Научно-практический журнал
Scientific and Practical Journal

ISSN 2307-9266
e-ISSN 2413-2241

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

PHARMACY & PHARMACOLOGY

2024 Том / Volume XII

№ 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

Scientific and Practical Journal

PHARMACY & PHARMACOLOGY

(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Frequency of 6 issues per year

Volume XII, Issue 2, 2024

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
Publisher and 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 "Buro novostey" in accord with provided materials. 278A, Serova Str., Stavropol, 355000

Научно-практический журнал

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

(PHARMACY & PHARMACOLOGY)

Периодичность 6 номеров в год

Том 12, Выпуск 2, 2024

Журнал зарегистрирован Федеральной службой
по надзору в сфере связи, информационных технологий
и массовых коммуникаций (Роскомнадзор):
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» Министерства здравоохранения Российской Федерации (сокращенное наименование: ФГБОУ ВО ВОЛГГМУ МИНЗДРАВА РОССИИ).
400131, Россия, г. Волгоград, площадь Павших Борцов, д. 1
Адрес издательства и редакции: 357532, Россия, г. Пятигорск, пр-кт Калинина, д. 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. Дата подписания в печать 25.11.2024; выход в свет 10.12.2024

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 «Бюро новостей», 355000, Россия, г. Ставрополь, ул. Серова, д. 278А

16+

CONTENS / СОДЕРЖАНИЕ

RESEARCH ARTICLE / ОРИГИНАЛЬНЫЕ СТАТЬИ

REVIEWS / ОБЗОРЫ

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Effect of *C-344T* polymorphism of aldosterone synthase gene on variability of antihypertensive therapy with angiotensin II receptor blockers: open randomized controlled clinical trial

E.V. Rebrova, E.V. Shikh, N.B. Lazareva

Sechenov First Moscow State Medical University,
Bld. 2, 8, Trubetskaya Str., Moscow, Russia, 119991

E-mail: katrina1987@rambler.ru

Received 01 Feb 2024 After peer review 30 May 2024 Accepted 15 Aug 2024

The effectiveness of the antihypertensive therapy may be associated with genetic factors that affect not only the degree of a blood pressure elevation but also predetermine an interindividual variability in response to the antihypertensive treatment.

The aim of the work was to study pharmacodynamic indices of the effectiveness of therapy with angiotensin II receptor blockers (ARBs) in the form of monotherapy and as a part of combined drugs in patients with an arterial hypertension (AH) depending on genetic features of patients – a polymorphism of the gene encoding aldosterone synthase, the *C-344T* polymorphism.

Materials and methods. The study included 179 patients of the Moscow region with a newly diagnosed 1–2-degree AH (141 (78.8%) women and 38 (21.2%) men) aged from 32 to 69 years who had been randomly allocated to treatment groups with irbesartan and valsartan in the form of the mono- or combined therapy with hydrochlorthiazide by simple randomization. After 3 weeks of pharmacotherapy, the presence of the genetic *rs1799998 (C-344T)* polymorphism of the aldosterone synthase gene, *CYP11B2*, and the minimum equilibrium concentration of angiotensin receptor blockers (ARBs) were determined.

Results. *TT* homozygotes in the irbesartan group were characterized by a lower level of the blood pressure (BP) target achievement after 3 weeks of pharmacotherapy and a higher frequency of the need to intensify the antihypertensive therapy compared with *CT* and *TT* genotypes. Among the patients taking valsartan, the carriers of the *TT* genotype were characterized by a higher frequency of achieving the target BP after 3 weeks of pharmacotherapy compared to the *CC* (p <0.001) and *CT* genotypes (p=0.084). Herewith, at the end of the study, according to the results of the office BP measurement and daily BP monitoring (DBPM), the achievement of the target BP values was not significantly associated with *CYP11B2 C-344T* genotype in both irbesartan (p >0.999) and valsartan (p=0.149). There was a trend toward a slightly more pronounced decrease in the daytime HR in the heterozygotes receiving irbesartan by a mean of 1.9 bpm compared to the *CC* homozygotes (p=0.059). The *CT* heterozygotes taking valsartan, were characterized by a less pronounced decrease in the HR by a mean of 1.4 bpm compared to the *TT* homozygotes (p=0.045). Moreover, the minimum drug concentration was not a statistically significant mediator of the effects (p=0.484 and p=0.736, respectively).

Conclusion. When personalizing the AH therapy in the patients of the Moscow region, to optimize the achievement of the target BP, the carriers of the *TT* genotype *C-344T* on the *CYP11B2* gene should be recommend valsartan as the starting therapy of ARBs in the form of the mono- or bicomponent therapy depending on the AH degree.

Keywords: arterial hypertension; aldosterone synthase; *CYP11B2; C-344T* polymorphism

Abbreviations: AH – arterial hypertension; C-344T – genetic polymorphism rs*1799998* of the aldosterone synthase *CYP11B2* gene; ARBs – angiotensin II receptor blockers; BP – blood pressure; DBPM – Daily BP monitoring; HR – heart rate; AHDs – antihypertensive drugs; SNP – single nucleotide polymorphism; GWAS – genome-wide association studies; SBP – systolic blood pressure; DBP – diastolic blood pressure; I/D polymorphism – insertion-deletion polymorphism; RAAS – renin-angiotensin-aldosterone system; ACE – angiotensin-converting enzyme; ECG – electrocardiography; BMI – body mass index; BA – bronchial asthma; AHT – antihypertensive therapy; ADR – adverse drug reaction.

For citation: E.V. Rebrova, E.V. Shikh, N.B. Lazareva. Effect of C-344T polymorphism of aldosterone synthase gene on variability of antihypertensive therapy with angiotensin II receptor blockers: open randomized controlled clinical trial. *Pharmacy & Pharmacology.* 2024;12(2):92-104. **DOI:** 10.19163/2307-9266-2024-12-2-92-104

Для цитирования: Е.В. Реброва, Е.В. Ших, Н.Б. Лазарева. Влияние полиморфизма С-344Т гена альдостерон синтазы на вариабельность антигипертензивной терапии блокаторами рецептора ангиотензина II: открытое рандомизированное контролируемое клиническое исследование. *Фармация и фармакология.* 2024;12(2):92-104. **DOI:** 10.19163/2307-9266-2024-12-2-92-104

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2024-12-2-92-104

Влияние полиморфизма *C-344T* гена альдостерон синтазы на вариабельность антигипертензивной терапии блокаторами рецептора ангиотензина II: открытое рандомизированное контролируемое клиническое исследование

Е.В. Реброва, Е.В. Ших, Н.Б. Лазарева

Федеральное государственное автономное образовательное учреждение высшего образования «Первый Московский государственный медицинский университет имени И.М. Сеченова» Министерства здравоохранения Российской Федерации (Сеченовский Университет), 119991, Россия, г. Москва, ул. Трубецкая, д. 8, стр. 2

E-mail: katrina1987@rambler.ru

Получена 01.02.2024 После рецензирования 30.05.2024 Принята к печати 15.08.2024

Эффективность антигипертензивной терапии может быть ассоциирована с генетическими факторами, которые влияют не только на степень повышения артериального давления, но и предопределяют межиндивидуальную вариабельность ответа на антигипертензивное лечение.

Цель. Изучить фармакодинамические показатели эффективности терапии блокаторами рецепторов ангиотензина II в виде монотерапии и в составе комбинированных препаратов у пациентов с артериальной гипертензией (АГ) в зависимости от генетических особенностей пациентов – полиморфизма гена, кодирующего альдостерон синтазу, или *C-344T* полиморфизм.

Материалы и методы. В исследование включено 179 пациентов Московского региона с впервые выявленной АГ 1–2 степени (141 (78,8%) женщина и 38 (21,2%) мужчин) в возрасте от 32 до 69 лет, которые были случайным образом распределены по группам лечения ирбесартаном и валсартаном в виде моно- или комбинированной терапии с гидрохлортиазидом методом простой рандомизации. Через 3 недели фармакотерапии определяли наличие генетического полиморфизма *rs1799998* (*C-344T*) гена альдостерон синтазы *CYP11B2* и определения минимальной равновесной концентрации блокаторов рецептора ангиотензина II (БРА).

Результаты. Гомозиготы *TT* в группе ирбесартана характеризовались более низким уровнем достижения целевых цифр артериального давления (АД) через 3 недели фармакотерапии и более высокой частотой возникновения необходимости интенсификации антигипертензивной терапии по сравнению с генотипами *CT* и *TT*. Среди пациентов, принимавших валсартан, носители генотипа *TT* характеризовались более высокой частотой достижения целевых цифр АД через 3 недели фармакотерапии по сравнению с генотипами *CC* (p <0,001) и *CT* (p=0,084). При этом достижение целевых цифр АД по результатам показателей измерения офисного АД и суточного мониторирования АД (СМАД) на момент окончания исследования не было достоверно взаимосвязано с генотипом *CYP11B2 C-344T* как при назначении ирбесартана (p >0,999), так и валсартана (p=0,149). Выявлена тенденция к несколько более выраженному уменьшению дневной ЧСС у гетерозигот, принимавших ирбесартан, в среднем на 1,9 уд/мин по сравнению с гомозиготами *CC* (p=0,059). Гетерозиготы *CT*, принимавшие валсартан, характеризовались менее выраженным уменьшением ЧСС в среднем на 1,4 уд/мин по сравнению с гомозиготами *TT* (p=0,045). При этом минимальная концентрация препарата не была статистически значимым медиатором эффектов (p=0,484 и p=0,736 соответственно).

Заключение. При персонализации терапии АГ пациентам Московского региона, носителям генотипа *TT* по *C-344T* гена *CYP11B2* для оптимизации достижения целевых цифр АД целесообразно рекомендовать в качестве стартовой терапии БРА валсартан в виде моно- или двухкомпонентной терапии в зависимости от степени АГ.

Ключевые слова: артериальная гипертензия; альдостерон синтаза; *CYP11B2*; *C-344T* полиморфизм

Список сокращений: АГ – артериальная гипертензия; С-344Т – генетический полиморфизм *rs1799998* гена альдостерон синтазы *CYP11B2*; БРА – блокаторы рецептора ангиотензина II; АД – артериальное давление; СМАД – суточное мониторирование АД; ЧСС – частота сердечных сокращений; ЛС – лекарственное средство; АГП – антигипертензивные препараты; SNP – однонуклеотидный полиморфизм; GWAS – полногеномный поиск ассоциаций; САД – систолическое артериальное давление; ДАД – диастолическое артериальное давление; I/D полиморфизм – инсерционно-делеционный (I/D) полиморфизм; РААС – ренин-ангиотензин-альдостероновая система; СКФ – скорость клубочковой фильтрации; АПФ – ангиотензинпревращающий фермент; ЭКГ – электрокардиография; ИМТ – индекс массы тела; БА – бронхиальная астма; АГТ – антигипертензивная терапия; НЛР – нежелательная лекарственная реакция.

INTRODUCTION

The arterial hypertension (AH) is a polygenic inherited disease and one of the major modifiable risk factors for cardiovascular events [1–3]. The prevalence of AH is steadily increasing worldwide. According to different authors' estimates, the number of people suffering from AH is expected to increase by 60% over the next 20 years, which will amount to more than 1.5 billion people [4–8].

The genetic structure that can influence the blood pressure (BP) currently includes more than 30 genes, with rare variants resulting in monogenic forms of

hypertension or hypotension, and more than 1477 single nucleotide polymorphisms (SNPs) associated with the phenotypic effect on BP. The majority of SNPs identified in genome-wide association studies (GWAS) as associated with the BP phenotype, show pleiotropic associations, the study of which will help to understand the underlying biological pathways [9].

Aldosterone is the major mineralocorticoid of the adrenal cortex. It is synthesized from cholesterol in response to an increase in angiotensin II or plasma potassium levels. Aldosterone stimulates a tubule reabsorption of sodium cations, chloride anions, and an excretion of potassium cations, and increases a tissue water-holding capacity, which promotes fluid and sodium transfer from the vascular bed to the tissues. Aldosterone synthesis is carried out under the action of the aldosterone synthase enzyme encoded by the *CYP11B2* gene (cytochrome *P450*, family 11, subfamily B, polypeptide 2) localized on the 8th chromosome at locus 8q21–q22 [10, 11].

About 227 single nucleotide polymorphisms (SNPs) have been identified for the *CYP11B2* gene that may be associated with an increased *CYP11B2* transcription, an increased aldosterone production, and a progression of many cardiovascular diseases, but only a few SNPs have been studied at present. The most widely studied is the *C-344T* polymorphism (*rs id 1799998*; cytosine to the thymidine substitution in the 5′ promoter region of the *CYP11B2* gene, position –344) [12, 13]. This site is the binding site of the steroidogenic transcription factor SF-1, a regulator of the aldosterone synthase gene expression. The *T* allele leads to the increased aldosterone production, which, in turn, is associated with the AH, as well as with myocardial fibrosis and hypertrophy, with the risk of hypertensive complications of pregnancy, with the development of an endothelial dysfunction and cardiovascular complications in patients with a chronic kidney disease [14–16].

Personalized pharmacotherapy of AH based on genetic variations of genes responsible for the function of metabolic enzymes of drugs, the genes that are involved in the pathogenetic mechanisms of the AH development and alter the pharmacodynamic effects of drugs, the genes associated with drug transporters, will improve the effectiveness of the AH pharmacotherapy in patients who regularly receive antihypertensive drugs (AHDs) in accordance with clinical recommendations at the same time not reaching the target values of BP [17–20].

THE AIM of the work was to study pharmacodynamic parameters of the efficacy of therapy with angiotensin II receptor blockers as monotherapy and as a part of combined drugs in patients with AH depending on the genetic features of patients – the gene polymorphism encoding aldosterone synthase, or C-344T polymorphism.

MATERIALS AND METHODS

Study Design

An open randomized controlled clinical study was performed. The study included 179 patients living in the Moscow region with a newly diagnosed 1–2-degree AH. The patients were 141 (78.8%) women and 38 (21.2%) men aged from 32 to 69 years (mean age – 58.2±6.4, the median age – 60 (57–63 years) [21, 22].

Randomization procedure

All the patients included in the study were allocated to groups by a simple randomization (an envelope method).

Eligibility criteria

The patients met the following *inclusion criteria*: the 1–2-degree AH; the age from 18 to 74 years; a signed informed consent of the patient for the participation in the study and a publication of personal medical information.

The *non-inclusion criteria* of patients in the study were: the 3rd degree AH; uncontrolled AH; arterial hypotension; hypersensitivity to irbesartan and valsartan or auxiliary components of the drug; an active liver disease or an increase in the serum transaminase activity more than 3 times; hepatic insufficiency (classes A and B on the Child-Pugh scale); a stage 4–5 chronic kidney disease (glomerular filtration rate less than 30 ml/min/1.73 m^2; creatinine clearance <30 ml/min); decompensated diabetes mellitus; pregnancy and lactation; the age under 18 years and over 75 years; patients with primary hyperaldosteronism, angioedema, including Quincke's edema; during the treatment with drugs affecting renin-angiotensin-aldosterone system (RAAS), including angiotensin-converting enzyme (ACE) inhibitors; a concomitant use of aliskiren and drugs containing aliskiren in patients with diabetes mellitus and/or a moderate or severe renal dysfunction (glomerular filtration rate, GFR) less than 60 ml/min/1.73 m^2 body surface area); a concomitant use of inhibitors with ACE in patients with diabetic nephropathy; galactose intolerance, lactase insufficiency and glucose-galactose malabsorption syndrome; established diagnosis of malignant tumors; diabetes mellitus, lactose intolerance, lactase deficiency and glucose-galactose malabsorption syndrome; an established diagnosis of malignant neoplasm at the time of the inclusion in the study; the need for a continuous intake of non-steroidal anti-inflammatory drugs and/or drugs metabolized by cytochrome *P-450 CYP2C9*, which may affect the effectiveness and safety profile of irbesartan.

The *exclusion criteria:* no patients were excluded during the study [21, 22].

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-92-104

Conditions and duration of the study

Patients were recruited in the period from July 1, 2021 to August 28, 2022. The search for study participants was carried out in the outpatient treatment and preventive care institutions of Moscow, clinical bases of the Department of Clinical Pharmacology and Propaedeutics of Internal Medicine of Sechenov First Moscow State Medical University (Sechenov University): Mukhin City Clinical Hospital, Hospital for War Veterans No. 3, Central Clinical Hospital of Civil Aviation, State Polyclinic No. 2.

Ethical approval

The study was approved by the Local Ethical Committee of Sechenov First Moscow State Medical University (Sechenov University), Protocol No. 05-21 dated March 10, 2021.

Medical intervention

The program of a clinical and instrumental examination of the patient included was: a collection of patients' complaints, anamnesis (presence of risk factors for the AH development, concomitant diseases), a physical examination, a biochemical blood analysis, an office BP measurement, electrocardiography (ECG) to exclude patients with rhythm disturbances or concomitant heart diseases, echo-CG, daily BP monitoring (DBPM). The BP was measured in both arms using the Korotkoff method after a 10-minute rest of the patient in the sitting position, and was determined as the mean of three measurements taken at 1-minute intervals. DBPM was performed when patients were enrolled in the study and after 3 months of therapy, and the values of the standard daytime and nighttime parameters were evaluated: a mean value, a variability of systolic BP (SBP), diastolic BP (DBP), BP, and heart rate (HR).

Three weeks after the inclusion in the study, the blood of patients was collected to determine the genetic *rs1799998 (C-344T)* polymorphism of the aldosterone synthase *CYP11B2* gene and to determine the minimum equilibrium concentration of ARBs. The office BP measurement was performed at each visit: at the inclusion in the study, at the intermediate stage after 3 weeks and after 3 months of therapy. DBPM was performed in patients at the inclusion in the study and after 3 months of therapy [21, 22].

Methods of outcomes registration

The genetic polymorphism *rs1799998 (C-344T)* of the aldosterone synthase *CYP11B2* gene was determined using the reagent kit for a polymorphism detection in the human genome SNP-screen (Syntol, Russia).

The determination of irbesartan and valsartan concentrations in the blood plasma was performed by HPLC on an Agilent 1290 Infinity II LC coupled with the 6470 Triple Quadrupole LC/MS liquid chromatograph (Agilent Technologies, USA), using standard calibration solutions with concentrations of 2500, 1000, 1000, 500, 250, 250, 100, 50, 25 and 10 ng/mL, trifluoroacetic acid, acetonitrile and Milli-Q purified water for HPLC. The additional equipment included ME54 analytical scales (Mettler Toledo, Sweden), single-channel mechanical pipettes with variable volumes of 100–1000 µL and 20–200 µL (Thermo Scientific Black series, USA), Eppendorf centrifuge (Germany), ZORBAX Eclipse plus C-18 HPLC column, 50×2.1 mm, 1.7 µm for HPLC (Agilent Technologies, USA).

Study groups

All the patients included in the study had not previously received regular antihypertensive therapy (AHT) and were randomly allocated to the irbesartan and valsartan groups by simple randomization (an envelope method). The study participants received ARB (irbesartan and valsartan) in monotherapy or in a combination with hydrochlorthiazide for 3 months. So, 83 patients included in the study, received irbesartan 150 mg once a day, 32 of them were on the irbesartan monotherapy, and 51 patients received the combination therapy: irbesartan 150 mg+hydrochlorthiazide 12.5 mg. 96 patients were prescribed valsartan, 8 of them received the valsartan 80 mg monotherapy once daily, and 88 patients received the valsartan 80 mg+hydrochlorthiazide 12.5 mg combination therapy. When the target BP numbers were achieved after 3 weeks of therapy (<140/90 mm Hg, if well tolerated <130/80 mm Hg, but not <120/70 mm Hg), the patients continued to adhere to their therapy for 3 months of treatment. In case of an insufficient BP control, the intensification of therapy was performed by doubling the dose of irbesartan or valsartan as part of the mono- or combination therapy.

Statistical processing

The sample size was not pre-calculated. The statistical analysis and visualization of the obtained data were performed using the R 4.3.1 statistical computing environment (R Foundation for Statistical Computing, Vienna, Austria).

Descriptive statistics for quantitative variables without a pronounced asymmetry of conditional sampling distributions are presented as a mean (±standard deviation, SD), for quantitative variables with a pronounced asymmetry (absolute values of the asymmetry coefficient >1.96) – as a median [Q1; Q3]. Descriptive statistics for qualitative variables are presented as a number of observations (a relative frequency). The Fisher's exact test was used to compare

the groups with respect to qualitative variables (including the Holm correction for multiple pairwise comparisons). The Welch's *t*-test and Mann–Whitney test were used to compare two independent groups with respect to quantitative variables, and the Kraskell–Wallis test and Dunn's test with the Holm correction for pairwise post-hoc comparisons were used to compare three groups. The differences were considered statistically significant at p <0.05.

The chi-square test (χ^2) was used to analyze the correspondence of the empirical distribution of genotypes to the theoretical one defined by the Hardy–Weinberg equilibrium.

For a correlation analysis, the Spearman's rank correlation coefficient ρ with corresponding 95% confidence intervals (95% CI) was used, and the regression coefficients (with a corresponding 95% CI) in single-factor regression models were estimated if there was a statistically significant correlation between quantitative indicators. To assess the strength and statistical significance of the association of quantitative predictors with binary outcomes, single-factor logistic regression models with coefficients estimated with the Firth (1993) correction for rare outcomes were used.

In the comparative analysis of genotype effects with changes in SBP, DBP, and HR, linear regression models were used with the inclusion of an interaction term between the genotype and drug used and robust standard errors of the regression coefficients. To assess the relative contribution of the drug concentration as a mediator of the identified genotype effects, two linear regression models were constructed: a two-factor outcome model including the genotype and the concentration and a single-factor genotype-dependent concentration model, which had been used to estimate the total and partial genotype effects and the ratio of coefficients (the sum of coefficients) to calculate the proportion of the genotype effect mediated by the concentration (the standard error was estimated using a nonparametric analysis). The association was considered statistically significant at p <0.05.

RESULTS AND DISCUSSION

Characteristics of study groups

Table 1 shows the demographic and anamnestic characteristics of the patient groups.

In the comparative analysis, it was found out that valsartan was statistically significantly more often prescribed to male patients (p <0.001). The patients were comparable in age (p=0.24). The patients receiving valsartan also had a statistically significantly higher BMI and were more likely to have a grade 3 obesity (p <0.001). In addition, the patients in this group were statistically significantly more likely to have bronchial asthma (BA; p=0.012) and statistically significantly less likely to have a history of Crohn's disease (p=0.044).

Main study result

All patients were genotyped according to the *C-344T* polymorphism of the aldosterone synthase *CYP11B2* gene. According to the data obtained, the frequency of the polymorphic allele *T* carriage by the polymorphism *C-344T* of the aldosterone synthase *CYP11B2* gene was 192 (53,6%), while 51 patients (28,5%) were homozygotes for the mutant allele and carriers of the *TT* genotype, and 90 (50,3%) patients were heterozygous representatives of the *CT* genotype. There were no statistically significant deviations of the observed frequency of genotypes for the *C-344T* polymorphism of the *CYP11B2* aldosterone synthase gene from the theoretical one determined by the Hardy-Weinberg equilibrium (χ^2=0.003, p=0.96).

Values of the minimum equilibrium irbesartan concentration in patients with the *CC* genotype by the *C-344T* polymorphism of the aldosterone synthase *CYP11B2* gene were determined at the level of 1788 (1633-2341) ng/mL, with the *CT* genotype – 1909 (1707–2346) ng/mL, with the *TT* genotype – 2476 (1969–2672) ng/mL. The mean values of the minimal equilibrium concentration of valsartan in patients with the *CC* genotype by the *C-344T* polymorphism of the aldosterone synthase *CYP11B2* gene were determined at the level of 1380 (1172–1568) ng/mL, with the *CT* genotype – at 1095 (740–1428) ng/mL, with the *TT* genotype – at 688 (519–1562) ng/mL.

Table 2 summarizes the results of SBP, DBP and HR dynamics in patients with different genotypes for the *C-344T* polymorphism of the aldosterone synthase *CYP11B2* gene.

The *TT* homozygotes at the *C-344T* polymorphic locus of the *CYP11B2* gene taking irbersartan, were characterized by a statistically significantly less pronounced reduction in the office SBP after 3 weeks of therapy by a mean of 5.5 [95% CI: 0.2; 10.9] mmHg (p=0.042); this effect was not associated with the concentration magnitude (p=0.708).

At the time of the interim phase of the study, the heterozygotes taking valsartan had a statistically significantly greater mean reduction in the SBP of 15.7 [95% CI: –21.9; –9.5] mmHg compared to the *CC* homozygotes (p <0.001) and 7.5 [95% CI: –13.4; –1.6] mmHg with the *TT* homozygotes (p=0.009). The *TT* homozygotes were characterized by a more pronounced reduction in the SBP by a mean of 8.2 [95% CI: –15; –1.4] mmHg compared to the *CC* homozygotes (p=0.014); this effect was not associated with the concentration value (p=0.538).

