID-19 was diagnosed in accordance with the current Temporary Guidelines for the prevention, diagnosis and treatment of the new coronavirus infection[2].

The study included patients of both sexes aged 50-65 years with laboratory and clinically confirmed mild and moderate forms of the novel coronavirus infection, in combination with metabolic syndrome (AH, the increased body mass index), established before the SARS-CoV-2 infection. Previously, the blood pressure control had been reached with antihypertensive drugs and low-density lipoproteins (LDL) levels – with drugs from the statin group, in case the duration of COVID-19 before the prescription of treatment had lasted no more than 5 days. Two groups were formed as follows: the patients who, along with the anti-inflammatory, anticoagulant and symptomatic therapy, were administrated with the antiviral drug Areplivir® at the outpatient stage during the acute course of COVID-19; and a comparison group – the patients who, according to the Temporary Guidelines[3], were administrated with the basic anti-inflammatory, anticoagulant, symptomatic (antibacterial) therapy for the coronavirus infection, and did not receive, for various reasons, antiviral drugs.

The favipiravir based drug was administered 30 minutes before meals p. o., according to the following scheme. For the patients weighing less than 75 kg – 1600 mg (8 tablets) twice on the 1st day of therapy, then 600 mg (3 tablets) twice per day from the 2nd to the 10th day; for the patients weighing more than 75 kg – 1800 mg (9 tablets) twice on the 1st day of therapy, then, in accordance with the instructions for use of the medicinal product, 800 mg (4 tablets) twice per day from the 2nd to the 10th day of therapy)[4].

The exclusion criteria were: associated clinical conditions in past medical history – acute cerebrovascular accident (CVA), myocardial infarction (MI), angina pectoris, coronary revascularization, renal failure, type 1 diabetes mellitus, autoimmune, allergic diseases, symptomatic hypertension, use of glucocorticosteroids, hydroxychloroquine, other antiviral drugs (except Areplivir®) and / or immunomodulators at the outpatient stage, a history of vaccination for the prevention of COVID-19, a patient's refusal to a long-term participation in the study.

Within one year (once every 2 months), at the post-COVID stage, 170 patients of these groups were interviewed with the registration of the post-COVID period features according to the developed questionnaire and the verification of the changes based on the outpatient records analysis. Within one year on days 10, 30 and 180 after a clinical and laboratory recovery (2 negative PCR test results for coronavirus RNA), all patients underwent blood sampling with the entry into the outpatient cards to determine the levels of ALT, AST, blood creatinine to calculate glomerular filtration, as well as the LDL control. The patients' characteristics at the time of the acute period of COVID-19 are presented in Table 1. The mean age of patients was 59 (95% CI [50–65]) years.

Obtaining biological material (blood) for the study, was carried out taking into account the provisions of World Medical Association's Declaration of Helsinki[5] (2013) and the protocol of the Convention of the Council of Europe on Human Rights and Biomedicine (1999), taking into account the additional protocol to the Convention on Human Rights and Biomedicine in the field of biomedical testing[6] (2005). Additional blood sampling in this category of patients was carried out after two negative PCR results on the presence of SARS CoV-2 virus RNA on days 10, 60, 180 in the morning on an empty stomach

[2] Interim guidelines "Prevention, diagnosis and treatment of a new coronavirus infection (COVID-19)". Version No.13.1 of 09 Nov 2021). Available from: https://static-0.minzdrav.gov.ru/system/attachments/attaches/000/058/211/original/BMP-13.pdf. Russian

3 Ibid.

[4] State Register of Medicinal Products of the Russian Federation. Areplivir®. Avaialable from: https://grls.rosminzdrav.ru/ЛП-007609-171121.

[5] World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013 Nov 27;310(20):2191-4. DOI: 10.1001/jama.2013.281053.

[6] Kholodova EI, Turshuk LD. Bioethics and Human Rights: International Legal Regulation and Ways of its Implementation. Actual Problems of Russian Law. 2017;(3):193-198. DOI: 10.17803/1994-1471.2017.76.3.193-198. Russian

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-217-228

(12 hours without eating). The blood was centrifuged, followed by separation of the serum and storage in labeled test tubes at -30°C for no more than 45 days. Then, the parameters were analyzed, their choice had been justified by the data of the previously conducted study of their own, including 32 cytokines and 5 vasoactive substances (NO, SDMA, ADMA, iNOS, eNOS). They demonstrated the significance of the blood levels role of IL-6 family members in the pathogenesis of cardiovascular complications [5], as well as the literature data on the pathogenetic role of representatives of the IL-6 family in the pathogenesis of COVID-19 [18].

The levels of cytokines (IL-6, sIL-6r, LIF, sLIFr), as well as vasoactive substances (NO, SDMA, ADMA, iNOS, eNOS) were determined by the enzyme-linked immunoelectrodiffusion essay (ELISA) in the laboratory of the Department of Immunology, Microbiology, Virology with a course of clinical immunology and Allergology at the National Research Ogarev Mordovia State University on the enzyme immunoassay analyzer "Personal Lab TM" (Adaltis, Italy). The following test systems were used: LIF (eBioscience (Bender MedSystems, Austria) – the analytical sensitivity of the test system: 0.66 pg/ml, the Detection interval: 0.66–200 pg/ml; sLIF-R/gp190 (eBioscience (Bender MedSystems, Austria) Austria) – the analytical sensitivity of the test system: 0.052 ng/ml, the detection interval: 0.052–5 ng/ml; IL-6 (eBioscience (Bender MedSystems, Austria) – the detection interval: 0.92–100 pg/ml, the analytical sensitivity test system: 0.92 pg/ml; sIL-6R (eBioscience (Bender MedSystems, Austria) – the analytical sensitivity of the test system: 0.01 ng/ml, the detection interval: 0.01–5 ng/ml; NO (R&D Systems, USA) – the detection interval: 0.78–200 µmol/L, the analytical sensitivity of the test system: 0.78-200 µmol/L; iNOS (USCN Life Science, Malaysia) – the detection interval: 0.064–10 ng/L ml, the analytical sensitivity of the test system: 0.064 ng/ml; eNOS (USCN Life Science, Malaysia) – the detection interval: 5.5–1000 pg/ml, the analytical sensitivity of the test system: 5.5 pg/ml; ADMA (Imm undiagnostik, Germany) – the detection interval: 0.0–2 µmol/l, the analytical sensitivity of the test system: 0.04 µmol/l; SDMA (Immundiagnostik, Germany) – the detection interval: 0.05-4 µmol/l, the sensitivity: 0.05 µmol/l.