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-92-104

Table 1 – Demographic and anamnestic characteristics of patient groups

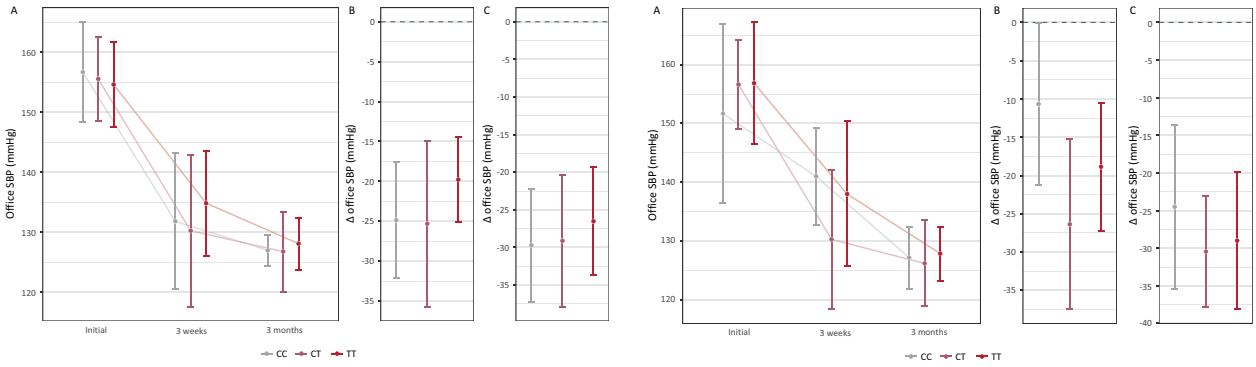
Characteristics		Irbesartan (*n*=83)	Valsartan (*n*=96)	*p*
Gender, *n* (%)		–	–	<0,001
	female	53 (63.9%)	88 (91.7%)	–
	male	30 (36.1%)	8 (8.3%)	–
Age, years		57 (±7.5)	59.3 (±5.1)	0.24
BMI, kg/m^2		29.4 (±5.4)	33.4 (±6.8)	<0.001
Obesity		–	–	<0.001
None		38 (45.8%)	44 (45.8%)	–
	Degree 1	29 (34.9%)	16 (16.7%)	–
	Degree 2	16 (19.3%)	20 (20.8%)	–
	Degree 3	0 (0%)	16 (16.7%)	–
Chronic gastritis, *n* (%)		10 (12%)	16 (17%)	0.382
Chronic tonsillitis, *n* (%)		7 (8.4%)	4 (4.2%)	0.236
Bronchial asthma, *n* (%)		2 (2.4%)	12 (13%)	0.012
Varicose veins of lower limbs, *n* (%)		8 (9.6%)	4 (4.2%)	0.144
Migraine, *n* (%)		2 (2.4%)	4 (4.2%)	0.687
Crohn's disease, n (%)		4 (4.8%)	0 (0%)	0.044
Psoriasis, *n* (%)		2 (2.4%)	0 (0%)	0.214
Osteochondrosis, *n* (%)		13 (16%)	12 (13%)	0.543

Note: BMI – body mass index; BA – bronchial asthma.

Table 2 – SBP, DBP and HR in patients with different genotypes for the C-344T polymorphism of the CYP11B2 gene in the irbesartan and valsartan patient groups

Characteristics	Irbesartan				Valsartan			
	CC	*CT*	*TT*	*p*	*CC*	*CT*	*TT*	*p*
Δ office SBP after 3 weeks	–24.9 (±7.3)	–25.3 (±10.4)	–19.8 (±5.3)	**0.026**	–10.7 (±10.5)	–26.4 (±11.1)	–18.9 (±8.4)	**<0.001**
Δ office SBP at the end of the study	–29.7 (±7.5)	–29.1 (±8.8)	–26.5 (±7.2)	0.287	–24.5 (±10.9)	–30.5 (±7.3)	–29 (±9.1)	**0.039**
Δ office DBP after 3 weeks	–18.3 (±7.6)	–20.2 (±7.9)	–14.6 (±9.3)	**0.071**	–10.8 (±14.5)	–16.1 (±12.2)	–13.9 (±7.7)	**0.073**
Δ office DBP at the end of the study	–23.9 (±9.8)	–25.4 (±7.3)	–23 (±8.8)	0.583	–23.3 (±10.3)	–20.5 (±6.8)	–24.3 (±9.6)	0.271
Δ office HR after 3 weeks	–1.9 (±4.2)	–3.4 (±3.3)	–2.6 (±3.6)	0.412	–4.2 (±4.1)	–1.6 (±7)	–5.7 (±4.4)	**0.06**
Δ office HR at the end of the study	–2.3 (±5.2)	–3.6 (±3.6)	–2.7 (±4.6)	0.819	–5.5 (±5)	–4.5 (±4.1)	–5.7 (±4.3)	0.525
Achievement of target BP after 3 weeks	9/14 (64.3%)	30/46 (65.2%)	12/23 (52.2%)	0.53	4/24 (16.7%)	20/44 (45.5%)	20/28 (71.4%)	**<0.001**
Dose increase (AHT intensification)	5/14 (35.7%)	13/46 (28.3%)	11/23 (47.8%)	0.262	20/24 (83.3%)	20/44 (45.5%)	16/28 (57.1%)	**0.009**
Δ mean daytime SBP	–27.6 (±6.7)	–28.5 (±9.5)	–27 (±6.5)	0.879	–31 (±6.2)	–32 (±7.7)	–27 (±4.4)	**0.035**
Δ mean daytime DBP	–29.9 (±5.1)	–30.3 (±6.2)	–31.1 (±5.9)	0.706	–30 (±6.6)	–27.2 (±5.6)	–29.9 (±5.8)	0.174
Δ mean daytime HR	–0.4 (±2.4)	–2.3 (±2.2)	–1.3 (±3.4)	**0.072**	–3 (±2.1)	–1.9 (±2.4)	–3.3 (±2.5)	**0.042**
Δ mean nighttime SBP	–17.9 (±5.5)	–17.4 (±9.4)	–15 (±6.9)	0.359	–19.7 (±6.8)	–17.1 (±7.2)	–17.4 (±8.7)	0.461
Δ mean nighttime DBP	–24.9 (±5.3)	–25.8 (±5.6)	–25.1 (±5.9)	0.848	–27.3 (±6.5)	–24.5 (±6.2)	–28.7 (±3.6)	**<0.001**
Δ mean nighttime HR	–0.9 (±4.7)	–2.2 (±3.6)	–2.4 (±3.8)	0.742	–3.8 (±3)	–4.1 (±4.9)	–4.7 (±4.7)	0.408
Δ daytime SBP variability	–5.5 (±3.5)	–6.1 (±3.5)	–5.9 (±3.7)	0.419	–8.2 (±0.4)	–8.3 (±0.4)	–8.4 (±0.6)	0.626
Δ daytime DBP variability	–2.8 (±2.2)	–2.6 (±1.9)	–2.5 (±1.9)	0.756	–4.3 (±0.5)	–3.5 (±0.7)	–4 (±0.5)	**<0.001**
Δ nighttime SBP variability	–1.8 (±1.7)	–2 (±1.5)	–1.7 (±1.2)	0.713	–2.7 (±1)	–3 (±0.6)	–2.7 (±1.1)	0.196
Δ nighttime DBP variability	–0.8 (±0.7)	–0.7 (±0.7)	–0.7 (±0.8)	0.775	–1 (±0.4)	–1.3 (±0.6)	–1.4 (±0.7)	0.2
Achievement of target BP at the end of the study	14/14 (100%)	43/44 (97.7%)	23/23 (100%)	>0.999	24/24 (100%)	40/44 (90.9%)	28/28 (100%)	0.149
ADR (arterial hypotension)	0/14 (0%)	3/46 (6.5%)	0/23 (0%)	0.741	0/24 (0%)	4/44 (9.1%)	0/28 (0%)	0.149

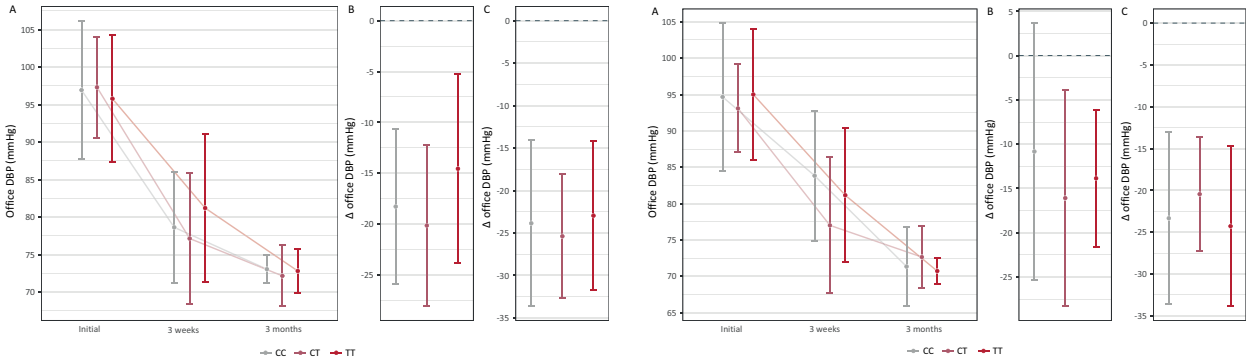
Note: * Statistically significant values are highlighted in bold at p <0.05. SBP – systolic blood pressure; DBP – diastolic blood pressure; HR – heart rate; BP – blood pressure; AHT – antihypertensive therapy; ADR – adverse drug reaction.



A **B**

Figure 1 – Comparative analysis of the office SBP dynamics in patients with different genotypes for the *C-344T* polymorphism of the *CYP11B2* gene in the irbesartan and valsartan patient groups

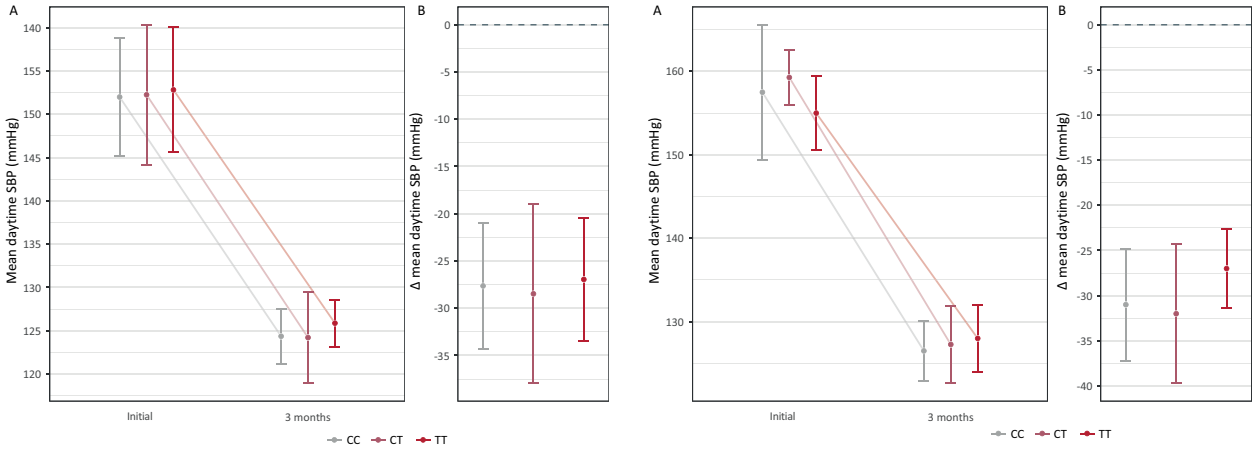
Note: A – irbesartan group; B – valsartan group; SBP – systolic blood pressure.



A **B**

Figure 2 – Comparative analysis of the office DBP dynamics in patients with different genotypes for the *C-344T* polymorphism of the *CYP11B2* gene in the irbesartan and valsartan patient groups

Note: A – irbesartan group; B – valsartan group; DBP – diastolic blood pressure.



A **B**

Figure 3 – Comparative analysis of the dynamics of the mean daytime SBP in patients with different genotypes for the *C-344T* polymorphism of the *CYP11B2* gene in the irbesartan and valsartan patient groups

Note: A – irbesartan group; B – valsartan group; SBP – systolic blood pressure.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-92-104

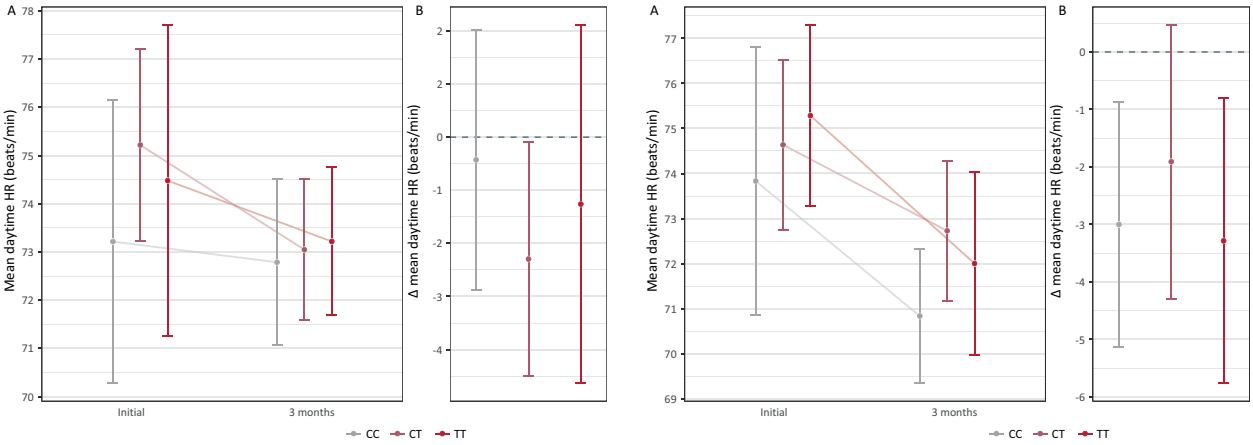


Figure 4 – Comparative analysis of the dynamics of mean daily HR in patients with different genotypes for the *C-344T* polymorphism of the *CYP11B2* gene in the irbesartan and valsartan patient groups
Note: A – irbesartan group; B – valsartan group; HR – heart rate.

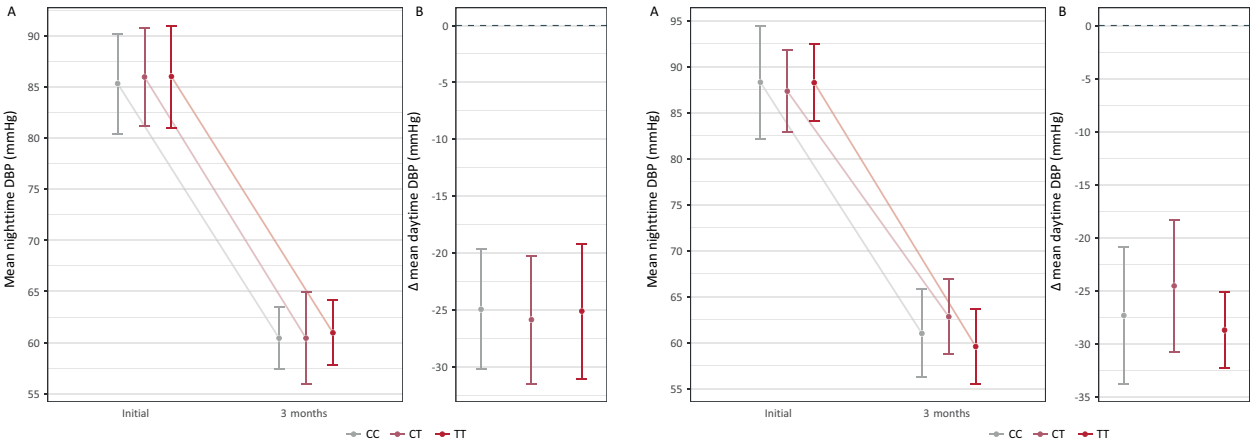


Figure 5 – Comparative analysis of mean nighttime DBP dynamics in patients with different genotypes for the *C-344T* polymorphism of the *CYP11B2* gene in the irbesartan and valsartan patient groups
Note: A – irbesartan group; B – valsartan group; DBP – diastolic blood pressure.

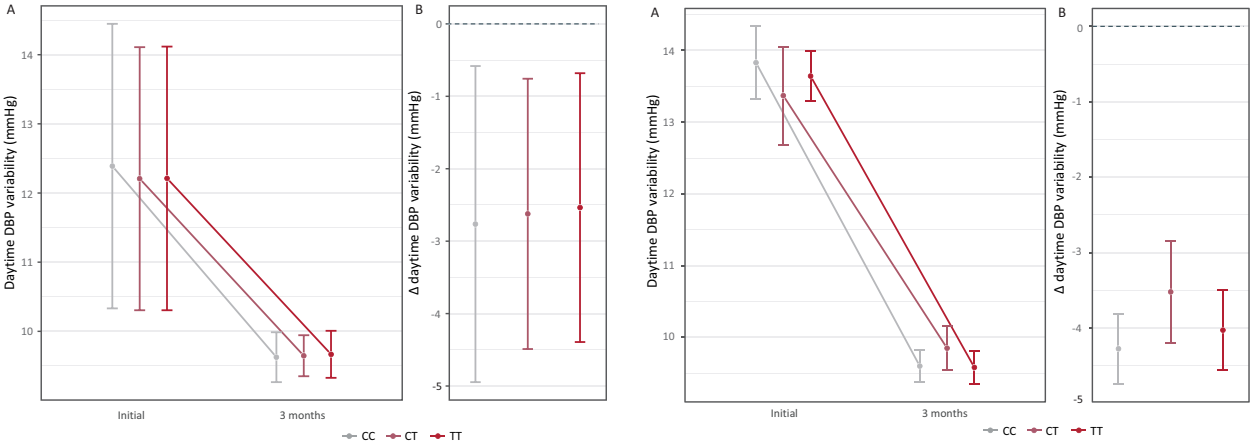


Figure 6 – Comparative analysis of the dynamics of daytime DBP variability in patients with different genotypes for the *C-344T* polymorphism of the *CYP11B2* gene in irbesartan and valsartan patient groups
Note: A – irbesartan group; B – valsartan group; DBP – diastolic blood pressure.

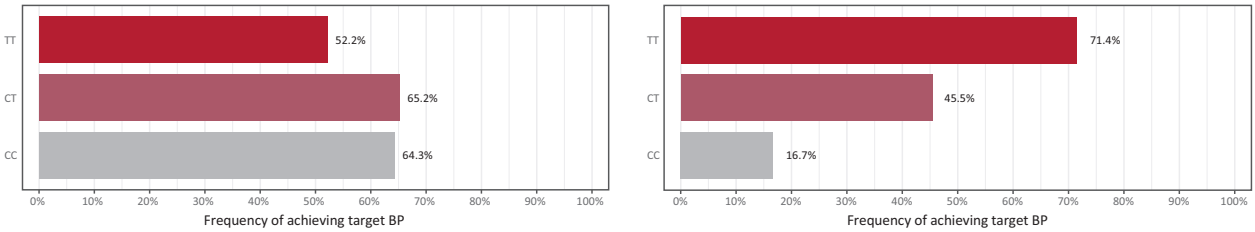


Figure 7 – Comparative analysis of the frequency of achieving the target BP in patients with different genotypes for the *C-344T* polymorphism of the *CYP11B2* gene in irbesartan and valsartan patient groups

Note: A – irbesartan group; B – valsartan group; BP – blood presure.

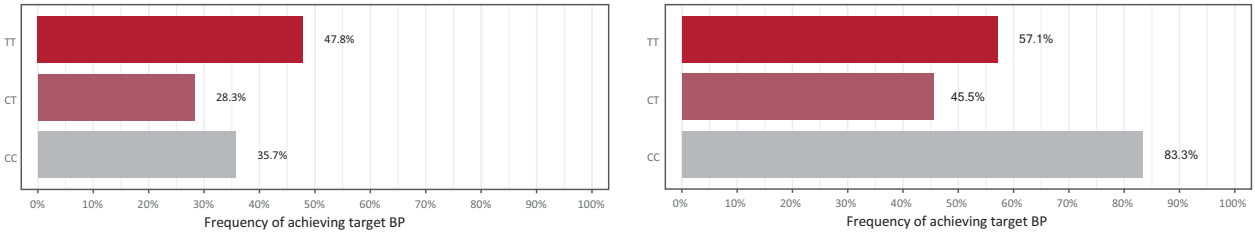


Figure 8 – Comparative analysis of the necessity incidence to intensify antihypertensive therapy in patients with different genotypes for the *C-344T* polymorphism of the *CYP11B2* gene in the irbesartan and valsartan patient groups

Note: A – irbesartan group; B – valsartan group; BP – blood presure.

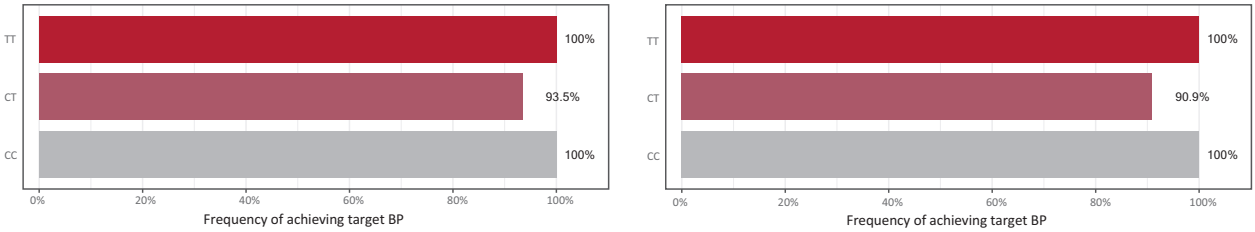


Figure 9 – Comparative analysis of the frequency of reaching the target BP at the end of the study in patients with different genotypes for the *C-344T* polymorphism of the *CYP11B2* gene in the irbesartan and valsartan patient groups

Note: A – irbesartan group; B – valsartan group; BP – blood presure.

There was no statistically significant association of the *CYP11B2 C-344T* genotype with the effect of irbersartan on the office SBP at the end of the study (p=0.287). The *TT* heterozygotes and homozygotes taking valsartan were characterized by a more pronounced reduction in the office SBP at the end of the study by a mean of 6 [95% CI: –11.3; –0.6] mmHg (p=0.026) and 4.5 [95% CI: –10.4; 1.4] mmHg (p=0.167) compared to the CC homozygotes, this effect was not associated with the concentration magnitude (p=0.574). The summarized data is shown in Figure 1.

At the interim assessment, there was a statistically significant less pronounced mean 5.6 [95% CI: 0.5; 10.6] mmHg reduction in the office DBP among the *TT* homozygotes receiving irbesartan compared to the *CT* heterozygotes (p=0.027); the effect was not associated with the magnitude of the equilibrium drug concentration (p=0.5). There was no statistically significant association of the drug effect on the office DBP with the genotype (p=0.583 and p=0.271, respectively; Fig. 2).

There were no statistically significant differences regarding the effect of irbesartan on the mean daytime SBP depending on the *CYP11B2 C-344T* genotype *(p=0.879).* The *TT* homozygotes receiving valsartan were characterized by a less pronounced reduction in the daytime SBP by a mean of 4 [95% CI: –0.3; 8.3] mmHg compared to the *CC* homozygotes (p=0.075) and 5 [95% CI: 1.3; 8.7] mmHg compared to the heterozygotes (p=0.006). This effect was not associated with the equilibrium drug concentration value (p=0.868; Fig. 3).

There was a trend towards a slightly more

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-92-104

pronounced reduction in the daytime HR in the heterozygotes at the *CYP11B2 C-344T* locus taking irbesartan by a mean of 1.9 [95% CI: –3.8; 0.1] bpm compared to the *CC* homozygotes (p=0.059), this effect was not associated with the equilibrium drug concentration value (p=0.484). The *CT* heterozygotes taking valsartan were characterized by a less pronounced reduction in the HR by a mean of 1.4 [95% CI: 0; 2.7] bpm compared to the *TT* homozygotes (p=0.045), with the drug concentration also not being a statistically significant mediator of the effect (p=0.736; Fig. 4).

There was no statistically significant association of the irbesartan effect on the mean nighttime DBP with the genotype at the *CYP11B2 C-344T* locus (p=0.848). The *TT* homozygotes taking valsartan were characterized by a more pronounced reduction in the nighttime DBP by a mean of 4.2 [95% CI: –7.4; –0.9] mmHg compared to the heterozygotes (p=0.008). This effect was not associated with the value of the equilibrium drug concentration (p=0.7; Fig. 5).

Among the patients taking irbesartan, no statistically significant association of the daytime BP variability with the genotype at the *CYP11B2 C-344T* locus was found (p=0.756). The heterozygotes taking valsartan were characterized by a statistically significant smaller reduction in the daytime DBP variability by a mean of 0.8 [95% CI: 0.4; 1.1 mmHg compared to the *CC* homozygotes (p <0.001) and 0.5 [95% CI: 0.1; 0.9] mmHg compared to the *TT* homozygotes (p=0.003). This effect was not associated with the equilibrium value of the drug concentration (p=0.642; Fig. 6).

There was no statistically significant association of the frequency of reaching the target BP at the interim assessment and the need for a dose increase in patients taking irbesartan (p=0.53 and p=0.262, respectively). Among the patients taking valsartan, the *TT* genotype was characterized by a higher frequency of achieving the target BP at the interim evaluation compared to the *CC* (p <0.001) and *CT* (p=0.084) genotypes. The *CC* genotype was also characterized by a higher frequency compared to heterozygotes (p=0.059). The *CC* genotype was also characterized by the greatest need for the intensification of the AHT (p=0.009). The achievement of the target BP at the end of the study was not statistically significantly associated with the *CYP11B2 C-344T* genotype when irbesartan (p >0.999) and valsartan (p=0.149) were used (Fig. 7–9).

DISCUSSION

When studying the genotype frequency distribution for the genetic polymorphism *C-344T* of the aldosterone synthase *CYP11B2* gene, which catalyzes the reaction of the aldosterone synthesis, the following results were obtained: the *CC* genotype was determined in 38 patients, corresponding to 21.2% of the sample, the *CT* genotype – in 90 (50.3%) patients and the *TT* genotype – in 51 (28.5%). The frequency of the *C* allele was 166 (46.4%) and the frequency of the *T* allele was 192 (53.6%).

In the study by Ji X. et al. [23], conducted in China, which included 345 patients with AH and 157 control subjects, the frequency of genotypes by the genetic polymorphism *C-344T* of the aldosterone synthase *CYP11B2* gene was analyzed. The following results were obtained: the frequency of the *CC* genotype occurrence among the patients suffering from the AH was 15.1%, from the *CT* genotype – 51.9%, from the *TT* genotype – 33%. The distribution of the genotypes in the control group among the healthy individuals was as follows: 8.3% of the people carried the *CC* genotype, 46.8% – the *CT* genotype, and 44.9% – the *TT* genotype. Thus, the frequency of the *C* allele was significantly higher in the patients with the AH compared with the controls (p <0.05) in the Chinese population. Thus, the distribution of genotype frequencies for the *C-344T* genetic polymorphism of the aldosterone synthase *CYP11B2* gene among 179 patients with newly diagnosed AH of the 1–2-degree in the Moscow region corresponds to the distribution of the genotypes in the Chinese population.

In the controlled study by Li X. et al. [10], 2115 people, residents of Tibet and northwest China, participated. The authors determined a direct correlation in the female population between the *C-344T* polymorphism of the *CYP11B2* gene and the development of the essential hypertension (EH) – the frequencies of the *CC* and *CT* genotypes and *C* allele in the EH group were higher than in the control group (p <0.05).

In the study by Yakimenko O. et al [11], the aim of which was to study the prevalence of the *C-344T* polymorphism and the distribution of the aldosterone synthase (*CYP11B2*) gene genotypes, to analyze the associations of the left ventricular hypertrophy (LVH) with the aldosterone synthase genotypes in young age patients with AH, the authors found that the *TT*, *CT* genotypes of the *C-344T* polymorphism of the aldosterone synthase gene were associated with higher blood aldosterone concentrations and higher stages of LVH in young patients with AH.

In the study by Ji X. et al. [23], performed in China, among 502 examined patients, the AH was detected in 34. The frequency of the *CC* and *CT* genotypes by the *C-344T* polymorphism of the *CYP11B2* gene, as well as the frequency of the *C*-allele, was significantly higher in patients with the AH compared to patients without the AH (p <0.05). In the prospective part of the study, 98 patients with the AH received the valsartan therapy for 4 weeks. The reduction of the SBP, DBP, as well as the SBP, DBP parameters obtained during a 24-hour BP monitoring in the group with the *CC* and *CT* genotypes, was significantly more pronounced than in the group with the *TT* genotype (p <0.05). The data obtained show that when assessing the effectiveness of AHT at the intermediate stage, the patients in the valsartan group, heterozygous for the *C-344T* polymorphism of the *CYP11B2* gene, reduced the office SBP more intensively by 15.7 [95% CI: –21.9; –9.5] mmHg. Hg compared with the *CC* genotype carriers (p <0.001) and by 7.5 [95% CI: –13.4; –1.6] mmHg compared with the *TT*

homozygotes (p=0.009). Herewith, the *TT* genotype carriers showed a significantly more intense reduction of 8.2 [95% CI: –15; –1.4] mmHg compared to the *CC* genotype carriers (p=0.014). However, when analyzing associations based on the results of the conducted DBPM, when included in the study and after 3 months of AHT, *TT* homozygotes for *C-344T* of the *CYP11B2* gene on average by 4 [95% CI: –0.3; 8.3] mmHg did not significantly reduce the average daily SBP compared with *CC* homozygotes (p=0.075) and by 5 [95% CI: 1.3; 8.7] mmHg compared with heterozygotes (p=0.006). The *TT* homozygotes for C-344T of the CYP11B2 gene had a mean 4.2 [95% CI: –7.4; –0.9] mmHg less pronounced reduction in the mean nighttime DBP compared to the heterozygotes (p=0.008). The obtained data may indicate a functional cumulation of drugs and the stabilization of the pharmacodynamic effect.

After 3 months of the irbesartan treatment, the SILVHIA study (The Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol) [24] showed a more pronounced decrease in the SBP compared to the patients with the *CC* and *TT* genotypes, in the patients with the *TT* genotype by the *C-344T* polymorphism of the *CYP11B2* gene. The authors' findings indicate, when assessed for the effectiveness 3 weeks after prescribed AHT, the patients who received the irbesartan therapy, the *TT* homozygotes for the *C-344T* of the *CYP11B2* gene, were statistically significantly less likely to reduce both the office SBP by 5.5 [95% CI: 0.2; 10.9] mmHg, and the office DBP by 5.6 [95% CI: 0.5; 10.6] mmHg compared with the heterozygotes (p=0.042 and p=0.027, respectively). In the group of patients taking irbesartan, no statistically significant DMBP results were obtained.

In the prospective study by Ortlepp J.R. et al. [25], the patients with the elevated DBP (>95 mmHg) received candesartan in high (16 mg) or low (8 mg) doses in addition to standard medications. Genotyping was performed in 116 patients, and the genotypes of the *CYP11B2* promoter polymorphism significantly predicted a positive response to the treatment: with the genotype *CC*, 67% of patients achieved a reduction in the DBP <85 mmHg, with *TC* – 34% and with *TT* – 21%; p=0.005).

Kurbanova D.R. et al. [26] studied the influence of the *I/D* polymorphism of the *ACE* gene, the *M235T* polymorphism of the *AGT* gene, the *A1166C* polymorphism of the *AGTR1* gene, the *C-344T* polymorphism of the *CYP11B2* gene on the effectiveness of the eprosartan therapy in 48 Uzbek men with AH. The study did not reveal a significant dependence of antihypertensive effectiveness of eprosartan on the carriage of the studied polymorphic genes. However, this may be due to the insufficient sample size.

Thus, a limited number of studies have been performed on the effect of the *C-344T* polymorphism of the *CYP11B2* gene on the effectiveness of AHT, and the results of these studies remain inconsistent.

Study limitations

This study focuses on the influence of a single polymorphism of a candidate gene responsible for the synthesis of aldosterone synthase, the enzyme that catalyzes the synthesis of aldosterone, on the risk of developing the AH and the effectiveness of AHDs. However, the risk of the AH and/or AHDs development may also be influenced by the polymorphisms in the genes involved in the AHDs metabolism, responsible for a certain link of RAAS, involved in pathogenetic mechanisms of the AH development and influencing pharmacodynamic effects of drugs, modifications of mechanical interactions between drugs and genes, as well as polymorphisms in genes related to drug transporters, which requires a further study. This study is also limited by the sample size, region and randomization method.

CONCLUSION

When personalizing the AH therapy in patients of the Moscow region, the carriers of the *TT* genotype *C-344T* of the *CYP11B2* gene, it is reasonable to recommend valsartan as a starting therapy of ARBs in the form of mono- or bicomponent therapy depending on the AH degree.

In this study, a single polymorphism responsible for a particular RAAS link and influencing the risk of the AH development and the response to the ARBs therapy, were analyzed. Herewith, the response to the AHT also depends on the genes, which are involved in pathogenetic mechanisms of the AH development, and modify pharmacodynamic effects of drugs, modify a mechanical interaction between drugs and genes, as well as polymorphisms in the genes related to drug transporters, which determines the need for a further study of the influence of polymorphisms in the panel of candidate genes.

FINDING

This study had no financial support from outside organizations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Ekaterina V. Rebrova – collection and processing of materials, writing the text; Evgenia V. Shikh, Natalia B. Lazareva – collection and processing of materials, editing the text of the article.
All the authors confirm their authorship compliance with the ICMJE international criteria (all authors made a significant contribution to the conceptualization, research and preparation of the article, read and approved the final version before publication).

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-92-104

REFERENCES

1. Rysz J, Franczyk B, Rysz-Górzyńska M, Gluba-Brzózka A. Pharmacogenomics of hypertension treatment. Int J Mol Sci. 2020;21(13):4709. DOI: 10.3390/ijms21134709
2. van Oort S, Beulens JWJ, van Ballegooijen AJ, Grobbee DE, Larsson SC. Association of cardiovascular risk factors and lifestyle behaviors with hypertension: A mendelian randomization study. Hypertension. 2020;76(6):1971–9. DOI: 10.1161/HYPERTENSIONAHA.120.15761
3. Hengel FE, Sommer C, Wenzel U. Arterielle Hypertonie – Eine Übersicht für den ärztlichen Alltag [Arterial Hypertension]. Dtsch Med Wochenschr. 2022;147(7):414–28. German. DOI: 10.1055/a-1577-8663
4. Siedlinski M, Carnevale L, Xu X, Carnevale D, Evangelou E, Caulfield MJ, Maffia P, Wardlaw J, Samani NJ, Tomaszewski M, Lembo G, Holmes MV, Guzik TJ. Genetic analyses identify brain structures related to cognitive impairment associated with elevated blood pressure. Eur Heart J. 2023;44(23):2114–25. DOI: 10.1093/eurheartj/ehad101
5. Laffin LJ, Rodman D, Luther JM, Vaidya A, Weir MR, Rajicic N, Slingsby BT, Nissen SE; Target-HTN Investigators. Aldosterone synthase inhibition with lorundrostat for uncontrolled hypertension: The Target-HTN Randomized Clinical Trial. JAMA. 2023;330(12):1140–50. DOI: 10.1001/jama.2023.16029
6. Xie M, Tang T, Liang H. Efficacy of single-pill combination in uncontrolled essential hypertension: A systematic review and network meta-analysis. Clin Cardiol. 2023;46(8):886–98. DOI: 10.1002/clc.24082
7. Zhu ML, Sun RL, Zhang HY, Zhao FR, Pan GP, Zhang C, Song P, Li P, Xu J, Wang S, Yin YL. Angiotensin II type 1 receptor blockers prevent aortic arterial stiffness in elderly patients with hypertension. Clin Exp Hypertens. 2019;41(7):657–61. DOI: 10.1080/10641963.2018.1529781
8. Zennaro MC, Boulkroun S, Fernandes-Rosa FL. Pathogenesis and treatment of primary aldosteronism. Nat Rev Endocrinol. 2020;16(10):578–89. DOI: 10.1038/s41574-020-0382-4
9. Naito T, Inoue K, Sonehara K, Baba R, Kodama T, Otagaki Y, Okada A, Itcho K, Kobuke K, Kishimoto S, Yamamoto K; BioBank Japan; Morisaki T, Higashi Y, Hinata N, Arihiro K, Hattori N, Okada Y, Oki K. Genetic risk of primary aldosteronism and its contribution to hypertension: A cross-ancestry meta-analysis of genome-wide association studies. Circulation. 2023;147(14):1097–109. DOI: 10.1161/CIRCULATIONAHA.122.062349
10. Shah WA, Jan A, Khan MA, Saeed M, Rahman N, Zakiullah, Afridi MS, Khuda F, Akbar R. Association between Aldosterone Synthase (CYP11B2) Gene Polymorphism and Hypertension in Pashtun Ethnic Population of Khyber Pakhtunkwha, Pakistan. Genes (Basel). 2023;14(6):1184. DOI: 10.3390/genes14061184
11. Vamsi UM, Swapna N, Padma G, Vishnupriya S, Padma T. Haplotype association and synergistic effect of human aldosterone synthase (CYP11B2) gene polymorphisms causing susceptibility to essential hypertension in Indian patients. Clinical and Experimental Hypertension. 2016;38(8):659–65.
12. Bezerra KRV, Tanaka SCSV, Silva VRS, Paschoinni MC, Grecco RLDS, Soardi FC, Balarin MAS. Contribution of rs1799998 polymorphism in CYP11B2 gene in susceptibility to preeclampsia. Revista Brasileira de Saúde Materno Infantil. 2020;20(2):467–71. DOI: 10.1590/1806-93042020000200008
13. Bantis C, Heering PJ, Siekierka-Harreis M, Kouri NM, Schwandt C, Rump LC, Ivens K. Impact of aldosterone synthase gene C-344T polymorphism on IgA nephropathy. Ren Fail. 2011;33(4):393–7. DOI: 10.3109/0886022X.2011.568135
14. Shalaeva EV, Messerli FH. What is resistant arterial hypertension? Blood Press. 2023;32(1):2185457. DOI: 10.1080/08037051.2023.2185457
15. Al Ghorani H, Götzinger F, Böhm M, Mahfoud F. Arterial hypertension – Clinical trials update 2021. Nutr Metab Cardiovasc Dis. 2022;32(1):21–31. DOI: 10.1016/j.numecd.2021.09.007
16. Ott C, Schmieder RE. Diagnosis and treatment of arterial hypertension 2021. Kidney Int. 2022;101(1):36–46. DOI: 10.1016/j.kint.2021.09.026
17. Padmanabhan S, Dominiczak AF. Genomics of hypertension: the road to precision medicine. Nat Rev Cardiol. 2021;18(4):235–50. DOI: 10.1038/s41569-020-00466-4
18. Wang Z, Hou J, Zheng H, Wang D, Tian W, Zhang D, Yan J. Genetic and phenotypic frequency distribution of ACE, ADRB1, AGTR1, CYP2C9*3, CYP2D6*10, CYP3A5*3, NPPA and factors associated with hypertension in Chinese Han hypertensive patients. Medicine (Baltimore). 2023;102(10):e33206. DOI: 10.1097/MD.0000000000033206
19. Li X, Xie P, He J, Cai H, Yang R, Zhang Q, Li B, Qi W, Ma H. CYP11B2 gene polymorphism and essential hypertension among Tibetan, Dongxiang and Han populations from northwest of China. Clin Exp Hypertens. 2016;38(4):375–80. DOI: 10.3109/10641963.2015.1131287
20. Yakimenko O, Chernyshova K, Bondar V, Klochko V, Kolomiets S, Tbilieli V. Aldosterone synthase gene C-344T polymorphism as a risk factor of early left ventricular remodeling in young hypertensive patients with obesity. Georgian Med News. 2021;(320):77–85.
21. Rebrova EV, Shikh EV. The effect of genetic polymorphism of genes encoding the target of action on the variability of the response to antihypertensive therapy. Clin Pharmacol Ther 2024;33(1):59–66. DOI: 10.32756/0869-5490-2024-1-59-66
22. Rebrova EV, Shikh EV. Effect of insertion/deletion polymorphism of angiotensin-converting enzyme gene on efficacy of antihypertensive therapy with angiotensin II receptor blockers. Pharmacy & Pharmacology. 2023;11(6):494–508. DOI: 10.19163/2307-9266-2023-11-6-494-508
23. Ji X, Qi H, Li DB, Liu RK, Zheng Y, Chen HL, Guo JC. Associations between human aldosterone synthase CYP11B2 (-344T/C) gene polymorphism and antihypertensive response to valsartan in Chinese patients with essential hypertension. Int J Clin Exp Med. 2015;8(1):1173–7.
24. Kurland L, Melhus H, Karlsson J, Kahan T, Malmqvist K, Ohman P, Nyström F, Hägg A, Lind L. Aldosterone synthase (CYP11B2)–344 CT polymorphism is related to antihypertensive response: result from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. Am J Hypertens. 2002;15(5):389–93. DOI: 10.1016/s0895-7061(02)02256-2
25. Ortlepp JR, Hanrath P, Mevissen V, Kiel G, Borggrefe M,

Hoffmann R. Variants of the CYP11B2 gene predict response to therapy with candesartan. Eur J Pharmacol. 2002;445(1-2):151–2. DOI: 10.1016/s0014-2999(02)01766-1

26. Kurbanova DR, Srozhidinova NZ, Tursunova NB, Eliseeva MR. Pharmacogenetic aspects of eprosartan therapy and polymorphic markers of renin-angiotensin-aldosterone system genes in Uzbek patients with essential arterial hypertension. Cardiovascular Therapy and Prevention. 2009;8(3):41–6.

AUTHORS

Ekaterina V. Rebrova – Candidate of Sciences (Medicine), Associate Professor, Associate Professor of the Department of Clinical Pharmacology and Propaedeutics of Internal Diseases, Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0002-4374-9754. E-mail: katrina1987@rambler.ru

Evgenia V. Shikh – Doctor of Sciences (Medicine), Professor, Head of the Department of Clinical Pharmacology and Propaedeutics of Internal Diseases, Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0001-6589-7654. E-mail: chih@mail.ru

Natalia B. Lazareva – Doctor of Sciences (Medicine), Associate Professor, Professor of the Department of Clinical Pharmacology and Propaedeutics of Internal Medicine, Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0001-6528-1585. E-mail: natalia.lazareva@gmail.com

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2024-12-2-105-116

Method for assessing myocardial damage under conditions of perfusion of an isolated heart according to the Langendorff method

Yi Wang[1], E.A. Smolyarchuk[2], D.A. Kudlay[2,3], V.S. Shchekin[4], K.A. Zavadich[2], S.S. Sologova[2], L.V. Kornopoltseva[2], I.D. Krylova[4], I.R. Abdurakhmonov[5], M.M. Galagudza[6], A.V. Samorodov[4]

[1] Hangzhou Normal University,
2318 Yuhangtan Str., Hangzhou, China 310030
[2] Sechenov First Moscow State Medical University (Sechenov University),
Bldg. 2, 8 Trubetskaya Str., Moscow, Russia, 119048
[3] Lomonosov Moscow State University,
1 Leninskie Gory, Moscow, Russia, 119991
[4] Bashkir State Medical University,
3 Lenin Str., Ufa, Russia, 450008
[5] Samarkand State Medical University,
18 Amir Temur Str., Samarkand, Republic of Uzbekistan, 140100
[6] Almazov National Medical Research Centre,
2 Akkuratov Str, St. Petersburg, Russia, 197341

E-mail: avsamorodov@gmail.com

Received 17 Oct 2024 | After peer review 01 Nov 2024 | Accepted 11 Nov 2024

One of the leading mechanisms for the development of a severe cardiovascular pathology is the intensification of free radical processes. With a decrease in the activity of the antioxidant defense, the accumulation of free radicals in the body and, as a consequence, the development of the oxidative stress is natural. The registration of the severity of processes that impair the effectiveness of the antioxidant protection, with the subsequent development of an oxidative stress, can serve as a new reliable method for assessing the degree of a myocardial damage.

The aim of the work was to develop a method for assessing the degree of ischemic and ischemia-reperfusion kinds of damage to the myocardium based on the activity of free radical processes in cardiomyocytes.

Materials and methods. All the experimental work under *in vivo* conditions was performed on 50 white sexually mature mongrel male rats. The physiological and morphological parameters of the hearts, biochemical parameters and the lipid peroxidation level of the perfusate were assessed. The changes in the level of the perfusate lipid peroxidation were assessed in a simple model system simulating the lipid peroxidation. The registration of luminescence was carried out using a chemiluminometer KHLM-003 (Russia). Luminol (5-amino-2,3-dehydro-4-phthalazinedione) was used to detect the reactive oxygen species.

Results. With an increase in the ischemia duration and, as a consequence, the degree of the myocardial damage, an increase in the values of the lipid peroxidation determined by chemiluminescence is observed. When simulating 30 minutes of ischemia, necrosis is formed; it accounts for 8.9% of the total heart volume. With an increase in the ischemia duration to 60 minutes, the necrosis zone increases by 1.4 times (p <0.05), and the light sum of luminescence increases by 9.4% (p <0.05) relative to the 30-minute ischemia. A maximum decrease in pH is recorded at the 5th minute of the reperfusion. Next comes the restoration of pH values, and at the 10th minute, there is no longer any statistical difference between the initial and reperfusion values (7.37 *vs* 7.04 at p >0.05). In turn, the activity indicators of cytolysis enzymes (lactate dehydrogenase [LDH] and creatine phosphokinase-MB [CPK-MB]) show a similar pH trend of the growth in the first minutes of the reperfusion, followed by a decrease in the initial values, which is most likely due to the "washing out" of metabolic products. At the same time, the "freeze–storage (14 days)–defrost" cycle does not affect the indicator of the lipid peroxidation activity.

Conclusion. A new method for assessing a myocardial damage during the perfusion of an isolated heart using the Langendorff method, based on the use of the luminol-dependent iron-induced chemiluminescence of the lipid peroxidation level of the perfusate obtained before and after the perfusion of an isolated heart, can become one of the most effective methods for assessing the damage to the myocardial structure.

Keywords: cardioprotection; oxidative stress; isolated heart; antioxidant protection; reperfusion syndrome

For citation: Yi Wang, E.A. Smolyarchuk, D.A. Kudlay, V.S. Shchekin, K.A. Zavadich, S.S. Sologova, L.V. Kornopoltseva, I.D. Krylova, I.R. Abdurakhmonov, M.M. Galagudza, A.V. Samorodov. Method for assessing myocardial damage under conditions of perfusion of an isolated heart according to the Langendorff method. *Pharmacy & Pharmacology.* 2024;12(2):105-116. **DOI:** 10.19163/2307-9266-2024-12-2-105-116

Для цитирования: Юи Ванг, Е.А. Смолярчук, Д.А. Кудлай, В.С. Щекин, К.А. Завадич, С.С. Сологова, Л.В. Корнопольцева, И.Д. Крылова, И.Р. Абдурахмонов, М.М. Галагудза, А.В. Самородов. Способ оценки повреждения миокарда в условиях перфузии изолированного сердца по методу Лангендорфа. *Фармация и фармакология.* 2024;12(2):105-116. **DOI:** 10.19163/2307-9266-2024-12-2-105-116

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Abbreviations: AHA – American Heart Association; CVD – coronary vascular diseases; MI – myocardial infarction; HFA – higher fatty acids; LDH – lactate dehydrogenase; CPK-MB – creatine phosphokinase-MB; VCPR – volumetric coronary perfusion rate; SBP – systolic blood pressure; DBP – diastolic blood pressure; PP – pulse pressure; HR – heart rate; EDP – end-diastolic pressure; LV – left ventricular; LP – lipid peroxidation; ROS – reactive oxygen species; HBFP – Haematoxylin-Basic Fuchsin-picric acid.