Based on the results of non-invasive arteriography, the following indicators were analyzed: SPVkf, as well as calculated hemodynamic parameters with the introduction of the EchoCG data: PVR (DIN * sec / ml) = 1332 * Mean BP / Minute blood volume[7].

Statistical processing of results

Statistical processing of the obtained data was carried out using Stat Soft Statistica 13.5. Results are shown with median (Me) and percentiles (Q 0.25–Q 0.75). The distribution of the indicators differed from the normal distribution of Gauss – Laplace, therefore, when comparing the dependent samples, the Wilcoxon test was used; for the unrelated samples, it was the Mann-Whitney U-test, the Spearman's correlation coefficient (significant at $p<0.05$). The absolute and relative risks were calculated with the determination of 95% confidence interval (CI), the sensitivity and specificity (χ^2). A multivariate correlation analysis was carried out based on the construction of the Cox regression model.

RESULTS

Within a year after suffering COVID-19, the observational analysis of patients with MS (AH, an increased body mass index) revealed significant differences in the incidence of complications depending on the intake of the drug from the favipiravir group. Thus, the patients whose therapy regimens included favipiravir at the outpatient stage were characterized by a decrease in the incidence of ACVE and ACS by 2.66 times (Table 2). Despite the risks of concomitant diseases complications due to timely viral load relief, in a greater number of cases, the patients in this group compared with the group without antiviral therapy, retained glucose tolerance without taking hypoglycemic drugs and control of blood pressure levels without changing antihypertensive drug regimens. The results obtained were statically confirmed based on the calculation of Pearson's coefficient and the assessment of the strength of the revealed correlations. The maximum strength of the correlation was determined between taking favipiravir and a decrease in the frequency of an increase in the LDL blood level when compared with the period before the SARS-CoV-2 infection (Table 2). That indicates a positive protective effect of favipiravir therapy on the patients' lipid profile. Herewith, there were no significant differences in the frequency analysis of the decrease in GFR and an increase in ALT, AST and bilirubin ($p>0.05$) in the patients of both groups. That confirms the available data on a favorable safety profile of the therapy even in comorbid patients.

In the authors' previous studies, a significant effect of changes in the level of cytokines on the pathogenesis of cardiovascular complications (ACVE, transient cerebrovascular accidents with a cognitive decline, acute coronary syndrome) in AH patients at stage II in the post-COVID period, was shown [5]. The recent publications also describe the role of members of the IL-6 family in the pathogenesis of COVID-19 [18]. To understand the immunopathogenesis of the above listed complications and reduce the risk of their development when using a favipiravir based drug in the patients in the acute stage of COVID-19, the content dynamics of IL-6 /sIL6r and LIF/sLIFr in the peripheral blood serum, was analyzed (Table 3).

[7] Savitsky NN. Biofizicheskie osnovy krovoobrashcheniya i klinicheskie metody izucheniya gemodinamiki [Biophysical bases of blood circulation and clinical methods for studying hemodynamics]. Medical Sciences Academy of the USSR. 3rd ed., rev. and addit. Leningrad: Medicine. Leningrad. dept. 1974: 311 p. Russian

Table 1 – Characteristics of COVID-19 patients (Me [$Q_{25\%}$–$Q_{75\%}$]) included in the study

Parameters of patients' anamnesis and conditions	Without taking antiviral drugs at the outpatient stage (n=64)	Taking favipiravir at the outpatient stage (n=68)
Illness-term before therapy (days)	4.16 [2.17–5.22]	4.17 [2.91–4.56]
Maximum percentage of lungs damage during disease period (%)	12.7 [6–28.2]	8.12 [5.24–27.2]
Presence of comorbid diseases	–	–
AH	100%	100%
Past medical history of type 2 diabetes mellitus	40%	100%
Obesity	100%	100%
BMI	37.4 [35.2–40.4]	38.9 [36.3–42.7]
SpO_2, %	97.98 [96.4–98.6]	98.1 [96.3–99]
C-, mg/l	6.43 [6.65–9.13]	5.46 [4.05–8.54]
D-dimer, ng/ml	238 [196–435]	223 [187–305]
Glucose, mmol/l	4.41 [2.8–5.2]	4.23 [2.72–5.92]
Hemoglobin, g/l	125 [112–137]	122 [117–141]

Table 2 – Analysis of correlation between complications development depending on the use of favipiravir based drug for COVID-19 (Me [$Q_{5\%}$–$Q_{95\%}$]) in MS patients, in post-COVID period

Indicators	Favipiravir (n=68)	No antivirals (n=64)
Numbers of ACVEs and ACSs	3	12
Relative Risk	2.66 [1.23–14.1] * (Se-0.8, Sp-0,55), χ^2=6.73 (p=0.01), medium correlation	
First recorded increase in blood glucose above 10 against the background of diet and / or use of hypoglycemic agents	5	13
Relative Risk	2.72 [1.03–7.2] * (Se-0.72, Sp-0.54) χ^2=4.7 (p=0.031), medium correlation	
Changing antihypertensive therapy regimen due to its inefficiency	9	27
Relative Risk	3.19 [1.63–6.25]* (Se-0.75, Sp-0.61) χ^2=13.9 (p<0.001), relatively strong correlation	
Increase in LDL levels	9	24
Relative Risk	2.83 [1.43–5.62]* (Se-0.73, Sp-0.6) χ^2=10.3 (p=0.002), medium correlation	
Increase in ALT, AST, bilirubin	6	5
Relative Risk	0.88 [0.28–2.76] (Se-0.45, Sp-0.51) χ^2=0.044 (p=0.83), insignificant correlation	
Decrease in GFR	6	4
Relative risk	0.7 [0.21–2.39] (Se-0.4, Sp-0.51) χ^2=0.31 (p=0.58), insignificant correlation	

Note: * – significant difference in the analysis of risk ratios.