Способ оценки повреждения миокарда в условиях перфузии изолированного сердца по методу Лангендорфа

Юи Ванг[1], Е.А. Смолярчук[2], Д.А. Кудлай[2,3], В.С. Щекин[4], К.А. Завадич[2], С.С. Сологова[2], Л.В. Корнопольцева[2], И.Д. Крылова[4], И.Р. Абдурахмонов[5], М.М. Галагудза[6], А.В. Самородов[4]

[1] Ханчжоуский педагогический университет,
310030, Китай, г. Ханчжоу, ул. Юхангтан, д. 2318
[2] Федеральное государственное автономное образовательное учреждение высшего образования «Первый Московский государственный медицинский университет имени И.М. Сеченова» Министерства здравоохранения Российской Федерации (Сеченовский Университет),
119048, Россия, г. Москва, ул. Трубецкая, д. 8, стр. 2
[3] Федеральное государственное бюджетное образовательное учреждение высшего образования «Московский государственный университет имени М.В. Ломоносова»,
119991, Россия, г. Москва, Ленинские Горы, д. 1
[4] Федеральное государственное бюджетное образовательное учреждение высшего образования «Башкирский государственный медицинский университет» Министерства здравоохранения Российской Федерации,
450008, Россия, г. Уфа, ул. Ленина, д. 3
[5] Самаркандский государственный медицинский университет,
140100, Республика Узбекистан, г. Самарканд, ул. Амира Темура, д. 18
[6] Федеральное государственное бюджетное учреждение «Национальный медицинский исследовательский центр имени В.А. Алмазова» Министерства здравоохранения Российской Федерации,
197341, Россия, г. Санкт-Петербург, ул. Аккуратова, д. 2

E-mail: avsamorodov@gmail.com

Получена 17.10.2024 После рецензирования 01.11.2024 Принята к печати 11.11.2024

Одним из ведущих механизмов развития тяжёлых сердечно-сосудистых патологий является интенсификация свободнорадикальных процессов. При снижении активности антиоксидантной защиты закономерным является кумуляция свободных радикалов в организме и, как следствие, развитие оксидативного стресса. Регистрация выраженности процессов нарушения эффективности антиоксидантной защиты с последующим развитием оксидативного стресса может послужить новым достоверным методом оценки степени повреждения миокарда.

Цель. Разработать способ оценки степени ишемического и ишемически-реперфузионного повреждения миокарда на основании активности свободнорадикальных процессов в кардиомиоцитах.

Материалы и методы. Вся экспериментальная работа в условиях *in vivo* была выполнена на 50 белых беспородных половозрелых крысах-самцах. Проводилась оценка физиологических и морфологических параметров сердец, биохимических показателей и уровня липидной пероксидации перфузата. Изменение уровня липидной пероксидации перфузата оценивали в простой модельной системе, имитирующей перекисное окисление липидов. Регистрацию свечения проводили на хемилюминомере «ХЛМ-003» (Россия). Для выявления активных форм кислорода использовали люминол (5-амино-2,3-дегидро-4-фталазиндион).

Результаты. При увеличении длительности ишемии и, как следствие, степени повреждения миокарда отмечался рост значений липидной пероксидации, установленных методом хемилюминесценции. При моделировании 30-минутной ишемии формировался некроз, составляющий 8,9% от общего объёма сердца. При увеличении длительности ишемии до 60 мин, зона некроза увеличивалась в 1,4 раза (p <0,05), а светосумма свечения – на 9,4% (p <0,05) относительно 30-минутной ишемии. Максимальное снижение pH регистрировали на 5-й мин реперфузии, а на 10-й мин статистической разницы между исходным и реперфузионным значением уже не наблюдали (7,37 *vs* 7,04 при p >0,05). В свою очередь показатели активности ферментов цитолиза (лактатдегидрогеназа и креатинкиназа – МВ) демонстрировали аналогичную pH тенденцию по росту в первые минуты реперфузии с последующим снижением исходных значений, что, вероятнее всего, связано с «вымыванием» продуктов обмена. При этом цикл «заморозка–хранение (14 сут)–разморозка» не сказывался на показателе активности липидной пероксидации.

Заключение. Новый способ оценки повреждения миокарда в условиях перфузии изолированного сердца по методу Лангендорфа, основанный на применении методики люминол-зависимой железоиндуцированной хемилюминесценции уровня липидной пероксидации перфузата, полученного до и после перфузии изолированного сердца, может стать одним из наиболее эффективных методов оценки нарушения структуры миокарда.

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-105-116

Ключевые слова: кардиопротекция; оксидативный стресс; изолированное сердце; антиоксидантная защита; реперфузионный синдром

Список сокращений: АНА – Американская кардиологическая ассоциация; ССЗ – сердечно-сосудистые заболевания; ССС – сердечно-сосудистая система; ИМ – инфаркт миокарда; ВЖК – высшие жирные кислоты; ЛДГ – лактатдегидрогеназа; КФК-МВ – креатинкиназа-МВ; ОСКП – объёмная скорость коронарной перфузии; САД – систолическое артериальное давление; ДАД – диастолическое артериальное давление; ПД – пульсовое давление; ЧСС – частота сердечных сокращений; КДД – конечное диастолическое давление; ЛЖ – левый желудочек; ПОЛ – перекисное окисление липидов; АФК – активные формы кислорода; ГОФП – гематоксилин, основный фуксин, пикриновая кислота.

INTRODUCTION

According to the American Heart Association (AHA), in the United States and Canada, the prevalence of cardiovascular diseases (CVD) ranges from 1.5 to 1.9% of the population, and up to 2% in Europe. [1]. In Russia, mortality rates from CVD among men and women of the working age, remain among the highest [2]. Therefore, in Europe, about 4 million people die annually, and the Russian population accounts for about 1 million of the above indicator. [3]. At the same time, one of the leading places in the structure of mortality from CVD is occupied by myocardial infarction (MI).

According to the data of the Department of Medical Statistics of the Central Research Institute of Health Care Organization and Informatization, in 2020, about 170,5 thousand cases of MI among the able-bodied population were registered, 33% of them were fatal outcomes. The presented statistical data cause the interest of many researchers and clinicians to the search for methods of an accurate early diagnostic assessment and prevention of CVD. Among other things, the development of methods to estimate the area of necrotized, stunned and hibernating myocardium in MI is of interest, as it directly correlates with both a high prognosis of a lethal outcome/disability of patients and the processes of myocardial remodelling, which, in turn, determines the preoperative selection of patients and the choice of the most effective cardiac surgical interventions [4].

To date, a number of markers of a cardiac muscle necrosis massiveness have been identified. However, disturbances in the regulation of free radical processes are an important basis for the development of severe cardiovascular pathologies and a number of other diseases. The antioxidant defense system provides an adequate regulation of a free radical formation in the cells, including cardiomyocytes. When its efficiency decreases, free radicals accumulate in the body and, as a consequence, an oxidative stress develops [5, 6]. In addition, ischemia leads to a decrease in the energy supply, which entails disturbances in the transmembrane concentration of Ca^{2+}, Na^{+}, and K^{+} [7–9]. A high concentration of Ca^{2+} inside the cell leads to the activation of a “lipid triad”, which is a source of active detergent substances (higher fatty acids [HFA], lysophospholipids). As a result, free radical oxidation processes increase, a phospholipases and lipases activity increases, free long-chain HFA accumulate [10]. That contributes to the damage of lysosome membranes and release of lysosomal proteases [11–13].

On the other hand, in ischemia, a high intracellular Ca^{2+} concentration is an independent factor of a myofibril damage and an impaired functional activity of mitochondria [14]. Thus, there are many reasons to believe that the registration of the violation processes severity of an antioxidant defense efficiency, with the subsequent development of the oxidative stress, can serve as a new reliable method of determining the degree of a myocardial damage.

THE AIM of the work was to develop a method for assessing a degree of ischemic and ischemia-reperfusion kinds of damage to the myocardium based on the activity of free radical processes in cardiomyocytes.

MATERIALS AND METHODS

Animals

The experimental work was performed on 50 white sexually mature mongrel male rats (225.7±22.4 g), obtained from the nursery of laboratory animals “Pushchino”, on the basis of the Department of Pharmacology of the Bashkir State Medical University (Russia), from January to February 2024. The study has been completed in accordance with the international recommendations of the European Convention for the Protection of Vertebrate Animals in Laboratory Conditions, Rules for Laboratory Preclinical Research in the Russian Federation (3 51000.3-96, 51000.4-96 and GOST 50258-92) and Order of the Ministry of Health and Social Development of Russia No. 708n (dated 23 Aug 2010) “On the approval of good laboratory practice rules (GLP)”.

The study was approved by the Ethical Committee of the Bashkir State Medical University (Protocol No. 1 dated 30 Jan 2024). The animals were kept in accordance with the rules of the European Convention for the Protection

of Vertebrate Animals (Directive 2010/63/EU) and the Guide for Keeping and Care of Laboratory Animals (GOST 33215-2014) in vivarium conditions in accordance with the sanitary and epidemic rules (SP 2.2.1.3218-14). The rats were kept no more than 5 heads in one cage, on an unlimited feed and water consumption, under a fixed light regime of 12 h/12 h. The temperature was maintained within 22–25°C, the relative humidity was 50–70%. The duration of quarantine for all the animals was 14 days, after which the rats were included in the study simultaneously. The animals were divided into 5 experimental groups of 10 rats each: group 1 – Control (without ischemia and reperfusion); group 2 – total ischemia modelling (without reperfusion), group 3 – 30-minute ischemia followed by a 2-hour reperfusion, group 4 – 45-minute ischemia followed by a 2-hour reperfusion, group 5 – 60-minute ischemia followed by a 2-hour reperfusion.

Perfusion of isolated heart according to the Langendorff method

Under the aseptic conditions, the animals were anesthetized using zoletil-xylazine anesthesia according to the following scheme: zoletil 0.3 mg i.m. ("Virbac", France)+xylanite 0.8 mg i.m. (NITA-FARM, Russia) per 100 g of an animal body weight [15, 16]. Further on, a thoracotomy was performed, the main vessels of the heart were cut off (above the capture site). The extracted hearts were placed in the Krebs–Henseleit solution (T_r=+4°C) in order to stop spontaneous contractions. The ascending part of the aorta was cannulated to ensure that the carbogen-saturated Krebs–Henseleit solution (95% O_2 and 5% CO_2) was delivered to the myocardium. To determine the myocardial contractility indices, a catheter with a balloon filled with purified water was inserted into the left ventricular (LV) cavity (volume of the liquid was sufficient to create an end-diastolic pressure; EDP) of 10–15 mmHg). After all the manipulation actions had been completed, a stabilization period of 5 minutes was maintained. Cardiophysiologic parameters were recorded using the PhysExp monitoring system (Cardiprotekt, Russia). The indices of the isolated myocardium contractility were recorded. After all the manipulation actions had been completed, a stabilization period of 5 min was maintained. Cardiophysiologic parameters were recorded using the PhysExp monitoring system (Cardiprotekt, Russia). The indices of the isolated myocardium contractility were recorded: the pressure developed by the LV; heart rate (HR, beats/min); EDP (mmHg).

Under the experimental conditions, the oxygen and plasma replacement solution (Krebs–Henseleit solution) supply to cardiomyocytes, as well as the outflow of the dissimilation products from them was completely stopped (total ischemia model); after the ischemia, the restoration of the cardiac perfusion was performed for 30, 45, and 60 minutes (depending on the tasks); it was followed by the assessment of the perfusate oxidative potential, a pH index, as well as a biochemical analysis and a morphologic evaluation of the myocardium. The perfusate was frozen and stored at -20–22°C, followed by thawing and the parameters re-evaluation to determine the stability of the lipid peroxidation level for 14 days.

Assessment of perfusate oxidative potential

The oxidative potential was assessed by luminol-dependent iron-induced chemiluminescence of the lipid peroxidation (LP) level of perfusate obtained before and after the perfusion of the isolated heart according to the Langendorff method. The changes in the level of the perfusate LP were evaluated in a simple model system simulating the lipid peroxidation. The luminescence was registered on a chemiluminometer HLM-003 (Russia). Luminol (5-amino-2,3-dehydro-4-phthalazindione) was used to registrate the ROS, which oxidizes and forms electronically excited carbonyl chromophores with a high quantum yield, resulting in a sharp increase in the intensity of luminescence associated with the formation of reactive oxygen species (ROS). Chemiluminescence was recorded for 3 min. One conditional unit of chemiluminescence was 5.1×105 quanta/sec. [17]. Luminescence intensity indices were recorded: a light sum and a slow flash amplitude of the model system in the presence of the perfusate obtained before the perfusion, and the luminescence intensity of the model system in the presence of the perfusate obtained after the perfusion. The intensity of the developing luminescence was used to evaluate the LP processes in the model system in the perfusate presence. The greater the values of the chemiluminescence intensity indices of the model system in the presence of the perfusate obtained after the perfusion in comparison with the initial indices are, the more massive the degree of the myocardial damage as a result of ischemic and ischemia-reperfusion factors of the myocardial damage is.

Evaluation of biochemical parameters creatine phosphokinase-MB

Biochemical indices (lactate dehydrogenase [LDH] and creatine phosphokinase-MB [CPhK-MB]) were determined by generally recognized methods on an automatic biochemical analyzer with an open reagent system Dirui CS-T240 (DIRUI Industrial Co., Ltd., China) using original reagent kits and their instructions.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-105-116

Morphologic evaluation of hearts

At the end of the reperfusion, half of the hearts in each group (5 hearts from each group) were stained with triphenyltetrazolium chloride followed by a macroscopic evaluation, the other half of the hearts were fixed in a 10% buffered neutral formalin solution. A macroscopic imaging of the hearts was performed using the LSCI laser speckle-imaging system (RWD, China) in the photoregistration mode of the speckle color pattern. The fixed hearts were transversely dissected into 4 parts from the base to the apex of the heart, so that the left, right ventricles and the interventricular septum were included in the slices. Next, a standard histological processing by alcohols of increasing concentrations was performed, after which the preparations were encapsulated in paraffin and 4 μ thick sections were made, which were stained with haematoxylin-eosin, haematoxylin-basic fuchsin-picric acid (HBFP). To improve the accuracy of the results, special staining for ischemia slices was done twice as much as haematoxylin-eosin, with each slice made in a paraffin tape. In this way, the possibility of staging false positive or false negative reactions was reduced. The prepared glass specimens were scanned on a Pannoramic 250 (3DHISTECH Ltd, Hungary) followed by the examination of histological sections under different magnifications using CaseViewer software (3DHISTECH Ltd., Hungary); the measurements were made with straight lines in micrometers (μm), after which the results were uploaded to Statistica 10 (StatSoft Inc., USA). The cardiomyocyte damage severity was assessed by a semi-quantitative method in glass specimens stained according to the HBFP method at a 10-fold magnification of a microscope objective at 10 fields of vision as follows: 0 points – intact myocardium, 1 point – a minimal damage (<26% of myocardium), 2 points – a minimal to moderate level (26–50% of myocardium), 3 points – a moderate level of damage (51–75% of myocardium), 4 points – severe damage (>75% of myocardium).

Statistical analysis

The results of the study were processed using a statistical package Statistica 10 (StatSoft Inc, USA). The test for the normality of distribution of the actual data was performed using the Shapiro–Wilk criterion. A median and an interquartile range were used to describe the groups. The analysis of variance was performed using the Kraskell–Wallis or Mann–Whitney criteria (for independent observations) and the Friedman one (for repeated observations). The critical significance level was taken as 0.05.

RESULTS

The study results of the perfusate collected before ischemia and after the reperfusion, as well as the measurements of cardiophysiological parameters and a histological examination in the experiment at 45 minutes of ischemia and the reperfusion are presented in Table 1.

The data of Table 1 show that the myocardial ischemia-reperfusion changes the indices of physiological constants. For example, systolic blood pressure (SBP) indices are characterised by a maximum 23.7% decrease after the reperfusion (15 min of reperfusion) with a subsequent recovery to 141.3 mmHg (86.6% of initial values) at 45 min of reperfusion. A diastolic blood pressure (DBP) values are characterised by an average increase of 40% (p <0.05) immediately after a blood flow start and during all 45 min of the reperfusion.

It should be noted that perfusate pH after 40 min ischemia is characterised by a 5.0% decrease (p <0.05) relative to the initial values. The maximum decrease in pH is registered at the 5th min of the reperfusion. Further on, there is a recovery of pH values, and at the 10th min, there is no statistical difference between initial and reperfusion values (7.37 *vs* 7.04 at p >0.05). In turn, the indices of the cytolysis enzymes activity (LDH and CPhK-MB) show a similar pH tendency to increase in the first minutes of the reperfusion with a subsequent decrease in the initial values. Evaluation results of the ischemia duration effect on the size of necrotic myocardium, as determined by a macroscopic evaluation by triphenyltetrazolium chloride staining (Fig. 1), and the level of the lipid peroxidation, as determined by chemiluminescence, 10 minutes after the end of the ischemia period, are presented in Table 2.

The data of Table 2 show that with increasing the ischemia duration there was registered an increase in the lipid peroxidation activity determined by the chemiluminescence method. When modelling a 30-minute ischemia, a necrosis was formed, accounting for 8.9% (p <0.05) of the total heart volume (Fig. 1). When the ischemia duration was increased 2-fold (60 min), the area of the necrosis increased 1.4-fold (p <0.05) and the luminescence increased by 9.5% (p <0.05) relative to the 30-minute ischemia.

The evaluation results of a single “freeze–store–thaw” cycle effect on the stability of lipid peroxidation level readings determined by chemiluminescence, are presented in Table 3.

According to the data of Table 3, the cycle “freezing–storage–thaw” did not affect the lipid peroxidation activity index. The storage for more than 14 days in a household freezer did not distort the data obtained.

The microscopic picture after the ischaemic-perfusion of the heart muscle on the isolated heart was characterised primarily by ventricular edema (Fig. 2), and predominantly, at the expense of its epicardial and myocardial layers.

Table 1 – Evaluation results of perfusate properties and morphological picture in 45-minute ischemia experiment and reperfusion, Me [Q1-Q3]

Indicator	Intact values	After stabilisation period (before ischemia)	Reperfusion, min					
			1	5	10	15	30	45
pH	7.37 (7.33–7.42)*	7.07 (6.98–7.12)	6.95 (6.87–7.08)*	6.75 (6.61–6.92)*	7.04 (6.89–7.13)	7.10 (7.01–7.15)	7.18 (7.09–7.21)†	7.31 (7.27–7.36)*, †
SBP, mmHg	–	163.2 (161.5–172.4)	143.5 (140.2–149.7)*	151.2 (147.3–158.4)*	149.4 (142.6–154.2)*	124.5 (120.5–134.9)*, †	144.5 (140.2–151.4)*	141.3 (137.8–145.4)*
DBP, mmHg	–	10.8 (9.1–11.6)	18.5 (17.4–19.3)*	17.4 (17.1–18.2)*, †	16.5 (15.7–17.8)*, †	17.1 (16.8–18.3)*, †	16.5(15.2–17.9)*, †	17.7 (16.7–18.5)*, †
PP, mmHg	–	106.1 (104.2–107.8)	56.1 (54.3–60.2)*	41.8 (38.5–42.7)*, †	42.4 (40.1–45.4)*, †	51.6 (49.3–54.8)*	64.3 (62.7–65.0)*, †	71.3 (70.2–73.5)*, †
HR, bpm	–	241.2 (237.8–245.3)	187.8 (180.2–192.4)*	290.4 (287.3–296.5)*, †	292.7 (284.5–300.2)*, †	252.2 (247.1–263.5)†	264.3 (251.6–274.8)†	231.9 (227.6–233.8)†
VCPR	–	13.8 (12.3–14.7)	19.8 (17.8–21.4)*	15.3 (14.2–17.8)†	14.4 (12.5–15.7)†	10.6 (8.6–12.7)*, †	9.2 (8.7–10.3)*, †	9.4 (8.5–11.2)*, †
CPhK-MB, MU/L	–	10.4 (9.8–11.7)	12.3 (10.7–13.8)	17.4 (15.8–19.2)*, †	21.3 (19.4–22.6)*, †	26.8 (24.3–29.5)*, †	12.8 (11.5–14.6)†	11.9 (10.2–12.5)
LDH, MU/L	–	16.2 (15.4–17.9)	18.3 (17.6–20.4)	28.4 (24.5–30.7)*, †	32.6 (28.4–34.7)*, †	29.3 (28.7–30.1)*, †	17.2 (16.4–18.9)	17.1 (15.7–19.3)
Light sum of luminescence, 5.1×105 quanta/sec	–	30.7 (26.5–33.8)	45.4 (38.1–47.3)*	104.5 (98.5–107.6)*, †	105.2 (96.3–102.4)*, †	104.7 (97.6–107.8)*, †	100.2 (97.3–104.5)*, †	110.3 (106.3–113.7)*, †
Amplitude of slow flash, 5.1×105 quanta/sec	–	16.6 (14.4–18.7)	24.5 (19.4–27.3)*	37.2 (34.4–42.3)*, †	38.7 (35.2–42.6)*, †	36.7 (33.4–40.2)*, †	36.2 (33.3–39.6)*, †	38.4 (36.1–44.3)*, †
Myocardial thickness, µm	11.8 (10.5–12.1)*	13.8 (12.4–14.2)	13.6 (13.1–14.9)	13.9 (12.7–15.2)	14.9 (14.5–17.1)*	16.7 (15.1–17.3)*, †	18.3 (16.8–19.1)*, †	19.1 (17.4–20.1)*, †

Note: VCPR – volumetric coronary perfusion rate; SBP – systolic blood pressure; DBP – diastolic blood pressure; PP – pulse pressure; HR – heart rate; LDH – lactate dehydrogenase; CPhK-MB – creatine phosphokinase-MB; * p <0.05 – values before ischemia *vs* after ischemia, or intact values; † p <0,05 – values immediately after ischemia (1 minute of reperfusion) *vs* the rest of the reperfusion period.

Table 2 – Lipid peroxidation levels determined by chemiluminescence and myocardial damage volume values determined macroscopically by triphenyltetrazolium chloride staining, Me [Q1-Q3]

Indicator	Krebs-Henseleit solution before perfusion	After stabilisation period (before ischemia)	Ischemia, min		
			30	45	60
Light sum of luminescence, 5.1†105 quanta/sec	53.76 (48.3–57.8)*, †	30.7 (26.4–33.6)	100.2 (97.3–102.5)*, †	106.3 (105.1–108.3)*	116.8 (114.7–122.5)*, †
Amplitude of slow flash, 5.1†105 quanta/sec	24.73 (20.1–27.6)*, †	16.6 (14.4–18.7)	35.1 (32.4 –37.2)*, †	38.4 (37.9–39.2)*	40.1 (39.6–44.8)*, †
Infarct area volume to total heart volume, %	–	0.0 (0.0–0.0)	8.9 (6.7–11.9)*, †	21.0 (17.9–23.5)*	39.4 (34.5–40.3)*, †

Note: * p <0,05 – values before ischemia *vs* after ischemia, or intact values, † p <0,05 – values of 45-minute ischemia *vs* intact values, or 30- and 60-minute ischemia.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-105-116

Table 3 – Evaluation results of perfusate properties of one “freeze–store–thaw” cycle, Me [Q1-Q3]

Indicator	Solution	Values before freezing	Period, days 1	3	7	14
Light sum of luminescence, 5.1×105 quanta/sec	I	100.2 (97.3–104.5)	101.5(97.5–106.6)†	100.5 (95.6–104.2)†	102.4 (98.4–106.2)†	101.3(96.2–103.4) †
	II	98.5 (96.3–101.4)*, †	99.8 (95.6–100.8)*, †	97.5 (93.1–100.2)*, †	98.6 (97.9–101.4)*, †	98.7 (97.7–101.5)*, †
Slow flash amplitude, 5.1×105 quanta/sec	I	36.2 (33.7 –39.6)	35.2 (32.6 –37.6)†	36.2 (33.5 –37.7)†	38.3 (34.6–41.5)†	36.4 (34.2 –38.6)†
	II	35.4 (32.4–37.3)*, †	33.5 (30.5–36.4)*, †	33.8 (30.2–35.3)*, †	35.2 (35.7–38.2)*, †	36.8 (36.4–39.3)*, †

Note: I – Krebs-Henseleit solution before perfusion; II – perfusate after 45 min of ischemia and 5 min of reperfusion of isolated heart. * p <0,05 – I *vs* II values for the corresponding unfrozen period; † p ≥0,05 – Krebs-Henseleit solution I and II values before freezing *vs* unfrozen samples.