Table 3 – Dynamics of changes in the content of IL-6 family cytokines depending on the intake of favipiravir based drug for COVID-19 Me [$Q_{25\%}$–$Q_{75\%}$], in MS patients in post-COVID period

Therapy	Favipiravir (n=68)			No antivirals (n=64)		
Period	In 10 days	In 30 days	In 180 days	In 10 days	In 30 days	In 180 days
IL-6, pg/ml	24.2 [22.8–27.3]	16.8*[1] [14.1–21.4]	13.1*[1,2] [10.7–16.3]	34.2 *[1] [28.5–36.7]	25.9*[2,4] [23.8–31.2]	25.1 *[3,4] [22.9–31.1]
sIL-6r, pg/ml	2160 [1548–2430]	1850'[1] [1240–2060]	1615*[1]^[2] [1470–1820]	3100 *[1] [2330–3470]	3620 *[2,4] [2980–4634]	2971 *[3,5] [2156–3365]
LIF, pg/ml	9.17 [8.23–11.3]	7.22 [6.2–9.24]*[1]	7.47 [6.15–9.12]*[1]	12.3 [10.3–14.8]*[1]	15.7*[2,4] [12.7–19.5]	15.5*[3,4] [11.2–18.7]
sLIFr, pg/ml	3520 [2980–4260]	4100'[1] [3420–4900]	2800 *[1,2] [2170–3120]	4810*[1] [3970–5530]	6200*[2,4] [4500–7610]	7460 *[3,4,5] [6120–9400]

Note: * – p<0.001, ^ – p<0.01, '– p<0.05 – significance level in accordance with the specified group based on the Wilcoxon test for related populations and the Mann-Whitney U-test for unrelated populations.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-217-228

Table 4 – Influence analysis of IL-6, sIL-6, LIF, LIFr contents in patients with stage II MS on the incidence of complications (95% CI) within 1 year after suffering COVID-19

Variables	Beta	Standard	t-value	Exponent Beta	Wald	P
IL-6 (>23.8 pg/ml)	1.07	0.63	1.69	2.17	2.73	0.062
sIL-6r (>2212 pg/ml)	1.17	0.67	1.77	1.38	2.34	0.072
LIF (>9.78 pg/ml)	1.12	0.79	1.95	2.04	1.6	0.093
sLIFr (>5074 pg/ml)	2.29	0.33	6.93	4.75	13.3	0.009

Note: Cox regression model and multivariate analysis are presented.

Table 5 – Correlation matrix of cytokine content in peripheral blood serum and hemodynamic parameters in MS patients in post-COVID period

Interleukine / Indicators	IL-6	sIL-6r	LIF	sLIFr
NO	0.64. p<0.05	0.46 p>0.05	0.49 p<0.05	–0.47 p<0.05
ADMA	0.52. p>0.05	0.4 p>0.05	0.58 p<0.05	0.86 p<0.001
SDMA	0.34. p>0.05	0.29 p>0.05	0.16 p>0.05	0.88 p<0.001
eNOS	–0.62 p<0.05	–0.67 p<0.05	–0.12 p>0.05	–0.72 p <0.001
iNOS	0.78 p<0.001	0.49 p>0.05	0.51 p<0.05	0.36 p>0.05
PVR	0.55 p<0.05	0.39 p>0.05	0.89 p<0.001	0.81 p<0.001
PWVcf	0.51 p<0.05	0.34 p>0.05	0.51 p<0.05	0.87 p<0.001

The table shows that the patients administrated with Areplivir at the early stage of infection, were characterized by lower serum levels of the four members of the IL-6 family (IL-6, sIL6r and LIF, sLIFr) 10 days after their clinical and laboratory recovery (p<0.001). This pattern persisted 30 and 180 days after COVID-19. Significant differences in the dynamics of cytokine parameters were revealed. In the patients administrated with Areplivir in past medical history, a dynamic decrease in IL-6 and its soluble receptor is recorded both in the interval from day 10 to day 30 (by 31 and 25%, respectively) and from day 30 to day 180 (by 19 and 23%, respectively) after suffering COVID-19. In the persons not administrated with antiviral drugs, the dynamics was different: a decrease in IL-6 from day 10 to day 30 by 24% (p<0.01), but there was no dynamics from day 30 to day 180 (p>0.05). The concentration of sIL-6r in the blood was characterized by an increase by 24% in the interval from day 10 to day 30 (p<0.001) and a decrease up to the level of day 10 in the period from day 30 to day 180 (Table 3). The presented average statistical patterns of changes in the IL-6 /sIL6r system are typical for 90% (77 people out of 86) from the group taking the favipiravir based drug during the COVID-19 period, and 85% (54 people out of 64) from the group not taking antiviral drugs. The differences were statistically significant (p<0.01).

Thus, the advisability of the early etiotropic favipiravir based therapy was also shown in terms of reducing the risk of developing long-term consequences of the coronavirus infection.

In the comparison of groups, the indicators analysis of the LIF system and its soluble receptor revealed multidirectional dynamics of changes. The patients taking the favipiravir-based drug are characterized by a decrease in serum LIF from day 10 to day 30 by 22% (p<0.001), but there was no further decrease from day 30 to day 180. The patients without taking antiviral drugs in past medical history were characterized by an increase in peripheral serum LIF content in the period from day 10 to day 30 by 22% (p<0.001), without dynamics – from day 30 to day 180. In the favipiravir group, there was a slight increase in the sLIFr levels from day 10 to day 30 by 24%, with a pronounced decrease by 32% (p<0.001) from day 30 to day 180. In the group without favipiravir in past medical history, there was an increase in the content of sLIF in the blood serum from day 10 to day 30 by 23% and from day 30 to day 180 by 17%, (p<0.001). It is important to note the importance of the analysis of patients' individual indicators. Thus, in the group with an official favipiravir intake in past medical history, 4 out of 68 patients were characterized by individual dynamics corresponding to the patients without antiviral drugs. There was an increase in the sLIFr serum by 23% from day 10 to day 30 and by 21% from day 30 to day 180; 3 of them suffered a stroke during the next 6 months of the observation. Herewith, in 10 patients from the group without favipiravir in past medical history, there was an increase in sLIFr by 54% from day 10 to day 30 and by 48% from day 30 to day 180; 8 of them suffered ACVE and ACS in the subsequent observation period. The data obtained confirm the hypothesis of a negative impact of the growth of the above listed indicators on the prognosis, and serve as a rationale for the positive pathogenetic effect of the early favipiravir therapy on reducing the risk of ACVE and ACS in the post-COVID period even in the patients with comorbid conditions.