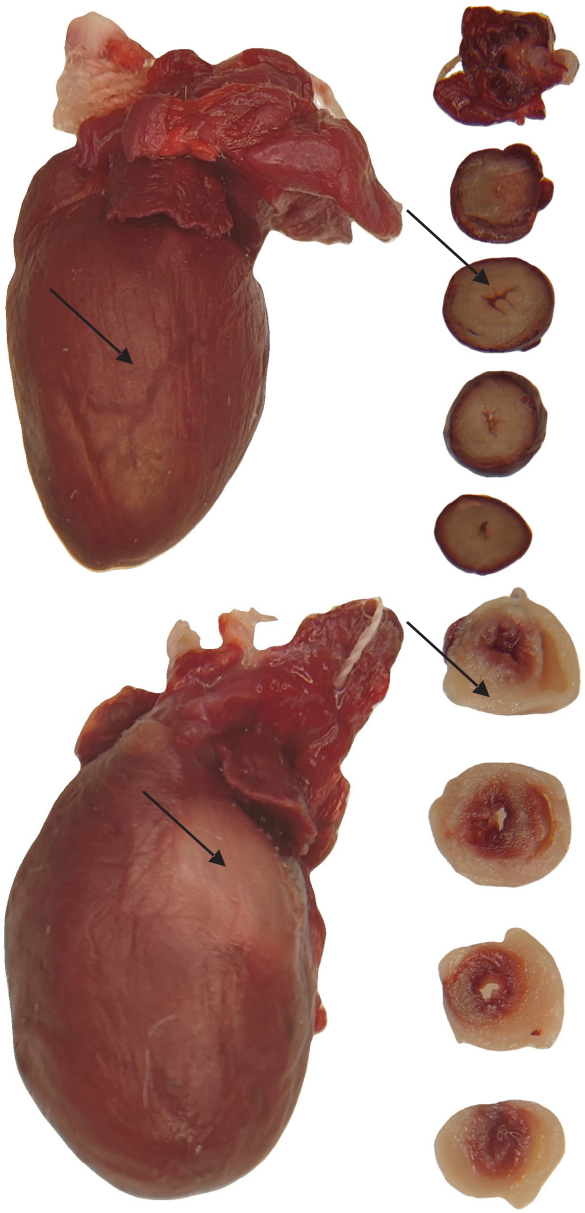


Figure 1 – Pictures of hearts staining with triphenyltetrazolium chloride
Note: areas of viable part of myocardium are coloured red; ischemic white parts are marked with arrows. Macroscopically ischemia was assessed on section with identification of pathology localisation.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

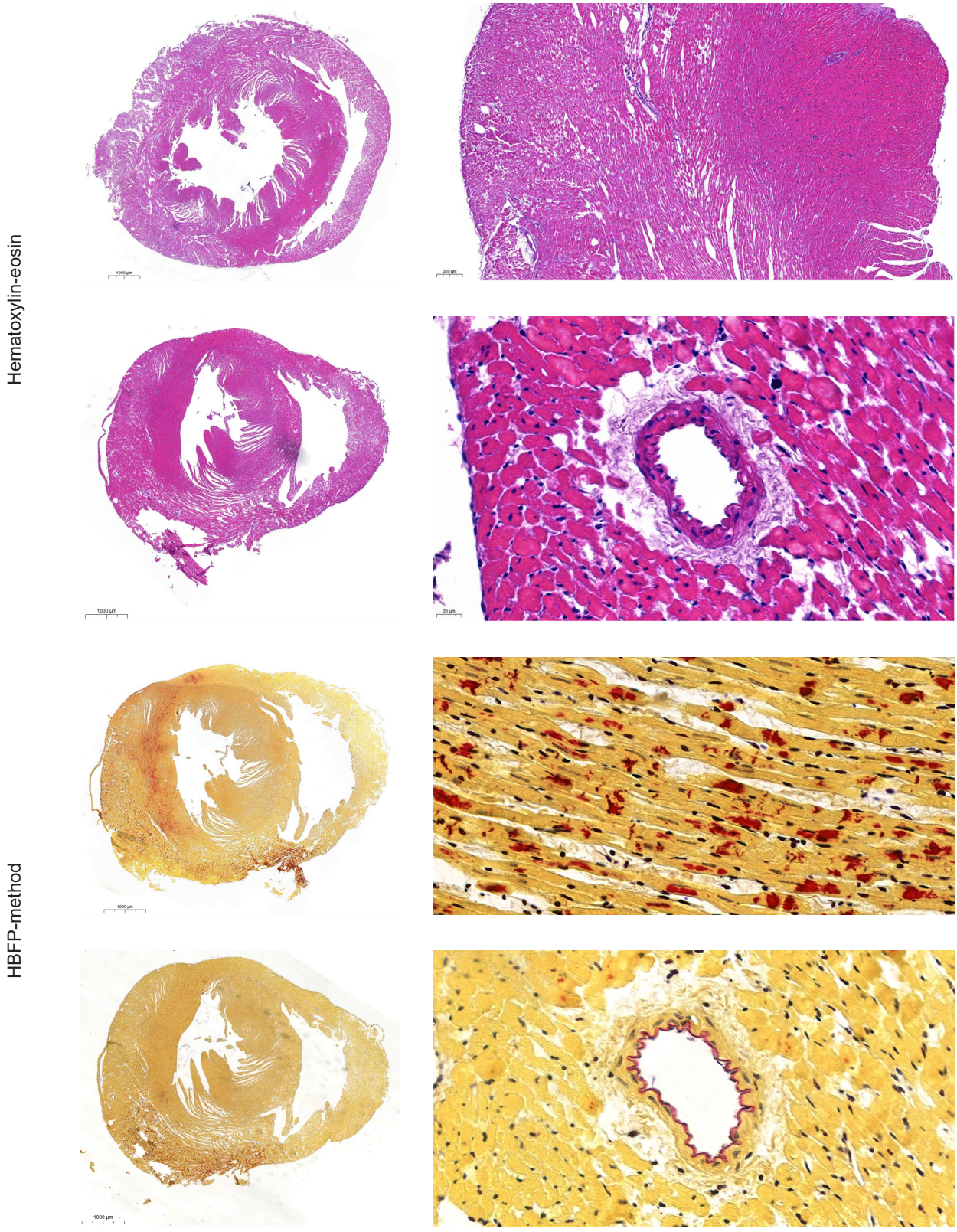


Figure 2 – Micropicture of myocardial cross-section

Note: Ventricular and paravasal edema in the organ is visualised; foci of damaged cardiomyocytes and endothelial cells are noted in specialized staining for ischemia. Hematoxyin-eosin staining, HBFP method; magnification ×15 and ×400.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-105-116

It should be noted that the very fact of the heart perfusion under artificial conditions leads to myocardial edema, thus increasing a myocardial thickness by 16.9% (p <0.05) compared to the hearts not subjected to the Langendorff perfusion. A blood flow restoration after 45-minute ischemia resulted in a gradual increase of edema. The median myocardial thickness reached 14.9 µm already at the 10th min of the reperfusion, which was 26.5 (p <0.05) and 7.9% (p <0.05) higher than the values of intact and non-ischaemic hearts, respectively. Edema reached its maximum values at the 45th min of the reperfusion, when the median value of the myocardial thickness reached 19.1 µm (p <0.05), which was 1.3 times (p <0.05) higher than in the hearts without ischemia. At the same time, the arterial wall thickened, the endothelial cells swelled. The application of specialised HBFP staining indicated the areas of myocyte ischemia also in the epicardial and myocardial part of the ventricle, which had previously been visualised macroscopically by the application of triphenyltetrazolium chloride staining. As a part of HBFP staining, basic fuchsin interacted with the breakdown products and picric acid got stained in a different colour. The appearance of a fuchsinophilic substrate was first observed in the nucleus area and then spread throughout the cytoplasm and muscle fibre. Thus, semi-quantitative scoring of a cardiomyocyte damage yielded the following results: control – 0.24±0.43 points; perfusion – 1.78±0.51 points (p <0.05 compared to control) and an ischemia-reperfusion group – 1.54±0.5 points (p <0.05 compared to control).

It should be noted that prolonged ischemia with lysis of cardiomyocytes resulted in either a decreased visualisation of the fuchsinophilic substrate or in the fact that the staining became background due to the dye release into the intercellular space. Thus, a macroscopical comparison was carried between the ischemia areas stained with triphenyltetrazolium chloride, contractural changes in the course of the muscle fibers on haematoxylin-eosin, and positive areas revealed by the HBFP staining. The determination of the lipid peroxidation in the perfusate served as an additional criterion when specialised methods of staining for ischemia become uninformative.

DISCUSSION

To date, there are several ways of modelling a myocardial injury simulating an acute coronary syndrome. There is a known method of studying the degree of a myocardial damage in laboratory rats by a direct ligation of coronary artery branches after thoracotomy with a subsequent wound closure and an elimination of pneumothorax, or a thermal coagulation of coronary arteries using an instrument / electrocoagulator heated with an alcohol burner [18]. However, all methods for *in vivo* studies have a number of drawbacks that limit a widespread use of these techniques. For example, after a coronary artery ligation or thermocoagulation, an assessment of the myocardial necrosis degree by analyzing biochemical markers of cytolysis (troponin, myoglobin, CFK, CFK-MB, ALT, AST, etc.) is insufficiently informative due to the insufficient specificity and the possibility of only indirectly assessing the damage degree. Moreover, a direct method of a myocardial ischemic damage modelling is rather difficult to reproduce due to high requirements to researchers' skills and possible peculiarities of coronary vessels in animals [19, 20].

A more standardized model of myocardial ischemia with a subsequent assessment of the degree of cardiac tissue ischemia is the Langendorff method of an isolated heart perfusion, which consists in a perfusion of an isolated rat heart with an oxygenated Krebs–Henseleit solution [21–24]. The assessment of a myocardial damage in this technique is based on the analysis of the LV contractility by an insertion of a measuring balloon into the LV cavity and the determination of the perfusate biochemical properties. Despite the advantages of this method – a complete control of the ischemia period, an exclusion of the influence of endogenous factors, as well as individual anatomical features of the animals – its significant disadvantage is the analysis of the obtained data. They rely solely on indirect biochemical indicators of the myocardial damage as well as on the contractility of the heart, resulting in the analysis of only a diastolic dysfunction of the heart contractility which is not a reliable and adequate method of assessing the degree of myocardial ischemia either.

It should be noted that the informativity of cardiomyocyte cytolysis markers dynamics in relation to the myocardial necrosis massiveness assessment has been studied for a long time with different approaches to the assessment of these markers. For example, for troponin, the assessment according to the reference values is recommended, while for CPhK-MB, there are no such recommendations, and there are separate proposals to assess CPhK-MB relative to baseline values or the upper limit of normal [25]. The increase in CPhK-MB is not a specific and highly sensitive indicator of a myocardial damage, especially during the cardiac surgery, due to the influence of many factors on this index. It should be emphasized that an important criterion reducing the diagnostic value of this enzyme detection is the possibility of its identification in blood only in the first 9 hours after the first signs of MI. After the above time interval, there is no reliable correlation between the degree of a myocardial damage and quantitative indices of CPhK-MB [26, 27]. The application of the CPhK-MB level estimation in relation to the necrotic changes in myocardium is most reliable from the diagnostic point of view, in case of the detection of large necrosis zones in myocardium with characteristic changes on the ECG [28, 29]. In such a situation, the appearance of an additional parameter allowing to detect a myocardial

damage and its degree in preclinical studies will help to objectify the picture.

Thus, it has been established that the level of lipid peroxidation changes in a regular way in accordance with the results of a biochemical analysis and a morphological study, but persists longer in the perfusate during the reperfusion, which shows the possibility of using the proposed method in order to determine the fact and degree of an ischemic and ischemic-perfusion myocardial damage.

Study limitations

The processes of the interrelation of the antioxidant defense impairment and the severity of necrotic changes in the cardiac muscle in the isolated heart according to the Langendorff method, have not been widely reported in the scientific literature. Within the framework of this study, the sample size is sufficient to confirm the presence of a regularity between the changes in the level of the LP and the degree of the myocardial damage, but within the framework of this paper, it is impossible to fully speak about the presence of strong relationships. This pattern requires a further in-depth study, including the prospect of a further application in the clinical practice as one of the signs of a myocardial ischemic damage in patients with an acute coronary syndrome. Other mechanisms, the use of which can be evaluated as markers of necrotic changes, have not been considered in the present study.

CONCLUSION

The assessment method of a myocardial damage under the conditions of a perfusion of an isolated heart according to the Langendorff method, based on the application of the luminol-dependent iron-induced chemiluminescence technique of the lipid peroxidation level of the perfusate obtained before and after the perfusion of an isolated heart, is one of the most effective methods of the assessment of the myocardial structure disturbance, as the level of the lipid peroxidation was comparable with the results of a biochemical analysis and a morphological study.

FUNDING

This research was supported by the Russian Science Foundation, grant No. 24-45-00071 (NSFC partner grant: No. 82361138563).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Wang Yi, Elena A. Smolyarchuk, Dmitry A. Kudlai, Ilkhomjon R. Abdurakhmonov, Mikhail M. Galagudza, Alexander V. Samorodov – development of the concept of the experiment; Vlas S. Shchekin, Ksenia A. Zavadich, Susanna S. Sologova, Lyubov V. Kornopoltseva, Irina D. Krylova – conducting the study; Ilkhomjon R. Abdurakhmonov, Ksenia A. Zavadich, Susanna S. Sologova, Lyubov V. Kornopoltseva – research literature search; Elena A. Smolyarchuk, Alexander V. Samorodov, Mikhail M. Galagudza – writing the manuscript. All the authors participated in the preparation and editing the text of the article. All the authors confirm their authorship compliance with the ICMJE international criteria (all the authors have made a significant contribution to the conceptualisation, research and preparation of the article, read and approved the final version before the publication).

REFERENCES

1. Roger VL. Epidemiology of heart failure: A contemporary perspective. Circ Res. 2021;128(10):1421–34. DOI: 10.1161/CIRCRESAHA.121.318172
2. Drapkina OM, Shalnova SA, Imaeva AE, Balanova YuA, Maksimov SA, Muromtseva GA, Kutsenko VA, Karamnova NS, Evstifeeva SE, Kapustina AV, Yarovaya EB, Litinskaya OA, Pokrovskaya MS, Efimova IA, Borisova AL, Doludin YuV, Kontsevaya AV. Epidemiology of cardiovascular diseases in regions of Russian Federation. Third survey (ESSE-RF-3). Rationale and study design. Cardiovascular Therapy and Prevention. 2022;21(5):3246. DOI: 10.15829/1728-8800-2022-3246
3. [Nauchno-organizatsionnyĭ komitet proekta ÉSSE-RF. Epidemiology of cardiovascular diseases in different regions of Russia (ESSE-RF). The rationale for and design of the study]. Russian Journal of Preventive Medicine. 2013;16(6):25–34. (In Russ).
4. Shigotarova EA, Galimskaja VA, Golubeva AV, Oleynikov VE. The myocardial infarction size measuring using modern methods. Terapevticheskii arkhiv. 2020;92(4):105–10. DOI: 10.26442/00403660.2020.04.000571
5. Anikin DA, Solovyeva IA, Demko IV, Sobko EA, Kraposhina AYu, Gordeeva NV. Free-radical oxidation as a pathogenetic factor of metabolic syndrome. Obesity and metabolism. 2022;19(3):306–16. DOI: 10.14341/omet12804
6. Levchenko IN, Vladimirov GK, Volodyaev IV. [Izmenenie struktury i funktsii tsitokhroma s pri vzaimodeystvii s kardiolipinom: struktura belka, ego peroksidaznaya aktivnost' i svobodno radikal'nye protsessy]. Genes & Cells. 2022;17(3):133–4. (In Russ).
7. Rybakova LP, Aleksanian LR, Kapustin SI, Bessmeltsev SS. Human oxidant-antioxidant system, role in the pathological process and its correction. Bulletin of Hematology. 2022;(4):52–6.
8. Galeeva NV, Valeeva IKh, Fazylova YuV. Correlation between the number and aggregation capacity of platelets

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-105-116

and the process of lipid peroxidation in patients with chronic hepatitis C. Practical medicine. 2022;20(1):37–43. DOI: 10.32000/2072-1757-2022-1-37-43

9. Fenouillet E, Mottola G, Kipson N, Paganelli F, Guieu R, Ruf J. Adenosine Receptor Profiling Reveals an Association between the Presence of Spare Receptors and Cardiovascular Disorders. Int J Mol Sci. 2019;20(23):5964. DOI: 10.3390/ijms20235964
10. Peng ML, Fu Y, Wu CW, Zhang Y, Ren H, Zhou SS. Signaling Pathways Related to Oxidative Stress in Diabetic Cardiomyopathy. Front Endocrinol (Lausanne). 2022;13:907757. DOI: 10.3389/fendo.2022.907757
11. Xu X, Jin K, Bais AS, Zhu W, Yagi H, Feinstein TN, Nguyen PK, Criscione JD, Liu X, Beutner G, Karunakaran KB, Rao KS, He H, Adams P, Kuo CK, Kostka D, Pryhuber GS, Shiva S, Ganapathiraju MK, Porter GA Jr, Lin JI, Aronow B, Lo CW. Uncompensated mitochondrial oxidative stress underlies heart failure in an iPSC-derived model of congenital heart disease. Cell Stem Cell. 2022;29(5):840–855.e7. DOI: 10.1016/j.stem.2022.03.003
12. Gumpper-Fedus K, Park KH, Ma H, Zhou X, Bian Z, Krishnamurthy K, Sermersheim M, Zhou J, Tan T, Li L, Liu J, Lin PH, Zhu H, Ma J. MG53 preserves mitochondrial integrity of cardiomyocytes during ischemia reperfusion-induced oxidative stress. Redox Biol. 2022;54:102357. DOI: 10.1016/j.redox.2022.102357
13. Wang J, Li S, Yu H, Gao D. Oxidative stress regulates cardiomyocyte energy metabolism through the IGF2BP2-dynamin2 signaling pathway. Biochem Biophys Res Commun. 2022;624:134–40. DOI: 10.1016/j.bbrc.2022.07.089
14. Harvey F, Aromokunola B, Montaut S, Yang G. The Antioxidant Properties of Glucosinolates in Cardiac Cells Are Independent of H_2S Signaling. Int J Mol Sci. 2024;25(2):696. DOI: 10.3390/ijms25020696
15. Nuzhny VP, Kibler NA, Tsvetkova AS, Kharin SN, Barkhaev AB, Shmakov DN. Influence of atrial electrical stimulation on the transmural sequence of depolarization of ventricular walls in rat heart under zolethyl-xylazine anesthesia. Journal of Evolutionary Biochemistry and Physiology. 2023;59(3):190–7. DOI: 10.31857/S0044452923030075
16. Kadomtsev DV, Pasechnikova EA, Golubev VG. Zoletil-xylazine anesthesia experiments in rats. International journal of applied and fundamental research. 2015;(5):56–7.
17. Khaliullin FA, Mamatov ZhK, Timirkhanova GA, Samorodov AV, Bashirova LI. Synthesis, antiaggregation and antioxidant activity of salts of 2-[(1-iso-butyl-3-methyl-7-(thietanyl-3)xanthine-8-yl)thio]-acetic acid. Chemical-Pharmaceutical Journal. 2020;54(9):9–14. DOI: 10.1007/s11094-020-02293-w
18. Rantsev MA, Sarapultsev PA, Dmitriev AN, Plaksin KV, Malkov AP, Medvedeva SY, inventors; Patent RU 2407062. A method for modeling myocardial infarction in rats. 2010 Dec 20.
19. Plitt A, Dangas G. Cardiac enzyme elevation after coronary revascularization. Catheter Cardiovasc Interv. 2018;91(2):224–5. DOI: 10.1002/ccd.27502
20. Fan J, Ma J, Xia N, Sun L, Li B, Liu H. Clinical Value of Combined Detection of CK-MB, MYO, cTnI and Plasma NT-proBNP in Diagnosis of Acute Myocardial Infarction. Clin Lab. 2017;63(3):427–33. DOI: 10.7754/Clin.Lab.2016.160533
21. Minasyan SM, Galagudza MM, Sonin DL, Bobrova EA, Zverev DA, Korolev DV, Dmitriev YuV, Vasilyeva MS, Grigorova YuN, Vlasov TD. Perfusion technique of isolated rat heart. Regional blood circulation and microcirculation. 2009;8(4(32)):54–9. (In Russ).
22. Liang T, Zhang D, Hu W, Tian C, Zeng L, Wu T, Lei D, Qiang T, Yang X, Sun X. A dual lock-and-key two photon fluorescence probe in response to hydrogen peroxide and viscosity: Application in cellular imaging and inflammation therapy. Talanta. 2021;235:122719. DOI: 10.1016/j.talanta.2021.122719
23. Dreischmeier E, Fahl WE. Determination of plasma levels of the active thiol form of the direct-acting PrC-210 ROS-scavenger using a fluorescence-based assay. Anal Biochem. 2021;616:114100. DOI: 10.1016/j.ab.2021.114100
24. Cabello MC, Bartoloni FH, Bastos EL, Baader WJ. The Molecular Basis of Organic Chemiluminescence. Biosensors (Basel). 2023;13(4):452. DOI: 10.3390/bios13040452
25. Semenyuta VV, Maksimov NI, Anisimov SV, Rykov VV, Mykolnikov AV, Nazarov SB. Changes of creatine phosphokinase MB levels in the context of myocardial reperfusion injury. Russian Journal of Cardiology. 2022;27(10):4954. DOI: 10.15829/1560-4071-2022-4954
26. Darenskaya MA, Kolesnikova LI, Kolesnikov SI. COVID-19: oxidative stress and the relevance of antioxidant therapy. Annals of the Russian academy of medical sciences. 2020;75(4):318–25. DOI: 10.15690/vramn1360
27. Vasiliev DK, Rudenko BA, Feshchenko DA, Shukurov FB, Shanoyan AS. Clinical case of IVUS-guided coronary artery stenting in a patient with chronic renal failure. Creative surgery and oncology. 2023;13(4):342–47. DOI: 10.24060/2076-3093-2023-13-4-342-347
28. Vasiliev DK, Rudenko BA, Feshchenko DA, Shukurov FB, Shanoyan AS. Endovascular Treatment of a Patient with Multivessel Decease Combined with Chronic Occlusion of the Right Coronary Artery. Creative surgery and oncology. 2022;12(3):217–23. DOI: 10.24060/2076-3093-2022-12-3-217-223
29. Poleschenko YaI, Protsak ES, Druzhininsky DA, Galagoudza MM, Minasian SM, Borshchev YuYu, Kurilov AB, Sonin DL. Methodology of asystolic donor heart's condition study in an experiment using small laboratory animals. Translational Medicine. 2021;8(5):50–6. DOI: 10.18705/2311-4495-2021-8-5-50-56

AUTHORS

Wang Yi – PhD, Professor, Hangzhoui Normal University, Institute of Pharmacy, China. ORCID ID: 0000-0001-9048-0092. E-mail: yi.wang1122@hznu.edu.cn

Elena A. Smolyarchuk – Candidate of Sciences (Medicine), Associate Professor, Head of the Department of Pharmacology, A.P. Nelyubin Institute of Pharmacy of Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0002-2615-7167. E-mail: smolyarchuk@mail.ru

Dmitry A. Kudlai – Doctor of Sciences (Medicine), Professor of the Department of Pharmacology, A.P. Nelyubin Institute of Pharmacy of Sechenov First

Moscow State Medical University (Sechenov University); Professor of the Department of Pharmacognosy and Industrial Pharmacy, Faculty of Fundamental Medicine of Lomonosov Moscow State University; Corresponding Member of the Russian Academy of Sciences. ORCID ID: 0000-0003-1878-4467. E-mail: kudlay_d_a@staff.sechenov.ru

Vlas S. Shchekin – Head of the Scientific and Morphological Laboratory of Bashkir State Medical University. ORCID ID: 0000-0003-2202-7071yu. E-mail: vlas-s@mail.ru

Ksenia A. Zavadich – Candidate of Sciences (Medicine), Associate Professor of the Department of Pharmacology, A.P. Nelyubin Institute of Pharmacy of Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0002-4792-7132. E-mail: kzavadich@mail.ru

Susanna S. Sologova – Candidate of Sciences (Biology), Associate Professor of Department of Pharmacology, A.P. Nelyubin Institute of Pharmacy of Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0000-0002-8526-7147. E-mail: sologova_s_s@staff.sechenov.ru

Lyubov V. Kornopoltseva – 4th year student of the Faculty of Pharmacy of Sechenov First Moscow State Medical University (Sechenov University). ORCID ID: 0009-0005-7264-6438. E-mail: kornopoltseva_l_v@staff.sechenov.ru

Irina D. Krylova – 6th year student of the paediatric faculty of Bashkir State Medical University. ORCID ID: 0000-0001-8979-9135. E-mail: i.krylova16@yandex.ru

Ilkhomjon R. Abdurakhmonov – PhD, Head of the Department of Clinical Pharmacology of Samarkand State Medical University, Republic of Uzbekistan. ORCID ID: 0000-0003-4409-0186. E-mail: makval81@rambler.ru

Mikhail M. Galagudza – Doctor of Sciences (Medicine), Professor, Director of the Institute of Experimental Medicine, Chief Researcher of the Research Institute of Microcirculation and Myocardial Metabolism, Head of the Department of Pathology of the Institute of Medical Education, Almazov National Medical Research Center; Corresponding Member of the Russian Academy of Sciences. ORCID ID: 0000-0001-5129-9944. E-mail: galagoudza@mail.ru

Alexander V. Samorodov – Doctor of Sciences (Medicine), Professor, Head of the Department of Pharmacology with a Course of Clinical Pharmacology of Bashkir State Medical University. ORCID ID: 0000-0001-9302-499X. E-mail: avsamorodov@gmail.com

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2024-12-2-117-130

Development and validation of methods for quantitative determination of α-solanine, α-chaconine, solanidine in extracts from potato tuber peels BY High-performance liquid chromatography–tandem mass spectrometry

T.O. Ostrikova, N.G. Bogomolov, P.Y. Mylnikov, A.V. Shchulkin, I.V. Chernykh

Ryazan State Medical University named after academician I.P. Pavlov,
9 Vysokovoltnaya Str., Ryazan, Russia, 390026

E-mail: tostrikova0@gmail.com

Received 26 March 2024 After peer review 28 Aug 2024 Accepted 10 Oct 2024

Promising metabolites of potato tuberosum *(Solanum tuberosum* L., f. *Solanaceae)* are α-solanine, α-chaconine and their aglycone solanidine.