Taking into account the identified potential correllations between the degree of increase in the content of IL-6 family cytokines in the blood serum and the risk of complications in the post-COVID period, a multivariate correlation analysis was carried out in order to identify the most significant marker for the development of cardiovascular complications of the coronavirus infection. The critical levels for entering the analyses system were determined based on the interquartile analysis data (Table 4). The obtained results demonstrate an increase in the blood serum of MS patients in the post-COVID period of the sLIFr level, which has the greatest influence (among the IL-6representatives) on the increase in the risk of developing cardiovascular complications during a year after COVID-19. The above-described pathological processes are observed in the group of patients without taking antiviral drugs in past medical history. In order to construct a potential pathogenetic scheme, taking into account MS as a symptom complex on the basis of which, after COVID-19, cardiovascular complications developed in some patients, an analysis of the correlations of the analyzed cytokines with vasoactive substances (NO ADMA, SDMA, eNOS, iNOS) and indicators, reflecting peripheral vascular resistance – OPSS and PWVcf, was made (Table 5). ADMA and SDMA are markers of the endothelial dysfunction and blockers of the NO synthesis by reducing the eNOS formation [19]. ADMA acts as an oxidative stress mediator by downregulating eNOS and uncoupling NO synthesis pathways [20], increasing the expression of inflammatory genes. It was fouare nd out that in MS patients in the post-COVID period, the most pronounced ($p<0.001$) relationships are between an increase of sLIF in the peripheral blood serum and the levels of conditioned vasopressors ADMA and SDMA with a secondary decrease in the vasorelaxant eNOS, as well as an increase in the functional parameters of TPVR and PWVcf. Symmetrical correlation lines but of lesser strength ($p<0.05$) were registered for LIF. It is important to note that IL-6 blood levels are directly associated with an increase in iNOS ($p<0.001$) without correlation with ADMA and SDMA ($p>0.05$). The presented data demonstrate the potential of the early antiviral therapy in terms of blocking the post-COVID immunopathogenetic vector – "COVID-19 in anamnesis-growth of IL-6 family members-increase in vasopressors-decrease in-eNOS induced nitric oxide."

DISCUSSION

The problem of complications development in comorbid patients in the post-COVID period is becoming increasingly relevant [21]. At the same time, persistent endotheliopathy during recovery is not limited to those who have experienced severe COVID-19 [21], which updates the information obtained in the present study. An important aspect is the analysis aimed at identifying the factors of the acute infectious period, such as components of therapy and / or the use of additional methods for assessing the laboratory / functional characteristics of the pathological process, which are associated with a change in the incidence of complications after suffering COVID-19. A dynamic observation of patients for 1 year in the post-COVID period, demonstrates a decrease in the frequency of both cardiovascular complications (ACVE, transient cerebrovascular accidents with a cognitive decline, acute coronary syndrome), and the risk of increased blood glucose and LDL levels, the growth of which is a prognostic marker for the development of cardiovascular complications. These effects are undoubtedly pathogenetically associated with a decrease in the viral replication and viral load [22–26] with a secondary blocking of the components of a potentially excessive cytokine response, which was demonstrated in this study using representatives of the IL-6 family as an example.

It is important to note that the study included only MS patients under clinically comparable COVID-19 medical treatment, regardless of taking an antiviral drug. But a deeper study of a cytokine regulation of the post-COVID period revealed fundamental differences in this group. Higher peripheral blood serum levels of both IL-6 and its soluble receptor (sIL-6r), which expands the spectrum of cells sensitive to this cytokine (endothelial cells are a significant component in the development of hypertension), express gp130 and not IL-6R. Therefore, these cells can respond to IL-6 only in the presence of sIL-6R [27], which is pathogenetically associated with a decrease in a significant NO vasorelaxant that determines the progression of atherosclerosis and an endothelial dysfunction with changes in the vascular tone (the study revealed the relationship of this cytokine with PVR and PWVcf). At the same time, it is important to note the change in the dynamic characteristics of IL-6: in the patients taking favipiravir during the acute infection period, a decrease in this cytokine in the blood is observed from day 10 to day 180 in the post-COVID period. At the same time, in the group without antiviral drugs, despite a decrease in the concentration of IL-6 in the first 30 days, a "plateau" is subsequently recorded, and the decrease in the level of the "dangerous" cytokine stops at the values that exceed those in the group with favipiravir. Moreover, persistent elevated levels of IL-6 are accompanied by an increase in soluble IL-6 receptor up to day 30 in the post-COVID period. Thus, the absence of the etiotropic therapy in the acute period of an infectious disease increases the risk of the components progression of the metabolic syndrome (AH, dyslipidemia, etc.).

The data on a long-term increase in IL-6 (3 or more months) in the post-COVID period and the association of this imbalance with symptoms of chronic fatigue, headaches, and changes in the metabolic processes of the brain indicating a greater degree of severity in women, have been published [28]. So, in the study by Durstenfeld M.S. et al., a hypothesis about the significance of the drugs aimed at blocking IL-6 in the post-

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-217-228

COVID period and indicating the importance of studying all immunopathophysiological processes associated with the IL-6 family, is expressed [29]. In particular, IL-6 itself promotes myocardial hypertrophy through the gp130 stimulation, activates the transcription through STAT3, causing adverse effects on the progression of a heart failure [30, 31] and hypertension as an MS component. A long-term pronounced physiological decrease in IL-6 observed in the patients taking Areplivir at the early stages of a COVID infection, indicates a decrease in the risk of developing not only life-threatening diseases (a heart failure, ACVE, etc.), but also the conditions that sharply worsen the quality of patients' life (chronic fatigue, headaches, etc.).

The data on changes in the LIF/sLIFr system due to their multidirectional dynamics in the post-COVID period in MS patients, depending on the past medical history of taking a favipiravir-based drug, are of great interest. LIF is a pleiotropic cytokine of the IL-6 family, the effect of which depends on the localization of the target cells [29]. The role of LIF in the regulation of the cardiovascular system has been described [29]. In the first month after COVID-19, an increase in the LIF and sLIFr peripheral blood serum is blocked in the group of patients taking a favipiravir-based drug, while in MS patients who did not undergo the antiviral therapy; an increase in the level of the above-mentioned indicators is recorded. At the same time, the data on the LIF effect on the vascular wall in AH are controversial [32,33], since against the background of the increased blood pressure, the LIF effects are distorted with the abolition of protection against the myocardium and endothelium. According to the data presented in the article, LIF correlates with the NO, SDMA level, thereby confirming the previously put forward hypothesis about the negative effect of this cytokine growth in the blood serum of patients with hypertension, including the ones within MS.

When analyzing the relationships between the studied cytokines and the frequency of cardiovascular complications, a multivariate correlation analysis revealed the priority of an increase in sLIFr in the peripheral blood serum as a risk marker of the developing ACVE and MI. sLIFr is a factor with a potentially antagonistic effect on LIF [34]. At the same time, in the earlier studies, there were the data on potential intrinsic sLIFr effects which have been undeservedly not studied [35,36] though their search is relevant. Previously, the research group published the data [37] demonstrating negative dose-dependent sLIF effects (at the level of more than 4800 pg/ml) on the AH progression, including the ones through positive correlations with SDMA and ADMA. That has also been confirmed in MS patients in the post-COVID period. In the patients without the antiviral therapy, the quantitative characteristics of the sLIF content in the post-COVID period correspond to a pathogenetically critical level (more than 4800 pg/ml) with a further increase and a correlation with an increase in SDMA and ADMA against the background of a decrease in NO and eNOS. That was clinically accompanied by a deterioration in the severity of the metabolic syndrome components.

It should be noted that an important component of the study was the analysis of hepatorenal complications. Their frequency did not differ in the groups of patients who received and the ones who did not receive favipiravir, which indicates a high safety profile of the therapy.

The most important protective effect of the early etiotropic therapy based on favipiravir is a significant reduction in the incidence of cardiovascular complications (ACS, ACVE) in the post-COVID period, which can be explained by blocking the components of the analyzed vector: "the iNO-dependent progression of the endothelial dysfunction – tissue remodeling of the cardiovascular complex – cardiovascular complications". According to Merkler A.E. et al. [38], the frequency of cerebral strokes in the post-COVID period reaches 1.6% and is 8 times higher than the frequency of similar complications in patients with influenza. It is assumed that this is due to the development of the acute endothelial dysfunction and a shift of hemostasis to the procoagulant side in COVID-19. Taking into account the number of patients who have survived a novel coronavirus infection, and the cost of one completed case of treating a patient with an ischemic stroke[8], the economic burden of ACVE alone associated with the suffered COVID-19 may be more than 20 billion rubles over the past pandemic period. Taking into account the above-mentioned results of the present study, the earlier administration of the favipiravir-based etiotropic therapy is appropriate not only clinically, but also pharmacoeconomically, by minimizing the risks of post-COVID complications, especially in comorbid patients.

CONCLUSION

It is important that the COVID-19 therapy regimen determines not only the risk of a severe and an extremely severe course of an infectious disease, but can directly affect the risk of both the progression of the patient's concomitant diseases and the development of new pathological conditions in the post-COVID period.

The Areplivir therapy in the acute period of the coronavirus infection allows a timely reduction of the viral load, which contributes to the correction of the pro-inflammatory vector of the immune response at the post-COVID stage. Accordingly, it reduces the risk of the atherosclerosis progression, transient cerebrovascular accidents with a cognitive decline, an endothelial dysfunction, and can be considered as a secondary prevention of cardiovascular complications.

The study provides valuable information on the complication patterns seen in after COVID-19 patients in

[8] Decree of the Government of the Russian Federation of December 7, 2019 No.1610 "On the Program of State Guarantees of Free Provision of Medical Care to Citizens for 2020 and for the Planning Period of 2021 and 2022" (with amendments and additions). Available from: https://base.gar. Russian

clinical practice, and strengthens the evidence that the favipiravir-based drug can be considered in the context of a positive effect on minimizing the symptoms in the post-COVID period.

Taking into account the frequency of decrease in IL-6 levels in the study drug group, it can be concluded that etiotropic therapy in the early stages helps to reduce the risk of life-threatening conditions and postcovid complications by 82% during the first year after COVID-19 in comorbid patients.

FUNDING

This study did not receive financial support from third parties.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Olga A. Radaeva – development and implementation of research design, research, aim setting, results analysis, text writing; Larisa A. Balykova – development and implementation of research design, text writing and editing; Kira Ya. Zaslavskaya – results analysis, text writing; Aleksey V. Taganov – results analysis, text editing; Petr A. Bely – results analysis; Yuliya A. Kostina – statistical processing of the experiment results; Elena V. Negodnova – statistical processing of the experiment results, study aim setting, results analysis; Svetlana V. Mashnina – statistical results processing, study aim setting, results analysis, Denis D. Bessheinov – statistical results processing, study aim setting, results analysis; Maria S. Iskandyarova – results analysis, text writing; Vitaliy V. Eremeev – control of material intake; Nikita M. Chumakov – statistical data processing.