The aim of the work was to develop and validate methods for a quantitative analysis of α-solanine, α-chaconine and solanidine in dry extracts from the potato tuber peels by a high-performance liquid chromatography with a tandem mass-selective detection (HPLC/MS/MS).

Materials and methods. The analysis was performed in a gradient mode on an Ultimate 3000 chromatograph (ThermoFisher, USA) with a TSQ Fortis tandem mass-selective detector and a 4.6 mm×100 mm, 5 µm, 100 Å UCT Selectra C18 column. An electrospray in a positive ionization mode was used in this work. The following mass transitions were used for the quantitative analysis: α-solanine, 868.4→398.3 m/z; α-chaconine, 853.4→706.3 m/z; solanidine, 398.3→98.1 m/z. The following mass transitions were used for the internal standard fexofenadine: 502.3→171 m/z and 502.3→466.2 m/z. The analysis time was 10 min. The developed chromatography conditions were validated for a suitability. The validation was performed according to the following parameters: specificity, analytical range, linearity, correctness, precision and a lower limit of quantification.

Results. The validation procedure showed that the methodology was selective, sufficiently sensitive for α-solanine, α-chaconine and solanidine (lower limits of the quantification were 50, 10 and 2 ng/mL, respectively), the linear in the concentration range of 50–5000, 10–5000 and 2–100 ng/mL, respectively; it was satisfactorily correct (RSD did not exceed 7% for each of the substances) and sufficiently sensitive (RSD for α-solanine did not exceed 5%, for α-chaconine and solanidine – not more than 10%).

Conclusion. A technique for a quantitative determination of *α*-solanine, *α*-chaconine and solanidine in dry extracts obtained from potato tuber peels by HPLC/MS/MS has been developed and validated. This technique can be used in the routine practice of the glycoalkaloids quantitative determination when analyzing their content in food products and combination medicines.

Keywords: validation; glycoalkaloids; *α*-solanine; *α*-chaconine; solanidine; tuberous potato; HPLC-MS/MS

Abbreviations: Gas – glycoalkaloids; HPLC/MS/MS – high-performance liquid chromatography with a mass-selective detection; DMSO – dimethyl sulfoxide; GPM – general pharmacopoeial monograph; SPh RF XV ed. – State Pharmacopoeia of the Russian Federation, XV edition; LLQ – Lower Level of Quantification; ELISA – enzyme-linked immunosorbent assay.

For citation: T.O. Ostrikova, N.G. Bogomolov, P.Y. Mylnikov, A.V. Shchulkin, I.V. Chernykh. Development and validation of methods for quantitative determination of α-solanine, α-chaconine, solanidine in extracts from potato tuber peels BY High-performance liquid chromatography–tandem mass spectrometry. *Pharmacy & Pharmacology.* 2024;12(2):117-130. **DOI:** 10.19163/2307-9266-2024-12-2-117-130

Для цитирования: Т.О. Острикова, Н.Г. Богомолов, П.Ю. Мыльников, А.В. Щулькин, И.В. Черных. Разработка и валидация методики количественного определения α-соланина, α-чаконина, соланидина в экстрактах из кожуры клубней картофеля клубненосного методом высокоэффективной жидкостной хроматографии и тандемной масс-спектрометрии. *Фармация и фармакология.* 2024;12(2):117-130. **DOI:** 10.19163/2307-9266-2024-12-2-117-130

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Разработка и валидация методики количественного определения α-соланина, α-чаконина, соланидина в экстрактах из кожуры клубней картофеля клубненосного методом высокоэффективной жидкостной хроматографии и тандемной масс-спектрометрии

Т.О. Острикова, Н.Г. Богомолов, П.Ю. Мыльников, А.В. Щулькин, И.В. Черных

Федеральное государственное бюджетное образовательное учреждение высшего образования
«Рязанский государственный медицинский университет имени академика И.П. Павлова»
Министерства здравоохранения Российской Федерации,
390026, Россия, г. Рязань, ул. Высоковольтная, д. 9

E-mail: tostrikova0@gmail.com

Получена 26.03.2024 После рецензирования 28.08.2024 Принята к печати 10.10.2024

Перспективными метаболитами картофеля клубненосного (*Solanum tuberosum* L., сем. *Solanaceae*) являются α-соланин, α-чаконин и их агликон соланидин.

Цель. Разработка и валидация методики количественного анализа α-соланина, α-чаконина и соланидина в сухих экстрактах из кожуры клубней картофеля клубненосного методом высокоэффективной жидкостной хроматографии с тандемным масс-селективным детектированием (ВЭЖХ/МС/МС).

Материалы и методы. Анализ выполнялся в градиентном режиме на хроматографе «Ultimate 3000» (ThermoFisher, США) с тандемным масс-селективным детектором TSQ Fortis и колонкой UCT Selectra C18 4,6 мм×100 мм, 3 мкм, 100 Å. В работе использовался электроспрей в положительном режиме ионизации. Для количественного анализа применялись следующие переходы масс: α-соланин – 868,4→398,3 m/z; α-чаконин – 853,4→706,3 m/z; соланидин – 398,3→98,1 m/z. Переходы масс для внутреннего стандарта фексофенадина: 502,3→171 m/z и 502,3→466,2 m/z. Время анализа составило 10 мин. Разработанные условия хроматографирования были проверены на пригодность. Валидацию проводили по следующим параметрам: специфичность, аналитическая область, линейность, правильность, прецизионность и нижний предел количественного определения.

Результаты. Процедура валидации показала, что методика была селективной, достаточно чувствительной в отношении α-соланина, α-чаконина и соланидина (нижний предел количественного определения составил соответственно 50, 10 и 2 нг/мл), линейна в интервале концентраций соответственно 50–5000, 10–5000 и 2–100 нг/мл, обладала удовлетворительной правильностью (RSD не превышали 7% для каждого из веществ), достаточной презиционностью (для α-соланина RSD не превышало 5%, для α-чаконина, соланидина – не более 10%).

Заключение. Разработана и валидирована методика количественного определения α-соланина, α-чаконина и соланидина в сухих экстрактах, полученных из кожуры клубней картофеля клубненосного, методом ВЭЖХ-МС/МС. Данная методика может быть использована в рутинной практике количественного определения гликоалкалоидов при анализе их содержания в пищевых продуктах и комбинированных лекарственных средствах.

Ключевые слова: валидация; гликоалкалоиды; α-соланин; α-чаконин; соланидин; картофель клубненосный; ВЭЖХ/МС/МС

Список сокращений: ГА – гликоалкалоиды; ВЭЖХ/МС/МС – высокоэффективная жидкостная хроматография с масс-селективным детектированием; ДМСО – диметилсульфоксид; ОФС – общая фармакопейная статья; ГФ РФ XV изд. – Государственная фармакопея Российской Федерации XV издания; НПКО – нижний предел количественного определения; ИФА – иммуноферментный анализ.

INTRODUCTION

Plant organisms are unique producers of biologically active substances with a wide range of pharmacological effects, i.e. antiseptic, antimicrobial, antitumor [1]. A secondary metabolism of plants makes it possible to obtain fundamentally new compounds characterized by their own mechanism of action. The complex of plant metabolites – flavonoids, alkaloids and terpenoids in combination with related substances – has an effective and often relatively safe therapeutic effect [2].

More than 90 different glycoalkaloids (GAs) have been described from 300 species of plants in family *Solanaceae)*. Their toxicity is reported in the scientific literature, but a number of useful pharmacological properties, including antitumor, antimicrobial, antifungal, etc., have also been reported. [3]. This fact actualizes the development of GAs extraction techniques, their analysis and a subsequent evaluation of their therapeutic potential with a subsequent determination of their activity.

As a source of raw materials for obtaining a GA pharmaceutical substance, it is promising to use a

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-117-130

popular food plant *Solanum tuberosum* L., which contains glycosylated alkaloids of the solanidine group: α-solanine and α-chaconine; their waste percentage occupies the third place after cereals and dairy products[1].

To select the optimal GAs extraction techniques, as well as the subsequent standardization of the extracts and evaluation of their pharmacological activity, it is necessary to develop a sensitive, reproducible and selective technique for the quantitative determination of α-solanine, α-chaconine and their aglycone solanidine in an appropriate matrix and to prove its suitability by analyzing its metrological characteristics.

A number of techniques for a GAs quantification have been described in the literature, but some studies focus on individual compounds [4, 5], other techniques are complicated in terms of a sample preparation (e.g., heterogeneous LLQ) [6, 7], some have a low selectivity (HPLC-UV) [8, 9] or an analytical area [10, 11]. The latter criterion is especially important in the selection of extraction techniques, since the GAs level depends both on the native state of the raw materials (a genetically programmed concentration, stress factors, etc.) and on the nature of the extractant used, an isolation mode, and additionally introduced modifications.

The validation method procedure of the quantitative determination of individual GAs in their combined presence in the extract from potato tuber peels according to the requirements of the State Pharmacopoeia of the Russian Federation, XV edition (SPh RF XV ed.) will make it possible to judge unambiguously about the acceptability of the chosen extraction method.

THE AIM of the work was to develop and validate methods for a quantitative analysis of α-solanine, α-chaconine and solanidine in dry extracts from the potato tuber peels (*Solanum tuberosum* L., family *Solanaceae*, by a high-performance liquid chromatography with a tandem mass-selective detection (HPLC/MS/MS).

MATERIALS AND METHODS

The following reagents and chemical substances were used in this work: standard samples of α-solanine, α-chaconine, solanidine (Sigma Aldrich, USA), fexofenadine (United States Pharmacopeia Reference Standard, USA; CAS No. 153439-40-8), methanol for gradient HPLC (Himmed, Russia), 98% formic acid for analytics (Panreac, Spain), HPLC-MS water (VWR, France), dimethyl sulfoxide (DMSO) (OOO SPE PanEco, Russia), a 5% solution of acetic acid (OOO Techplant, Russia), Hanks' Balanced Salt solution without phenol red (HBSS; OOO SPE PanEco, Russia).

The methods development was carried out using the following equipment: Ultimate 3000 chromatograph (ThermoFisher, USA) with a TSQ Fortis tandem mass-selective detector (ThermoFisher, USA) equipped with an autosampler and degasser, Selectra C18 4.6 mm×100 mm, 3 µm, 100 Å column with Selectra C18 SLC-18GDC46-3UM pre-column (UCT, USA). The following kinds of the auxiliary equipment were used: a centrifuge for microcuvettes (Elmi, Latvia); analytical scales LV 210-a (Sartogosm, Russia), a Vortex shaker (Heidolph, Germany). A control of the chromatographic system, as well as mathematical processing of the data were carried out using a Thermo Scientific Xcalibur program (ver. 4.2.47).

The methods development consisted in the selection of the fragmentation method, the choice of the internal standard and the mobile phase composition.

Fragmentation parameters were selected in a semiautomatic mode (fragmentation energies were selected automatically). The two most intense fragments were selected for the registration. Fragmentation conditions were selected under the argon supply at pressures of 1.5, 2.0, 2.5 and 3.0 mTorr.

The chromatographic technique was set up on a water-methanol phase with a C18 column. Initially, an isocratic elution mode with 25, 50 and 80% methanol was used to evaluate the influence of an organic solvent on the separation of the substances, their retention times and chromatographic parameters of the system. Based on the results obtained, the gradient elution profile was selected to optimize the chromatographic parameters. Since satisfactory chromatographic parameters and the sensitivity were already obtained at this stage, testing of the aqueous-acetonitrile phase was deemed unnecessary.

An internal standard was selected from 6 substances: fexofenadine, anastrazole, valsartan, amantadine, amlodipine, metoprolol in concentrations from 1 to 10 ng/mL. The best results in terms of the reproducibility were observed for fexofenadine, which had been selected as an internal standard at a concentration of 1 ng/mL.

A chromatography was carried out in a gradient elution mode: solvent A – 0.1% aqueous formic acid, solvent B – methanol according to Table 1.

The temperature of the test samples was 20°C, the temperature of the chromatography column maintained with a thermostat, was 35°C. The flow rate of the mobile phase was 400 µL/min. The volume sample injection was 20 µL; using an autosampler at 8°C.

The decay products of the molecular ion were recorded using a quadrupole mass detector when exposed to an electrospray in the positive ionization mode.

The flow rate of the Sheath Gas was 50 Arb, of the auxiliary gas (Aux Gas) – 10 Arb, of the Sweep Gas – 1 Arb; the ion transfer tube temperature was 300°C, and evaporator temperature was 350°C. The following mass transitions were used for the detection: α-solanine – 868.4→98.1 m/z, 868.4→398.3 m/z (this transition was used for the quantification) at a collision energy of

[1] Serpova OS, Borzenkov LA. Resource-saving technologies of potato processing: a scientific analytical review. Moscow: Rosinformagrotech; 2009. 84 p. Russian

60 V; α-chaconine – 853.4→398.3 m/z, 853.4→706.3 m/z (this transition was used for the quantification) at a collision energy of 60 V; solanidine – 398.3→98.1 m/z (this transition was used for the quantification) at the collision energy of 43 V, 398.3→382.3 m/z at the collision energy of 48 V; the source fragmentation was 20 V, CID gas 2 mTorr. Mass transitions for fexofenadine were: 502.3→171 m/z and 502.3→466.2 m/z at the collision energy of 27 V. The time per analysis was 10 min.

The structural formulas of the analyzed substances and fragmentation of their molecules are presented in Fig. 1–4.

Validation of analytical methodology

Validation of chromatographic techniques involves proving their suitability for specific purposes and is regulated by a number of domestic regulatory documents[2].

The method was evaluated according to SPh RF, XV ed. by the following parameters: a suitability of the chromatographic system[3], specificity, an analytical range, linearity, correctness, precision (repeatability, in-laboratory precision) and a lower limit of quantification.

Preparation of standard sample solutions

According to the requirements of SPh RF, XV ed., for the evaluation of the validation parameters of the analytical methodology (correctness, precision at the levels of repeatability and in-laboratory precision) it is necessary to use solutions with 100% of the nominal value of the investigated substance, but these concentrations in extracts obtained by different methods can vary significantly. Therefore, to select such concentrations, a preliminary study was carried out to evaluate the content of α-solanine, α-chaconine and solanidine extracted from the insolubilized potato tuber peels using different extractants (pyridine, methanol, ethanol, 5% aqueous acetic acid) according to the physicochemical properties of the substances. The analysis was carried out by HPLC/MS/MS using a partially validated technique (with a satisfactory linearity and reproducibility). The content of α-solanine in the 4 extracts obtained was 252.61±182.85 ng/mL, α-chaconine was 451.33±100.33 ng/mL, and solanidine was 4.60±1.72 ng/mL. The averaged concentrations were further used as theoretical (100%) for the calculation of metrological characteristics [12].

The solutions of α-solanine, α-chaconine and solanidine standard samples were prepared as follows: 1 mg of the substance was dissolved in 1 mL DMSO, incubated at 50°C in an ultrasonic bath for 60 min, diluted 10 times with a DMSO solution, then with Hanks Solution (HBSS) to the concentrations of 50, 250, 300, 500, 1000 and 5000 ng/mL (α-solanine); 10, 50, 50, 400, 450, 450, 500, 1000, 2000, and 5000 ng/mL (α-chaconine); 2, 3, 3.2, 4, 4.8, 5, 10 and 100 ng/mL (solanidine). The sample preparation was performed by a 10-fold dilution with methanol containing the internal standard, fexofenadine (1 ng/mL), followed by a centrifugation at 1500 g for 10 min.

The quantification was performed using a calibration plot, and a normalization of the analytical response was performed using an internal standard:

$$K = \frac{S_{[analyte]}}{S_{[internal\ standard]}},$$

where K – normalized analytical response, %; $S_{[analyte]}$ – peak area of the determined component, %; $S_{[internal\ standard]}$ – peak area of the internal standard, %.

Preparation of test solutions

To validate the developed HPLC methodology, a dry extract of potato tuber peel of the tuberous potato variety Gala (Ryazan region, Klepikovsky district, Tuma settlement, Russia) was analyzed. The tubers pre-treatment included a two-week insolation with a daylight to maximize the GAs accumulation. Then, the dry extract obtained by a 3-fold maceration of the dry peel with a 5% aqueous solution of acetic acid was analyzed.

1 mg of the dry extract was diluted in 1 mL of DMSO, incubated for 60 min at 50°C in an ultrasonic bath, centrifuged for 10 min at 1500 g; the supernatant was diluted 10 times with a DMSO solution, followed by 10 times with HBSS. The sample preparation and calculations were performed according to the methods described above.

Preparation of working solutions for the validation assessment according to the linearity criterion

Solutions of 6 concentrations for α-solanine (50, 250, 300, 500, 1000 and 5000 ng/mL), 8 points for α-chaconine (10, 50, 400, 450, 500, 1000, 2000 and 5000 ng/mL) and 7 for solanidine (2, 3, 3,2, 4, 4,8, 5, 10 and 100 ng/mL), respectively, 3 repetitions for each followed by a sample preparation, were used to determine the linearity criterion.

Preparation of working solutions for validation evaluation by precision, in-laboratory precision, and correctness

The precision validation included the standard solutions preparation of α-solanine (250 ng/mL), α-chaconine (450 ng/mL) and solanidine (4.0 ng/mL) in 6 replicates of each concentration.

[2] 1.1.0012 Validation of analytical techniques. State Pharmacopoeia of the Russian Federation XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-1/validatsiya-analiticheskikh-metodik/. Russian

[3] 1.2.1.2.0001 Chromatography. State Pharmacopoeia of the Russian Federation XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/1-2-1-2-khromatograficheskie-metody-analiza/khromatografiya/. Russian

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-117-130

The solutions for the evaluation of the "in-laboratory precision" index were prepared by another researcher using a similar methodology.

Concentrations of 200, 250 and 300 ng/mL of the test solutions were chosen for an α-solanine to assess correctness. Solutions of α-chaconine were prepared at the concentrations of 400, 450 and 500 ng/mL; solanidine at the concentrations of 3.2, 4.0 and 4.8 ng/mL. All the solutions were prepared in three repetitions. Thus, a total of 9 working solutions of each substance were analyzed.

Statistical processing

The data statistical processing was performed using Microsoft Office 2019 office suite (Microsoft Inc., USA) and Statistica 13.0 program (StatSoft, USA). The nature of the data distribution was determined using the Shapiro-Wilk criterion. Pearson correlation coefficient was used to assess the linearity. MS Excel 2019 program with PKSolver extension was used to construct the weighted regression equation. This parameter was used for a more accurate construction of the calibration plot in the region of low concentrations.

Fisher's criteria were used to prove the absence of variance differences in the analysis of the intra-laboratory precision. The Student's criterion was used to compare the mean values of the analysis results obtained by different researchers. The tables present the following metrological characteristics: arithmetic mean ($\bar{x}$), variance (S^2), standard deviation (SD, S_δ), a relative standard deviation (RSD, $S_{\delta,\%}$), a half-width of the confidence interval of the value (Δx), relative errors of the result of an individual determination (ε), boundary values of the confidence interval of an individual determination ($\bar{x}\pm\Delta x$) result.

Due to the fact that the obtained data had a normal distribution, the results in the text were presented as an arithmetic mean (a mean of 3 parallel measurements) ± standard deviation.

RESULTS

Suitability of chromatographic system

To evaluate the suitability of the chromatographic system, solutions of α-solanine, α-chaconine, and solanidine at concentrations of 250, 450 and 4.8 ng/mL, respectively, were analyzed sequentially in a six-fold replicate.

The main characteristics of the target substances peaks on the chromatograms of the standard sample solutions are presented in Table 2.

Based on the presented data, the RSD values for the peak areas of each of the analyzed substances and the internal standard did not exceed 2%. The number of theoretical plates exceeded 2000. The asymmetry factors corresponded to the acceptable range of 0.7–2.5 [13].

Thus, the developed system meets the requirements of SPh RF, XV ed. 1.2.1.2.2.0001 Chromatography[4].

Validation of the developed methods

Validation of the analytical method for the quantitative determination of α-solanine, α-chaconine, solanidine by HPLC/MS/MS in extracts was performed in accordance with the requirements of SPh RF, XV edition.

Fexofenadine in methanol with a concentration of 1 ng/mL was chosen as an internal standard because of the best reproducibility of the assay results.

The retention times of α-solanine, α-chaconine and solanidine in the dry extract containing the target substances used to test the methodology coincided with those in the chromatograms of the standard solutions (Fig. 5). No accompanying substances eluting at the corresponding time were observed, which confirmed the specificity of the technique.

It should be noted that in the analysis of the obtained plant extracts, the chromatograms showed a substance peak with retention times coinciding with those for α-solanine and α-chaconine (t_R=4.79±0.02 min and t_R=4.78±0.015 min), while the molecular weight corresponded to the aglycone – solanidine (m/z=398.3). Herewith, the retention time for solanidine was t_R=5.38±0.0098 min. Most likely, during the ionization of GAs in the mass detector there was a break of a glycosidic bond with the release of aglycone. The content of solanidine relative to the content of the glycosides sum in the sample was not more than 2% by mass.

The methods linearity was established in the range of the assumed analytical area of the target substances concentrations, including from 80 to 120% of the content of each component in the potato tuber peel extracts, according to Table 3. The calibration plots of the peak area dependence on the concentration of the components, the regression equations and correlation coefficients are presented in Fig. 6–8.

The correlation coefficients obtained were greater than 0.99, meeting the requirements of SPh RF XV ed.

The lower limit of quantification (LLQ) was calculated by comparing the maximum intensity of the detector response when injecting a blank sample (5% DMSO, 95 mL HBSS, 10 mL internal standard methanol) and a sample with minimum analyte concentrations. Concentrations of the target substances that gave a detector response

[4] Ibid.

of at least 10 times the noise level at the time interval t_R of the peak analyte±width at the base obtained after the injection of the sample with the maximum concentration of the working range were taken as LLQ. In addition, the repeatability and correctness of 5 injections of solutions with the concentrations corresponding to the LLQ did not exceed 20%. The LLQ was 50 ng/mL for α-solanine, 10 ng/mL for α-chaconine, and 2.0 ng/mL for solanidine.

The precision (repeatability) of the methodology was evaluated using 6 points of a single concentration equal to 100% of the nominal concentration (Table 4).

For α-solanine at the concentration of 250 ng/mL, RSD did not exceed 5%. For α-chaconine at 450 ng/mL and solanidine at 4 ng/mL, the RSD did not exceed 10%.

An intra-laboratory precision was assessed by 6 points of 100% of nominal concentrations of the test substances prepared by two investigators in the same laboratory, using the same equipment and materials. The data are presented in Table 5.

Fisher's criteria calculated for α-solanine, α-chaconine and solanidine were lower than the table values and were 1.18, 1.19 and 1.15, respectively. The calculated Student's criteria for the substances were 0.46, 0.42 and 1.41, respectively, at a significance level of 95%, the number of degrees of freedom was 10, which was also lower than the tabulated values.

To assess the correctness, the parameters of 9 points were analyzed (Table 6). An openability, a standard deviation, a mean-square deviation were calculated.

As a result, RSDs did not exceed 7% for each of the substances.

DISCUSSION

GAs are promising pharmacological agents due to their wide range of biological effects [14, 15]. The use of extracts from plant raw materials requires a preliminary standardization: first of all, proofs of the percentage content of target substances.

A number of methods for the determination of the GAs content in potato products have been described in the literature: a high-performance thin-layer chromatography [16, 17], a heterogeneous [6, 7] and homogeneous immunoassay (LLQ) combined with capillary electrophoresis [18], a high-performance liquid chromatography with a UV detection [8, 9] or a tandem mass spectrometry [19–21].

The disadvantages of solid-phase LLQ are a low reproducibility, a considerable duration, and a high cost of the analysis. A high-performance capillary electrophoresis in combination with a laser-fluorescence detection is of a limited use in the analysis of the substances poorly soluble in water and water-alcohol solutions. The use of a laser-fluorescence detector also implies a multistage preparation for the analysis (separation, purification, labeling) [22].