REFERENCES

1. Shah SM, Odanovic N, Kunnirickal S, Feher A, Pfau SE, Spatz ES. Chest pain and coronary endothelial dysfunction after recovery from COVID-19: A case series. Clin Case Rep. 2022 Apr 8;10(4):e05612. DOI: 10.1002/ccr3.5612.
2. Davis HE, Assaf GS, McCorkell L, Wei H, Low RJ, Re'em Y, Redfield S, Austin JP, Akrami A. Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. EClinicalMedicine. 2021 Aug;38:101019. DOI: 10.1016/j.eclinm.2021.101019.
3. Besnier F, Bérubé B, Malo J, Gagnon C, Grégoire CA, Juneau M, Simard F, L'Allier P, Nigam A, Iglésies-Grau J, Vincent T, Talamonti D, Dupuy EG, Mohammadi H, Gayda M, Bherer L. Cardiopulmonary Rehabilitation in Long-COVID-19 Patients with Persistent Breathlessness and Fatigue: The COVID-Rehab Study. Int J Environ Res Public Health. 2022 Mar 31;19(7):4133. DOI: 10.3390/ijerph19074133.
4. Sanyaolu A, Marinkovic A, Prakash S, Zhao A, Balendra V, Haider N, Jain I, Simic T, Okorie C. Post-acute Sequelae in COVID-19 Survivors: an Overview. SN Compr Clin Med. 2022;4(1):91. DOI: 10.1007/s42399-022-01172-7.
5. Radaeva OA, Simbirtsev AS, Kostina YuA, Iskandiarova MS, Mashnina SV, Bessheynov DD, Negodnova EV, Kulyapkin VV. Changes in blood levels of IL1 family cytokines in patients with essential hypertension after having COVID-19. Bulletin of RSMU. 2021; (3): 23–8. DOI: 10.24075/brsmu.2021.026.
6. Acosta-Ampudia Y, Monsalve DM, Rojas M, Rodríguez Y, Zapata E, Ramírez-Santana C, Anaya JM. Persistent Autoimmune Activation and Proinflammatory State in Post-COVID Syndrome. J Infect Dis. 2022 Jan 25:jiac017. DOI: 10.1093/infdis/jiac017.
7. Tserel L, Jõgi P, Naaber P, Maslovskaja J, Häling A, Salumets A, Zusinaite E, Soeorg H, Lättekivi F, Ingerainen D, Soots M, Toompere K, Kaarna K, Kisand K, Lutsar I, Peterson P. Long-Term Elevated Inflammatory Protein Levels in Asymptomatic SARS-CoV-2 Infected Individuals. Front Immunol. 2021 Sep 17;12:709759. DOI: 10.3389/fimmu.2021.709759.
8. Balykova LA, Radaeva OA, Zaslavskaya KY, Kostina YuA, Iskandyarova MS, Negodnova EV, Eremeev VV, Sabirov LF, Semeleva EV. Study of clinical and pathogenetic effects of anti-viral drug based on favipiravir in comorbid patients with COVID-19 at the outpatient stage of treatment. Pharmacy & Pharmacology. 2021;9(6):454–64. DOI: 10.19163/2307-9266-2021-9-6-454-464.
9. Amon E.P., Esaulenko E.V., Taganov A.V., Shiryaeva M.A., Malinnikova E.Y. Terapiya ostryh respiratornyh virusnyh infekcij v ambulatornoj praktike v usloviyah pandemii COVID-19 [Therapy of acute respiratory viral infections in outpatient practice during the COVID-19 pandemic]. Therapy. 2022; 8(3).16–28. DOI: 10.18565/therapy.2022.3.1426. Russian
10. Munblit D, Nekliudov NA, Bugaeva P, Blyuss O, Kislova M, Listovskaya E, Gamirova A, Shikhaleva A, Belyaev V, Timashev P, Warner JO, Comberiati P, Apfelbacher C, Bezrukov E, Politov ME, Yavorovskiy A, Bulanova E, Tsareva N, Avdeev S, Kapustina VA, Pigolkin YI, Dankwa EA, Kartsonaki C, Pritchard MG, Fomin V, Svistunov AA, Butnaru D, Glybochko P; Sechenov StopCOVID Research Team. Stop COVID Cohort: An Observational Study of 3480 Patients Admitted to the Sechenov University Hospital Network in Moscow City for Suspected Coronavirus Disease 2019 (COVID-19) Infection. Clin Infect Dis. 2021 Jul 1;73(1):1–11. DOI: 10.1093/cid/ciaa1535.
11. McArthur L, Sakthivel D, Ataide R, Chan F, Richards JS, Narh CA. Review of Burden, Clinical Definitions, and Management of COVID-19 Cases. Am J Trop Med Hyg. 2020 Aug;103(2):625–38. DOI: 10.4269/ajtmh.20-0564.
12. Granovskaya MV, Zaslavskaya KYa, Balykova LA, Pushkar DYu. COVID-19 – a set of symptoms or a systemic pathology? Clinical lecture. Part 2. Areplivir (favipiravir) in the treatment of patients with coronavirus infection: background of use and first results. Infektsionnye bolezni: novosti, mneniya, obuchenie [Infectious Diseases: News, Opinions, Training]. 2020; 9 (3): 10–7. DOI: 10.33029/2305-3496-2020-9-3S-10-17. Russian
13. Rocha-Pereira J, Jochmans D, Dallmeier K, Leyssen P, Nascimento MS, Neyts J. Favipiravir (T-705) inhibits in vitro nor-