GAs have close absorption maxima lying in the range of 200–208 nm, due to which the selectivity and sensitivity of the quantification with the use of an ultraviolet detector are reduced. A high-performance thin-layer chromatography can detect 10 ng of alkaloids, whereas the sensitivity of HPLC/MS/MS is often significantly higher [23, 24]. Also for the described methods (with the exception of the mass detection), the separation of the substances close in physicochemical properties, e.g. α-solanine and α-chaconine, is also a problem, which reduces their selectivity.

Thus, it is urgent to develop a highly sensitive technique that allows a simultaneous analysis of α-solanine, α-chaconine and their aglycone in multicomponent plant extracts with a minimal sample preparation and satisfactory metrological characteristics.

A number of questions about the validation of a methodology that is intended to be used for the quantification of substances in the samples with a potentially wide range of concentrations (using different extractants or extraction techniques) are discouraged.

A determination of the quantification limit is not regulated by SPh RF, XV ed., however, in view of the analysis of unknown concentrations of the target substances during the selection of the extraction technique it was necessary to determine this criterion.

The additive method, classically used in the validation of multicomponent systems [25], was not used in this study, since the tuber peel of tuberous potatoes not subjected to an insolation (assumed matrix) contained the tested substances: 30.74±26.0017 ng/mL α-solanine, 39.19±5.86 ng/mL α-chaconine, 1.71±0.37 ng/mL solanidine. At the same time, the initial level of GAs in the tuber peels of different potatoes varies considerably and can change under the influence of environmental factors (humidity, temperature, etc.) [26]. These peculiarities of the raw materials used served as a basis for the selection of an alternative method using an internal standard.

Study limitations

Changing the conditions of the chromatographic determination (e.g., using a different column or elution mode) can significantly affect the results of the study, and therefore the interlaboratory validation would be more appropriate.

In the present study, the validated methods was tested on the extracts obtained from the peels of potato tubers of the tuber-bearing variety Gala. In case of using another more highly productive potato variety, the GAs concentration values may be outside the working range of the developed methodology.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-117-130

Table 1 – Ratio of mobile phase components by volume as a function of elution time

Analysis time, min	0.1% aqueous formic acid	Methanol
1–5	65%	35%
6–8	30%	70%
9	10%	90%
10	1%	99%

Table 2 – Evaluation data of chromatography system suitability

Analyzed substance	Parameter			
	Retention time, min	RSD of peak area, %	Asymmetry factor, A_s	Number of theoretical plates, N
Fexofenadine	5,27±0,037	1,67	1,42	15620,64
α-solanine	4,85±0,0033	1,85	0,96	8827,11
α-chaconine	4,83±0,013	1,73	0,96	8790,29
Solanidine	5,38±0,0098	1,74	0,87	10644,34

Table 3 – Initial concentrations of substances to assess methodology linearity

Concentration of standard sample solution, ng/mL		Peak area of standard sample mAU×min	Peak area of standard sample in terms of internal standard, %	Calculated concentration of standard sample, ng/mL	R, %
α-solanine	50	8003	0.24	54.91	109.82
	250	20994	0.90	237.72	95.088
	300	25957	1.16	308.52	102.84
	500	40719	2.13	573.15	114.63
	1000	72572	3.95	1072.42	107.24
	5000	295813	17.73	4855.96	97.12
α-chaconine	10	124	0.04	8.50	85.00
	50	799	0.04	50.08	100.16
	400	5339	0.29	399.62	99.91
	450	6103	0.32	435.51	96.78
	500	8024	0.40	544.31	108.86
	1000	14759	0.84	1142.53	114.25
	2000	22347	1.44	1949.54	97.48
	5000	52103	3.60	4879.91	97.60
Solanidine	2.0	1355	0.05	2.54	117.00
	3.2	2840	0.11	3.48	111.88
	4.0	2924	0.12	3.65	93.75
	4.8	4093	0.18	4.39	93.54
	5.0	4064	0.19	4.68	95.40
	10.0	13746	0.46	8.62	87.10
	100.0	138737	6.75	101.64	101.37

Table 4 – Results of precision assessment of α-solanine, α-chaconine, solanidine quantitative determination method (repeatability level)

Analyzed component, ng/ml	Peak area of standard sample, mAU×min	Peak area of standard sample in terms of internal standard, %	Calculated concentration of standard sample, ng/mL	Metrological characteristics
α-solanine, 250	21945	0.92	242.33	$\bar{x}$=249.18 ng/mL S^2=136.33 ng/mL SD=11.68 ng/mL RSD=4.69% Δx=±10.12 ng/mL ε=±4.06% $\bar{x}$±Δx=249.18±10.12 ng/mL
	22258	0.90	237.49	
	22453	0.92	243.98	
	20138	0.92	243.41	
	23007	1.00	264.98	
	21626	0.99	262.88	
α-chaconine, 450	7790	0.36	484.60	$\bar{x}$=456.52 ng/mL S^2=1165.93 ng/mL SD=34.15 ng/mL RSD=7.48% Δx=±29.59 ng/mL ε=±6.48% $\bar{x}$±Δx=456.52±29.59 ng/mL
	7997	0.35	476.16	
	7532	0.33	455.45	
	7562	0.36	487.76	
	5339	0.29	399.62	
	6103	0.32	435.51	
Solanidine	3562	0.15	4.08	x=456.52 ng/mL S^2=013 ng/mL SD=036 ng/mL RSD=935% Δx =±032 ng/mL ε=±811% $\bar{x}$±Δx=389±032ng/mL
	2288	0.13	3.89	
	4093	0.18	4.49	
	3483	0.12	3.65	
	3615	0.12	3.75	
	4799	0.19	4.77	
	3562	0.15	4.08	

Table 5 – Results of precision assessment (intra-laboratory precision level) of α-solanine, α-chaconine, solanidine quantitative determination methods

Peak area of standard sample. mAU×min	Peak area of standard sample in terms of internal standard. %	Calculated concentration of standard sample. ng/mL	Peak area of standard sample. mAU×min	Peak area of standard sample in terms of internal standard. %	Calculated concentration of standard sample. ng/mL	Metrological characteristics	
Researcher I			Researcher II				
α-solanine $t_{calculated}$=0.46 <t (95%; 10), $F_{calculated}$=1.18 <F (95%; 5, 5) – differences between results are randomized							
21945	0.92	242.33	22848	0.97	257.67	$\bar{x}$=249.18 ng/mL S^2=136.33 ng/mL SD=11.68 ng/mL RSD=4.69% Δx=±10.12 ng/mL ε=±4.06% $\bar{x}$±Δx=249. 18±10.12 ng/mL	$\bar{x}$=245.91 ng/mL S^2=115.097 ng/mL SD=10.73 ng/mL RSD=4.36% Δx=±9.30 ng/mL ε=±3.78% $\bar{x}$±Δx= 245.91±3.78 ng/mL
22258	0.90	237.49	22635	0.96	253.65		
22453	0.92	243.98	22164	0.89	235.62		
20138	0.92	243.41	22258	0.90	237.72		
23007	1.00	264.98	22935	0.89	235.32		
21626	0.99	262.90	22872	0.97	255.51		
α-chakonine $t_{calculated}$=0.42 <t (95%; 10), $F_{calculated}$=1.19 <F (95%; 5, 5) – differences between results are randomized							
7790	0.36	484.60	8343	0.37	505.77	$\bar{x}$=456.52 ng/mL S^2=1165.93 ng/mL SD=34.15 ng/mL RSD=7.48% Δx=±29.59 ng/mL ε=±6.48% $\bar{x}$±Δx=456.52±6.48 ng/mL	$\bar{x}$=465.14 ng/mL S^2=976.47 ng/mL SD=31.25 ng/mL RSD=6.72% Δx=±27.08 ng/mL ε = ±5.82% $\bar{x}$±Δx=465.14±6.48 ng/mL
7997	0.35	476.16	6103	0.32	503.51		
7532	0.33	455.45	7532	0.33	435.51		
7562	0.36	487.76	2823	0.38	445.61		
5339	0.29	399.62	6318	0.33	444.99		
6103	0.32	435.51	7418	0.37	455.46		
Solanidine $t_{calculated}$=1.41 <t (95%; 10), $F_{calculated}$=1.15 <F (95%; 5, 5) – differences between results are randomized							
3562	0.15	4.08	3615	0.118	3.653	$\bar{x}$=3.89ng/mL S^2=0.13 ng/mL SD=0.36 ng/mL RSD=9.35 % Δx=±0.32 ng/mL ε=±8.11% $\bar{x}$±Δx=3.89±0.32 ng/mL	$\bar{x}$=3.86 ng/mL S^2=0.13 ng/mL SD=0.36 ng/mL RSD=9.16 % Δx=±0.31 ng/mL ε=±7.94% $\bar{x}$±Δx=3.86±0.31 ng/mL
2288	0.13	3.89	3483	0.120	3.681		
4093	0.18	4.49	3155	0.110	3.540		
3483	0.12	3.65	4697	0.147	4.075		
3615	0.12	3.75	4093	0.175	4.486		
4799	0.19	4.77	2921	0.124	3.749		

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-117-130

Table 6 – Results of correctness assessment of methodology for quantitative determination of α-solanine, α-chaconine, solanidine

Concentration of standard solution, ng/mL	Peak area of standard sample, mAU×min	Peak area of standard sample in terms of internal standard, %	Found, ng/mL	R, %	Metrological characteristics
α-solanine					
200	18565	0.817	214.44	107.22	$\bar{x}$=98.23%
200	19026	0.743	194.24	97.12	SD=5.43
200	19411	0.723	188.69	94.35	RSD=5.53%
250	21945	0.918	242.33	96.93	
250	20138	0.922	243.41	97.36	
250	22453	0.924	243.98	97.59	
300	42062	1.154	307.01	102.34	
300	23007	1.001	264.98	88.33	
300	25957	1.160	308.52	102.84	
α-chakonine					
400	6407	0.290	394.69	98.67	$\bar{x}$=101.25%
400	6545	0.285	388.71	97.18	SD=4.52
400	5339	0.293	399.62	99.91	RSD=4.47%
450	7532	0.334	455.45	101.21	
450	8063	0.370	503.51	111.89	
450	7002	0.320	435.51	96.78	
500	8343	0.371	505.45	101.09	
500	8223	0.371	504.89	100.98	
500	8027	0.381	517.86	103.57	
Solandinine					
3.2	3626	0.092	3.27	97.98	$\bar{x}$=96.81%
3.2	3278	0.082	3.12	102.60	SD=6.28
3.2	3455	0.082	3.13	102.33	RSD=6.49%
4.0	4987	0.147	4.075	98.17	
4.0	3615	0.118	3.65	109.51	
4.0	3483	0.120	3.68	108.67	
4.8	4064	0.194	4.77	100.59	
4.8	6965	0.210	5.01	95.80	
4.8	4697	0.147	4.075	117.81	

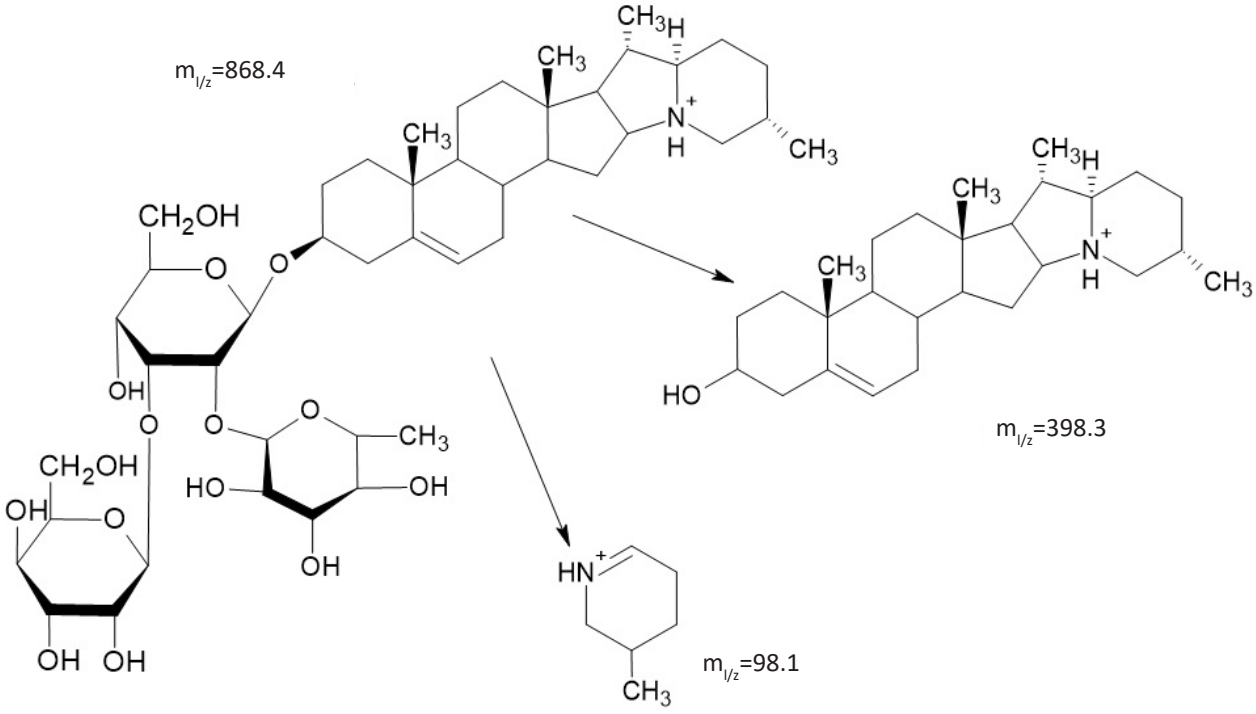


Figure 1 – Structural formula of α-solanine and fragmentation of its molecule

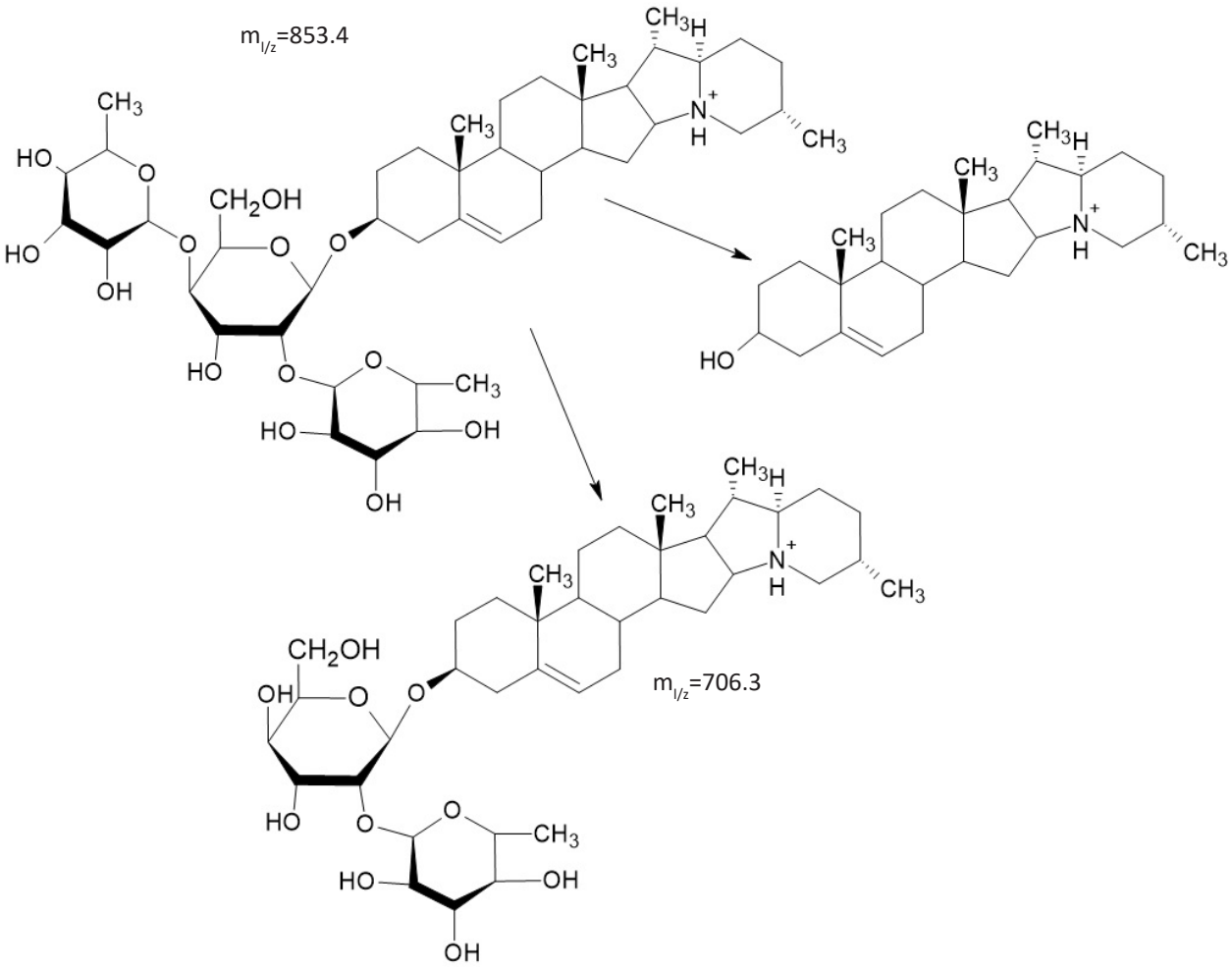


Figure 2 – Structural formula of α-chaconin and fragmentation of its molecule

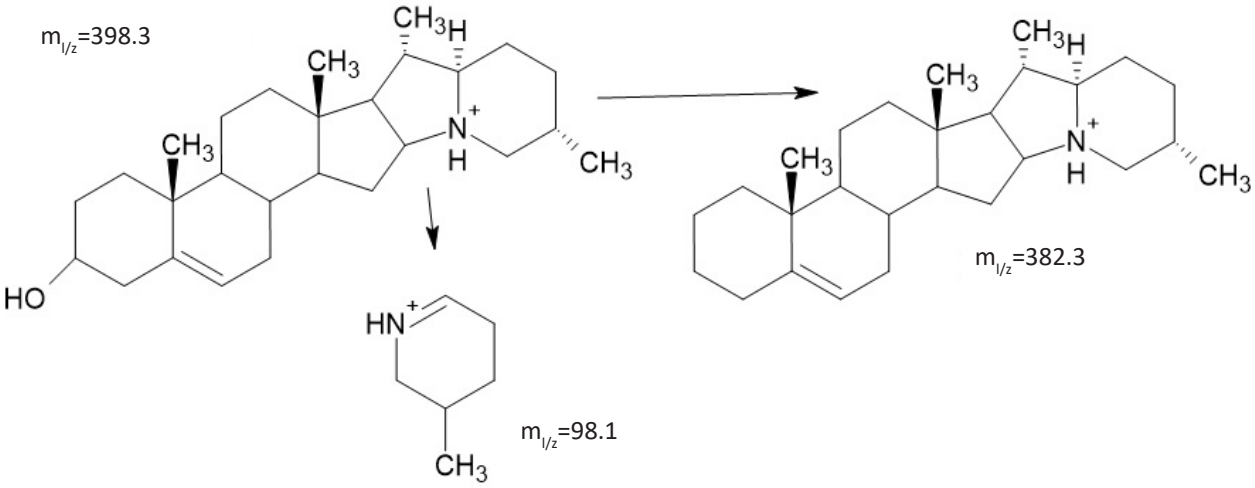


Figure 3 – Structural formula of solanidine and fragmentation of its molecule

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-117-130

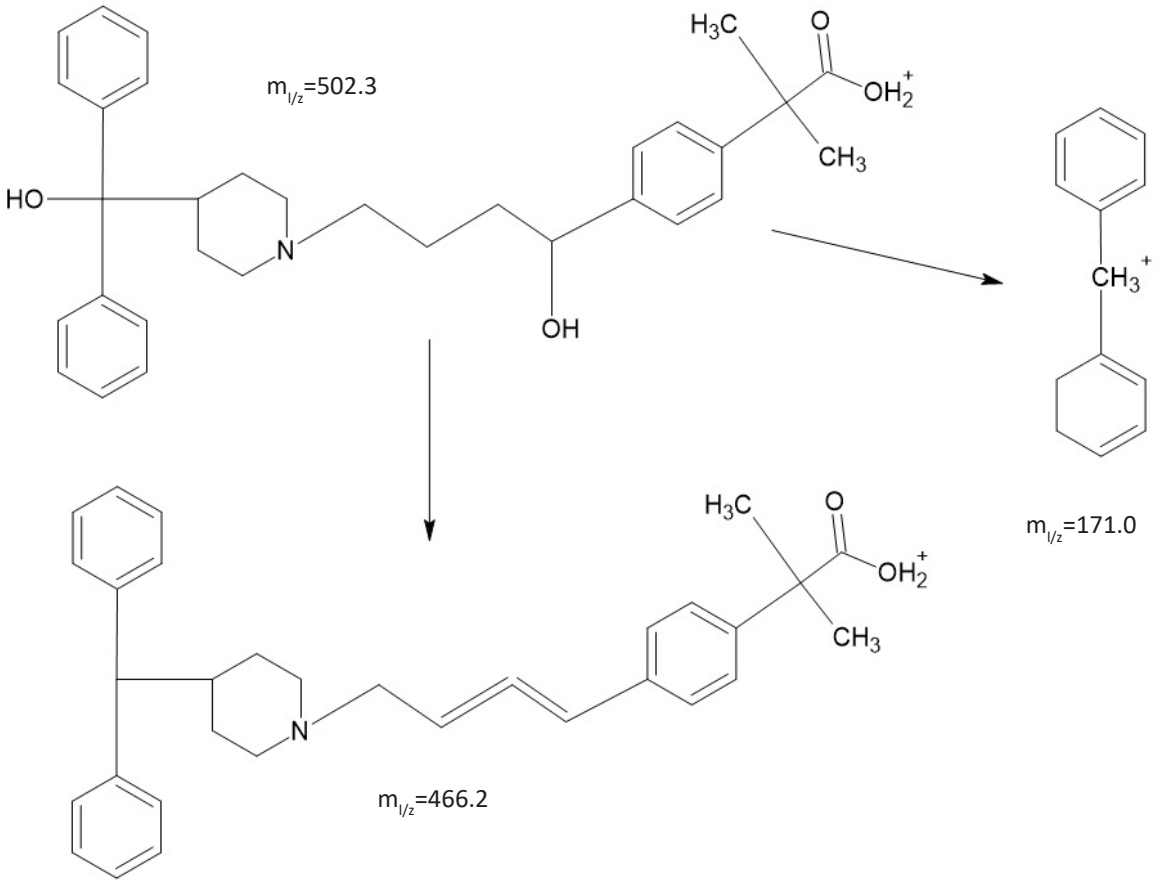


Figure 4 – Structural formula of fexofenadine and fragmentation of its molecule

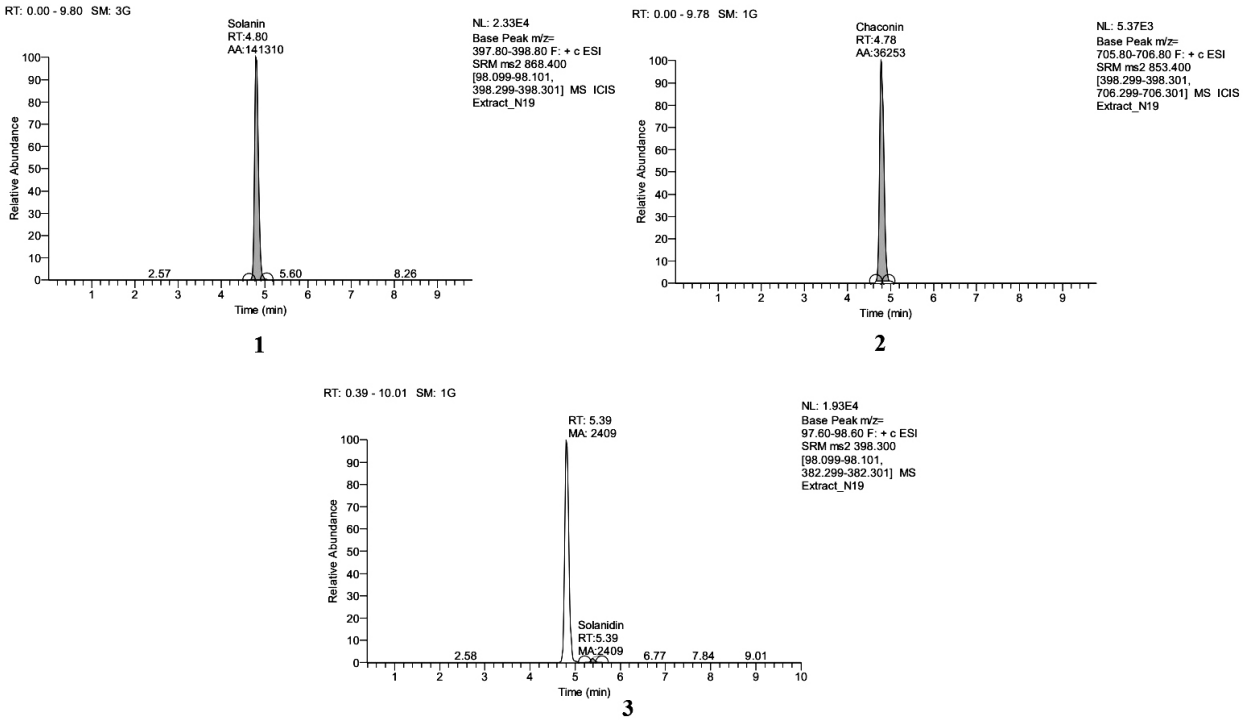


Figure 5 – Sample chromatograms of extract containing α-solanine (1), α-chaconine (2), solanidine (3)