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2022-10-2-217-228

ovirus replication. Biochem Biophys Res Commun. 2012 Aug 10;424(4):777–80. DOI: 10.1016/j.bbrc.2012.07.034.
14. Zmurko J, Marques RE, Schols D, Verbeken E, Kaptein SJ, Neyts J. The Viral Polymerase Inhibitor 7-Deaza-2'-C-Methyladenosine Is a Potent Inhibitor of In Vitro Zika Virus Replication and Delays Disease Progression in a Robust Mouse Infection Model. PLoS Negl Trop Dis. 2016 May 10;10(5):e0004695. DOI: 10.1371/journal.pntd.0004695.
15. Furuta Y, Takahashi K, Kuno-Maekawa M, Sangawa H, Uehara S, Kozaki K, Nomura N, Egawa H, Shiraki K. Mechanism of action of T-705 against influenza virus. Antimicrob Agents Chemother. 2005 Mar;49(3):981–6. DOI: 10.1128/AAC.49.3.981-986.2005.
16. Furuta Y, Komeno T, Nakamura T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. Proc Jpn Acad Ser B Phys Biol Sci. 2017;93(7):449–63. DOI: 10.2183/pjab.93.027.
17. Balykova LA, Granovskaya MV, Zaslavskaya KYa, Simakina EN, Agaf'ina AS, Ivanova AYu, Kolontarev KB, Pushkar DYu. New possibilities for targeted antiviral therapy for COVID-19. Results of a multicenter clinical study of the efficacy and safety of using the drug Areplivir. Infektsionnye bolezni: novosti, mneniya, obuchenie [Infectious Diseases: News, Opinions, Training]. 2020; 9 (3): 16–29. DOI: 10.33029/2305-3496-2020-9-3-16-29. Russian
18. Halim C, Mirza AF, Sari MI. The Association between TNF-α, IL-6, and Vitamin D Levels and COVID-19 Severity and Mortality: A Systematic Review and Meta-Analysis. Pathogens. 2022 Feb 1;11(2):195. DOI: 10.3390/pathogens11020195.
19. Neves JA, Oliveira RCM. Biomarkers of endothelial function in cardiovascular diseases: hypertension. J Vasc Bras. 2016; 15(3):224–33. DOI:10.1590/1677-5449.000316.
20. Chandra D, Poole JA, Bailey KL, Staab E, Sweeter JM, DeVasure JM, Romberger DJ, Wyatt TA. Dimethylarginine dimethylaminohydrolase (DDAH) overexpression enhances wound repair in airway epithelial cells exposed to agricultural organic dust. Inhal Toxicol. 2018 Feb;30(3):133–9. DOI: 10.1080/08958378.2018.1474976.
21. Fogarty H, Townsend L, Morrin H, Ahmad A, Comerford C, Karampini E, Englert H, Byrne M, Bergin C, O'Sullivan JM, Martin-Loeches I, Nadarajan P, Bannan C, Mallon PW, Curley GF, Preston RJS, Rehill AM, McGonagle D, Ni Cheallaigh C, Baker RI, Renné T, Ward SE, O'Donnell JS; Irish COVID-19 Vasculopathy Study (iCVS) investigators. Persistent endotheliopathy in the pathogenesis of long COVID syndrome. J Thromb Haemost. 2021 Oct;19(10):2546–53. DOI: 10.1111/jth.15490.
22. Doi Y, Hibino M, Hase R, Yamamoto M, Kasamatsu Y, Hirose M, Mutoh Y, Homma Y, Terada M, Ogawa T, Kashizaki F, Yokoyama T, Koba H, Kasahara H, Yokota K, Kato H, Yoshida J, Kita T, Kato Y, Kamio T, Kodama N, Uchida Y, Ikeda N, Shinoda M, Nakagawa A, Nakatsumi H, Horiguchi T, Iwata M, Matsuyama A, Banno S, Koseki T, Teramachi M, Miyata M, Tajima S, Maeki T, Nakayama E, Taniguchi S, Lim CK, Saijo M, Imai T, Yoshida H, Kabata D, Shintani A, Yuzawa Y, Kondo M. A Prospective, Randomized, Open-Label Trial of Early versus Late Favipiravir Therapy in Hospitalized Patients with COVID-19. Antimicrob Agents Chemother. 2020 Nov 17;64(12):e01897-20. DOI: 10.1128/AAC.01897-20.
23. Gao Y, Yan L, Huang Y, Liu F, Zhao Y, Cao L, Wang T, Sun Q, Ming Z, Zhang L, Ge J, Zheng L, Zhang Y, Wang H, Zhu Y, Zhu C, Hu T, Hua T, Zhang B, Yang X, Li J, Yang H, Liu Z, Xu W, Guddat LW, Wang Q, Lou Z, Rao Z. Structure of the RNA-dependent RNA polymerase from COVID-19 virus. Science. 2020 May 15;368(6492):779–82. DOI: 10.1126/science.abb7498.
24. Shiraki K, Daikoku T. Favipiravir, an anti-influenza drug against life-threatening RNA virus infections. Pharmacol Ther. 2020 May;209:107512. DOI: 10.1016/j.pharmthera.2020.107512.
25. Furuta Y, Gowen BB, Takahashi K, Shiraki K, Smee DF, Barnard DL. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antiviral Res. 2013 Nov;100(2):446–54. DOI: 10.1016/j.antiviral.2013.09.015.
26. Delang L, Abdelnabi R, Neyts J. Favipiravir as a potential countermeasure against neglected and emerging RNA viruses. Antiviral Res. 2018 May;153:85–94. DOI: 10.1016/j.antiviral.2018.03.003.
27. Rose-John S. Interleukin-6 Family Cytokines. Cold Spring Harb Perspect Biol. 2018 Feb 1;10(2):a028415. DOI: 10.1101/cshperspect.a028415.
28. Ganesh R, Grach SL, Ghosh AK, Bierle DM, Salonen BR, Collins NM, Joshi AY, Boeder ND Jr, Anstine CV, Mueller MR, Wight EC, Croghan IT, Badley AD, Carter RE, Hurt RT. The Female-Predominant Persistent Immune Dysregulation of the Post-COVID Syndrome. Mayo Clin Proc. 2022 Mar;97(3):454–64. DOI: 10.1016/j.mayocp.2021.11.033.
29. Durstenfeld MS, Hsue PY, Peluso MJ, Deeks SG. Findings From Mayo Clinic's Post-COVID Clinic: PASC Phenotypes Vary by Sex and Degree of IL-6 Elevation. Mayo Clin Proc. 2022 Mar;97(3):430-432. DOI: 10.1016/j.mayocp.2022.01.020.
30. Meléndez GC, McLarty JL, Levick SP, Du Y, Janicki JS, Brower GL. Interleukin 6 mediates myocardial fibrosis, concentric hypertrophy, and diastolic dysfunction in rats. Hypertension. 2010 Aug;56(2):225–31. DOI: 10.1161/HYPERTENSIONAHA.109.148635.
31. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014 Sep 4;6(10):a016295. DOI: 10.1101/cshperspect.a016295.
32. Wu R, Wyatt E, Chawla K, Tran M, Ghanefar M, Laakso M, Epting CL, Ardehali H. Hexokinase II knockdown results in exaggerated cardiac hypertrophy via increased ROS production. EMBO Mol Med. 2012 Jul;4(7):633–46. DOI: 10.1002/emmm.201200240.
33. Zgheib C, Zouein FA, Kurdi M, Booz GW. Differential STAT3 signaling in the heart: Impact of concurrent signals and oxidative stress. JAKSTAT. 2012 Apr 1;1(2):101–10. DOI: 10.4161/jkst.19776.
34. Rose-John S. Interleukin-6 signalling in health and disease. F1000Res. 2020 Aug 20;9:F1000 Faculty Rev-1013. DOI: 10.12688/f1000research.26058.1.
35. Horowitz MC, Levy JB. The LIF/IL-6 Subfamily of Cytokines Induce Protein Phosphorylation and Signal Transduction by Nonreceptor Tyrosine Kinases in Human and Murine Osteoblasts. Calcif Tissue Int. 1995; 56: 32–4. DOI: 10.1007/BF03354650.
36. Tomida M. Structural and functional studies on the leukemia inhibitory factor receptor (LIF-R): gene and soluble form of LIF-R, and cytoplasmic domain of LIF-R required for differentiation and growth arrest of myeloid leukemic cells. Leuk Lymphoma. 2000 May;37(5–6):517–25. DOI: 10.3109/10428190009058503.

37. Radaeva OA, Kostina YuA. Analysis data of mechanism of drugs recommended for the treatment of COVID-19. Infektsionnye bolezni: novosti, mneniya, obuchenie [Infectious Diseases: News, Opinions, Training]. 2021; 10 (3): 106–17. DOI: 10.33029/2305-3496-2021-10-3-106-117. Russian

38. Merkler AE, Parikh NS, Mir S, Gupta A, Kamel H, Lin E, Lantos J, Schenck EJ, Goyal P, Bruce SS, Kahan J, Lansdale KN, LeMoss NM, Murthy SB, Stieg PE, Fink ME, Iadecola C, Segal AZ, Cusick M, Campion TR Jr, Diaz I, Zhang C, Navi BB. Risk of Ischemic Stroke in Patients With Coronavirus Disease 2019 (COVID-19) vs Patients With Influenza. JAMA Neurol. 2020 Jul 2;77(11):1–7. DOI: 10.1001/jamaneurol.2020.2730.

AUTHORS

Olga A. Radaeva – Doctor of Sciences (Medicine), Associate Professor, Head of the Department of Immunology, Microbiology and Virology with a Course of Clinical Immunology and Allergology of the Medical Institute, N.P. Ogarevs Mordovia State University. ORCID ID: 0000-0003-1383-2474. E-mail: radaevamed@mail.ru

Larisa A. Balykova – Doctor of Sciences (Medicine), Professor, Corresponding Member of the Russian Academy of Sciences, Head of the Department of Pediatrics, Directress of Medical Institute, N.P. Ogarevs Mordovia State University. ORCID ID: 0000-0002-2290-0013. E-mail: larisabalykova@yandex.ru

Kira Ya. Zaslavskaya – Assistant of the Department of Biological and Pharmaceutical Chemistry with a course of organization and management of pharmacy of the Medical Institute, N.P. Ogarevs Mordovia State University. ORCID ID: 0000-0002-7348-9412. E-mail: kiryonok@yandex.ru

Aleksey V. Taganov – Doctor of Sciences (Medicine), Professor of the Department of Dermatovenereology with a course of cosmetology of the Faculty of Continuous Medical Education of the Medical Institute, Peoples' Friendship University of Russia. ORCID ID: 0000-0001-5056-374X. E-mail: matis87177@yandex.ru.

Petr A. Bely – Candidate of Sciences (Medicine), Senior Laboratory Assistant, Department of Propaedeutics of Internal Diseases and Gastroenterology, Moscow State University of Medicine and Dentistry n. a. A.I. Evdokimov. ORCID ID: 0000-0001-5998-4874. E-mail: pbely@ncpharm.ru.

Yuliya A. Kostina – Candidate of Sciences (Medicine), Associate Professor of the Department of Immunology, Microbiology and Virology with a course of clinical immunology and allergology of the Medical Institute, N.P. Ogarevs Mordovia State University. ORCID ID: 0000-0003-1383-2474. E-mail: bazunova.2013@mail.ru

Elena V. Negodnova – assistant of the Department of Immunology, Microbiology and Virology with a course of clinical immunology and allergology of the Medical Institute, N.P. Ogarevs Mordovia State University. ORCID ID: 0000-0002-1581-618X. E-mail: el.negodnova@gmail.com

Svetlana V. Mashnina – post-graduate student of the Department of Immunology, Microbiology and Virology with a course of Clinical Immunology and Allergology of the Medical Institute, N.P. Ogarevs Mordovia State University. ORCID ID: 0000-0001-7506-3688. E-mail: svetlana_belyaeva93@mail.ru

Denis D. Bessheinov – post-graduate student of the Department of Immunology, Microbiology and Virology with a course of clinical immunology and allergology of the Medical Institute, N.P. Ogarevs Mordovia State University. ORCID ID: 0000-0001-9722-7102. E-mail: bessheynovdd@mail.ru

Maria S. Iskandyarova – Assistant of the Department of Immunology, Microbiology and Virology with a course of clinical immunology and allergology of the Medical Institute, N.P. Ogarevs Mordovia State University. ORCID ID: 0000-0001-9465-7668. E-mail: iskandiarova.m@yandex.ru

Vitaliy V. Eremeev – post-graduate student of the Department of Immunology, Microbiology and Virology with a course of clinical immunology and allergology of the Medical Institute, N.P. Ogarevs Mordovia State University. ORCID ID: 0000-0002-2293-1987. E-mail: vital.eremeev2010@mail.ru

Nikita M. Chumakov – 6th year student, Faculty of Medicine of the Medical Institute, N.P. Ogarevs Mordovia State University. ORCID ID: 0000-0001-9834-591X, E-mail: chum9714akov@mail.ru

PROMOMED
ООО «ПРОМОМЕД ДМ», 129090 Москва, Проспект Мира, д. 13 стр.1
Тел.: +7 (495) 640-25-28, e-mail: reception@promo-med.ru

ЭСПЕРАВИР®

МОЛНУПИРАВИР

Новый препарат направленной противовирусной терапии

- После 5 дней элиминация (полное выведение) вируса из организма у 9 из 10 пациентов[1]
- Каждый второй пациент добился полного выздоровления*[1]
- Благоприятный профиль безопасности[1]
- Снижение риска госпитализации и ухудшения состояния пациентов[1]

! ВНЕСЕН В МЕТОДИЧЕСКИЕ РЕКОМЕНДАЦИИ МЗ РФ: «ПРОФИЛАКТИКА, ДИАГНОСТИКА И ЛЕЧЕНИЕ НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИИ (COVID-19)». ВЕРСИЯ 15 (22.02.2022)

ЛЕЧЕНИЕ НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИИ COVID-19 ЛЕГКОГО ИЛИ СРЕДНЕГО ТЕЧЕНИЯ У ВЗРОСЛЫХ

Продолжительность курса лечения — **5 суток**

*Лечение лекарственным препаратом ЭСПЕРАВИР® должно быть начато при появлении первых симптомов заболевания!***

**0 баллов по категориальной порядковой шкале клинического статуса ВОЗ*

***Лечение лекарственным препаратом ЭСПЕРАВИР® должно быть начато как можно раньше после постановки диагноза новой коронавирусной инфекции (COVID-19) и/или в течение 5 дней после появления первых симптомов заболевания*

[1]Открытое двухэтапное многоцентровое исследование по оценке основных фармакокинетических параметров, безопасности, а также эффективности в отношении COVID-19 лекарственного препарата JCBC00101, капсулы (ООО «ПРОМОМЕД РУС», Россия) у взрослой популяции, MOL-112021. 2022г

ИНФОРМАЦИЯ ПРЕДНАЗНАЧЕНА ДЛЯ СПЕЦИАЛИСТОВ ЗДРАВООХРАНЕНИЯ
ИМЕЮТСЯ ПРОТИВОПОКАЗАНИЯ ОЗНАКОМЬТЕСЬ С ИНСТРУКЦИЕЙ


1943
MENTE ET ANIMO
ПМФИ
Филиал ВолгГМУ