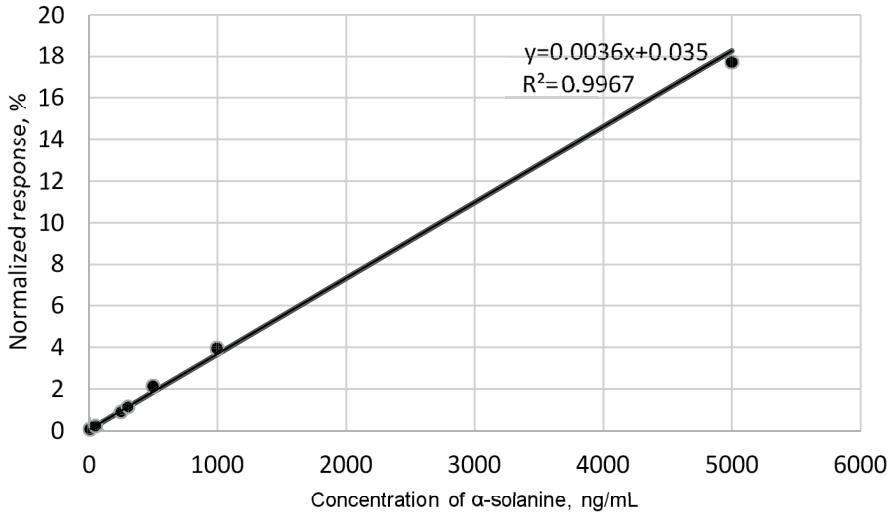


Figure 6 – Dependence graph of α-solanine normalized response on its concentration

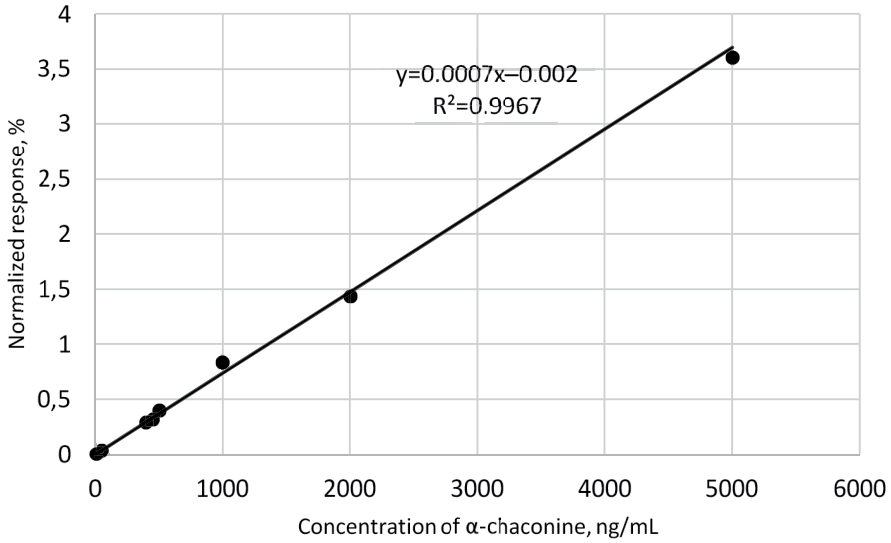


Figure 7 – Dependence graph of α-chaconin normalized response on its concentration

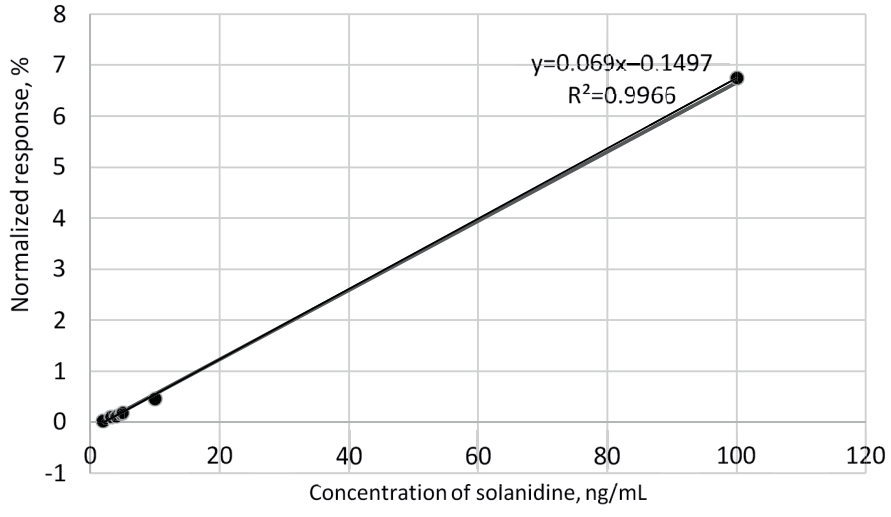


Figure 8 – Dependence graph of solanidine normalized response on its concentration

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-117-130

CONCLUSION

Thus, an analytical method for the quantitative analysis of α-solanine, α-chaconine and solanidine in the extracts obtained from potato tuber peels *(Solanum tuberosum*, family *Solanaceae)* by HPLC/MS/MS has been developed and validated. The method meets the requirements of the regulatory documentation in terms of the specificity, linearity in the analytical area, correctness, precision (at the levels of a repeatability, an intralaboratory precision).

This technique can be used in the routine practice of the GAs quantification when analyzing their content in food products and compounded pharmaceuticals.

FUNDINGS

This study had no financial support from outside organizations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Tatiana O. Ostrikova – conducting the experimental part of the work, statistical processing of the obtained results, preparation of the preliminary version of the manuscript; Nikita G. Bogomolov – conducting the experimental part of the work, review of the literature sources on the topic of the study; Pavel Yu. Mylnikov – conducting the experimental part of the work, statistical processing of the obtained results; Alexey V. Shchulkin – approval of the final version of the manuscript; Ivan V. Chernykh – development of the study concept, approval of the final version of the manuscript. All the authors confirm that their authorship complies with the ICMJE international criteria (all the authors have made a substantial contribution to the concept development, research and preparation of the article, read and approved the final version before publication).

REFERENCES

1. Shulkin AV, Popova NM, Chernykh IV. The original and generic drugs: current state of the problem. Nauka molodykh (Eruditio Juvenium). 2016;(2):30–5.
2. Chernykh IV, Kirichenko EE, Shchulkin AV, Popova NM, Kotlyarova AA. Possibilities of use of plant derived nonstarch polysaccharides in clinical practice. I.P. Pavlov Russian Medical Biological Herald. 2018;26(2):305–16. DOI: 10.23888/PAVLOVJ2018262305-316
3. Ostreikova TO, Kalinkina OV, Bogomolov NG, Chernykh IV. Glycoalkaloids of the Solanaceous Family (f. *Solanaceae*) Plants as Potential Drugs. Khimiko-Farmatsevticheskii Zhurnal. 2022;56(7):25–34. DOI: 10.30906/0023-1134-2022-56-7-25-34
4. Mokgehle TM, Madala M,·Gitari WM, Tavengwa NT. Effect of Microwave-Assisted Aqueous Two-Phase Extraction of α-Solanine from *S. retroflexum* and Analysis on UHPLC-qTOF-MS. Food Analytical Methods. 2022;15,(3):1–13. DOI: 10.1007/s12161-021-02224-9
5. Desai S, Tatke P, Gabhe S. A new validated HPLC-DAD method for estimation of solasodine from *Solanum nigrum* Linn. extracts and formulations. The Natural Products Journal. 2014;(3):196–200. DOI: 10.2174/2210315504666141017002102
6. Okada K, Matsuo K. Development of New Antibodies and an ELISA System to Detect the Potato Alkaloids α-Solanine and α-Chaconine. Foods. 2023;12(8):1621. DOI: 10.3390/foods12081621
7. Li P, Jin M. Application of Immunoassay Technology in Food Inspection. Foods. 2023;12(15):2923. DOI: 10.3390/foods12152923
8. Sotelo A, Serrano B. High-performance liquid chromatographic determination of the glycoalkaloids α-solanine and α-chaconine in 12 commercial varieties of Mexican potato. Journal of Agricultural and Food Chemistry. 2000;48(6):2472–5. DOI: 10.1021/jf990755t
9. Hussain MH, Hassoon AS, Abid FM. Reversed phase HPLC method for Separation and Identification Of steroidal glycoalkaloids (SGAs) from Form (Solanum) extract. Indian Journal of Public Health Research & Development. 2018; 9(8):1197. DOI: 10.5958/0976-5506.2018.00893.8
10. Martínez-García I, Gaona-Scheytt C, Morante-Zarcero S, Sierra I. Development of a Green, Quick, and Efficient Method Based on Ultrasound-Assisted Extraction Followed by HPLC-DAD for the Analysis of Bioactive Glycoalkaloids in Potato Peel Waste. Foods. 2024;13(5):651. DOI: 10.3390/foods13050651
11. Liu W, Zhang N, Li B, Fan S, Zhao R, Li LP, Wu GH, Zhao Y. Determination of α-chaconine and α-solanine in commercial potato crisps by QuEChERS extraction and UPLC-MS/MS. Chemical Papers. 2014;68(11):1498–504. DOI: 10.2478/s11696-014-0617-8
12. Hossain MB, Rai DK, Brunton NP. Optimisation and validation of ultra-high performance liquid chromatographic-tandem mass spectrometry method for qualitative and quantitative analysis of potato steroidal alkaloids. J Chromatogr B Analyt Technol Biomed Life Sci. 2015;997:110–15. DOI: 10.1016/j.jchromb.2015.05.033
13. Epshtein NA. Chromatographic system suitability testing: influence of correction factors and late eluting peaks of impurities on the requirement for the signal-to-noise ratio (Review). Drug development & registration. 2019;8(1):108–12. DOI: 10.33380/2305-2066-2019-8-1-108-112
14. Delbrouck JA, Desgagné M, Comeau C, Bouarab K, Malouin F, Boudreault PL. The Therapeutic Value of Solanum Steroidal (Glyco)Alkaloids: A 10-Year Comprehensive Review. Molecules. 2023;28(13):4957. DOI: 10.3390/molecules28134957
15. Hassan SH, Gul S, Zahra HS, Maryam A, Shakir HA, Khan M, Irfan M. Alpha Solanine: A Novel Natural Bioactive Molecule with Anticancer Effects in Multiple Human Malignancies. Nutr Cancer. 2021;73(9):1541–52. DOI: 10.1080/01635581.2020.1803932

16. Simonovska B, Vovk I. High-performance thin-layer chromatographic determination of potato glycoalkaloids. J Chromatogr A. 2000;903(1-2):219–25. DOI: 10.1016/s0021-9673(00)00900-6
17. Skarkova J, Ostry V, Ruprich J. Instrumental HPTLC determination of α-solanine and α-chaconine in peeled potato tubers. J PC–Journal of Planar Chromatography–Modern TLC. 2008;21(Issue 2):113–7. DOI: 10.1556/JPC.21.2008.2.7
18. Driedger DR, LeBlanc RJ, LeBlanc EL, Sporns P. A capillary electrophoresis laser-induced fluorescence method for analysis of potato glycoalkaloids based on a solution-phase immunoassay. 1. Separation and quantification of immunoassay products. J Agric Food Chem. 2000;48(4):1135-9. DOI: 10.1021/jf990680t
19. Wan L, Gao H, Gao H, Du R, Wang F, Wang Y, Chen M. Selective extraction and determination of steroidal glycoalkaloids in potato tissues by electromembrane extraction combined with LC-MS/MS. Food Chem. 2022;367:130724. DOI: 10.1016/j.foodchem.2021.130724
20. Shakya R, Navarre DA. Rapid screening of ascorbic acid, glycoalkaloids, and phenolics in potato using high-performance liquid chromatography. J Agric Food Chem. 2006;54(15):5253-60. DOI: 10.1021/jf0605300
21. Sheridan RS, Kemnah JL. Glycoalkaloid content in pet food by UPLC-tandem mass spectrometry. J Chromatogr Sci. 2010;48(10):790-4. DOI: 10.1093/chromsci/48.10.790
22. Razgonova M, Kulikova V, Khodaeva V, Bolotova L, Baigarashev T, Plotnikova N, Zakharenko A, Golokhvas K. Simultaneous Determination of Steroidal Alkaloids and Polyphenol Group from Eight Varieties of Siberian *Solanum tuberosum* L. through Tandem Mass Spectrometry. Agriculture. 2023;13(4):758. DOI: 10.3390/agriculture13040758
23. Taniguchi M, Takamura N, Watanabe T, Ishimaru R, Chinaka S, Miki A, Miyazaki H, Tsuchihashi H, Zaitsu K. Easily Operable Quantification Method of 21 Plant-Derived Alkaloids in Human Serum by Automatic Sample Preparation and Liquid Chromatography-Tandem Mass Spectrometry. Chromatographia. 2022;85(12):1051–63. DOI: 10.1007/s10337-022-04212-5
24. Larskiy MV, Pozdnyakova AE, Khadzhieva ZD, Pozdnyakov DI. Development and validation of methods for quantitative determination of active pharmaceutical substances in nasal spray. Pharmacy & Pharmacology. 2021;9(4):266–77. DOI: 10.19163/2307-9266-2021-9-4-266-277
25. Dhalsamant K, Singh CB, Lankapalli R. A Review on Greening and Glycoalkaloids in Potato Tubers: Potential Solutions. J Agric Food Chem. 2022;70(43):13819–31. DOI: 10.1021/acs.jafc.2c01169

AUTHORS

Tatiana O. Ostrikova – assistant of the Department of Pharmaceutical Chemistry and Pharmacognosy of Ryazan State Medical University n.a. I.P. Pavlov. ORCID ID: 0000-0002-7916-2269. E-mail: tostrikova0@gmail.com

Nikita G. Bogomolov – 5th year student of the Faculty of Pharmacy of Ryazan State Medical University n.a. I.P. Pavlov. ORCID ID: 0000-0002-3268-4174. E-mail: nikita.bogomolov50@gmail.com

Pavel Yu. Mylnikov – Candidate of Sciences (Biology), Assistant of the Department of Pharmacology with a course of pharmacy, Faculty of Additional Professional Education of Ryazan State Medical University n.a. I.P. Pavlov. ORCID ID: 0000-0001-7829-2494. E-mail: Pavelmylnikov@mail.ru

Alexey V. Shchulkin – Doctor of Sciences (Biology), Associate Professor, Professor of the Department of Pharmacology with a Course of Pharmacy, Faculty of Additional Professional Education of Ryazan State Medical University n.a. I.P. Pavlov. ORCID ID: 0000-0002-5618-7607. E-mail: ivchernykh88@mail.ru

Ivan V. Chernykh – Candidate of Sciences (Biology), Associate Professor, Head of the Department of Pharmaceutical Chemistry of Ryazan State Medical University n.a. I.P. Pavlov. ORCID: 0000-0002-5618-7607. E-mail: ivchernykh88@mail.ru

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2024-12-2-131-149

Analysis of Russian pharmaceutical specialists labor market for 2019–2022

D.V. Kurkin[1], Yu.S. Knyazeva[2], O.V. Ivanova[1], Yu.A. Kolosov[1], Yu.V. Gorbunova[1], D.A. Bakulin[1], I.S. Krysanov[1], D.L. Klabukova[1], M.A. Javakhyan[1], V.I. Zvereva[1], N.A. Lycheva[1], V.N. Shoich[1], V.V. Neyolova[1], E.V. Pavlova[1], E.I. Morkovin[2], E.A. Kalashnikova[2], A.B. Bosenko[3], D.Z. Zyazikova[2], R.E. Musaev[3], A.G. Gadzhiev[3], B.M. Gabrielyan[4], A.A. Agamirova[3], V.I. Petrov[2]

[1] Russian University of Medicine,
4, Dolgorukovskaya Str., Moscow, Russia, 127473
[2] Volgograd State Medical University,
1, Pavshikh Bortsov Sq., Volgograd, Russia, 400131
[3] Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University,
11, Kalinin Ave., Pyatigorsk, Russia, 357500
[4] Russian-Armenian University,
123, Hovsep Emin Str., Yerevan, Armenia, 0051

E-mail: strannik986@mail.ru

Received 27 Feb 2024 | After peer review 28 June 2024 | Accepted 15 July 2024

The higher education system is becoming more and more competitive every year. It faces ambitious tasks of training highly qualified personnel and ensuring technological sovereignty. A labor market analysis is an integral component of the early career guidance work with students and their navigation in the profession, which is especially important at the beginning of their employment.

The aim of the work was to conduct a comparative analysis of supply and demand indicators in the labor market of pharmaceutical specialists in nine Russian regions in 2019–2022.

Materials and methods. The research methodology included the collection, quantitative and qualitative analyses of the data on the SuperJob.ru website. The posted offers (vacancies) and CVs were analyzed on the portal SuperJob.ru (a large online service of CVs and vacancies from direct employers, agencies, employment centers) for 2019–2022 in nine regions and federal districts of Russia: Moscow and the Moscow region, St. Petersburg and the Leningrad region, the Central Federal District (without the Moscow region), the Northwestern Federal District (without the Leningrad Region), the Volga Federal District, the Urals Federal District, the Siberian Federal District, the Far Eastern Federal District, the Southern and North Caucasus Federal Districts.

Results. The analysis of the number of posted vacancies and CVs in the recruitment agency database for 2019–2022 shows a stable shortage of pharmacy professionals with both secondary (vacancies / CVs – 42112 / 41037) and higher professional education (vacancies / CVs – 36432 / 25149). The pointed out specialists are among the most required ones by employers, and pharmacy is one of the most popular areas for candidates. Cities with a high level of economy and population experience a greater deficit in specialists with pharmaceutical education. Salary levels of specialists with higher or secondary education are almost comparable on average from 50 to 75 thousand rubles, but employers generally prefer to hire specialists with higher education.

Conclusion. On average in the regions, the demand for specialists with higher education is lower than the demand for specialists with secondary education and the salary expectations of pharmacists coincide with the employers' offers, while the salary expectations of specialists with higher education are higher in the market. Specialists with secondary education are much more numerous, as they are trained by a much larger number of educational institutions. For pharmacy technicians, a pharmacy is often the only place of work in their specialty, while for pharmacists it is usually a starting point.

Keywords: pharmaceutical market; pharmaceutical specialists; labor market; pharmacist; pharmacy technician; vacancies

Abbreviations: MR – Moscow and the Moscow region; SPb – St. Petersburg and the Leningrad region; CFD – the Central Federal District (without the Moscow region); NWFD – the Northwestern Federal District (without the Leningrad Region); VFD – the Volga Federal District; UFD – the Urals Federal District; SFD – the Siberian Federal District; FEFD – the Far Eastern Federal District; SFD+NCFD – the Southern and North Caucasus Federal Districts.

For citation: D.V. Kurkin, Yu.S. Knyazeva, O.V. Ivanova, Yu.A. Kolosov, Yu.V. Gorbunova, D.A. Bakulin, I.S. Krysanov, D.L. Klabukova, M.A. Javakhyan, V.I. Zvereva, N.A. Lycheva, V.N. Shoich, V.V. Neyolova, E.V. Pavlova, E.I. Morkovin, E.A. Kalashnikova, A.B. Bosenko, D.Z. Zyazikova, R.E. Musaev, A.G. Gadzhiev, B.M. Gabrielyan, A.A. Agamirova, V.I. Petrov. Analysis of Russian pharmaceutical specialists labor market for 2019–2022. *Pharmacy & Pharmacology.* 2024;12(2):131-149. **DOI:** 10.19163/2307-9266-2024-12-2-131-149

Для цитирования: Д.В. Куркин, Ю.С. Князева, О.В. Иванова, Ю.А. Колосов, Ю.В. Горбунова, Д.А. Бакулин, И.С. Крысанов, Д.Л. Клабукова, М.А. Джавахян, В.И. Зверева, Н.А. Лычева, В.Н. Шоич, В.В. Неёлова, Е.В. Павлова, Е.И. Морковин, Е.А. Калашникова, А.Б. Босенко, Д.З. Зязикова, Р.Э. Мусаев, А.Г. Гаджиев, Б.М. Габриелян, А.А. Агамирова, В.И. Петров. Анализ российского рынка труда фармацевтических работников за 2019–2022 гг. *Фармация и фармакология.* 2024;12(2):131-149. **DOI:** 10.19163/2307-9266-2024-12-2-131-149

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Анализ российского рынка труда фармацевтических работников за 2019–2022 гг.

Д.В. Куркин[1], Ю.С. Князева[2], О.В. Иванова[1], Ю.А. Колосов[1], Ю.В. Горбунова[1], Д.А. Бакулин[1], И.С. Крысанов[1], Д.Л. Клабукова[1], М.А. Джавахян[1], В.И. Зверева[1], Н.А. Лычева[1], В.Н. Шоич[1], В.В. Неёлова[1], Е.В. Павлова[1], Е.И. Морковин[2], Е.А. Калашникова[2], А.Б. Босенко[3], Д.З. Зязикова[2], Р.Э. Мусаев[3], А.Г. Гаджиев[3], Б.М. Габриелян[4], А.А. Агамирова[3], В.И. Петров[2]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Российский университет медицины» Министерства здравоохранения Российской Федерации, 127473, Россия, г. Москва, ул. Долгоруковская, д. 4

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

[3] Пятигорский медико-фармацевтический институт – филиал федерального государственного бюджетного образовательного учреждения высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации, 357500, Россия, г. Пятигорск, пр-кт Калинина, д. 11

[4] Государственное образовательное учреждение высшего профессионального образования «Российско-Армянский (Славянский) университет», 0051, Армения, г. Ереван, ул. Овсепа Эмина, д. 123

E-mail: strannik986@mail.ru

Получена 27.02.2024 После рецензирования 28.06.2024 Принята к печати 15.07.2024

Система высшего образования с каждым годом становится более конкурентной. Перед ней стоят амбициозные задачи подготовки кадров высшей квалификации и обеспечение технологического суверенитета. Анализ рынка труда представляет собой неотъемлемый компонент ранней профориентационной работы со студентами и их навигации в профессии, что особенно актуально вначале трудовой деятельности.

Цель. Провести сравнительный анализ показателей спроса и предложения на рынке труда фармацевтических работников девяти российских регионов за период с 2019 по 2022 гг.

Материалы и методы. Методология исследования включала сбор, количественный и качественный анализ данных на сайте SuperJob.ru. Анализу подвергали размещенные предложения (вакансии) и резюме на портале SuperJob.ru (крупный онлайн-сервис резюме и вакансий от прямых работодателей, агентств, центров занятости) за 2019–2022 гг. в девяти регионах и федеральных округах России – г. Москва и Московская область, г. Санкт-Петербург и Ленинградская область, Центральный федеральный округ (без Московской области), Северо-Западный федеральный округ (без Ленинградской области), Приволжский федеральный округ, Уральский федеральный округ, Сибирский федеральный округ, Дальневосточный федеральный округ, Южный и Северо-Кавказский федеральные округи.

Результаты. Анализ количества размещённых вакансий и резюме в базе рекрутингового агентства в 2019–2022 гг. показывает стабильный дефицит фармацевтических работников как со средним (вакансии / резюме – 42112 / 41037), так и с высшим профессиональным образованием (вакансии / резюме – 36432 / 25149). Обозначенные специалисты входят в число самых востребованных среди работодателей, а фармация – одно из самых популярных направлений у кандидатов. Города с высоким уровнем экономики и численности населения испытывают больший дефицит в специалистах с фармацевтическим образованием. Уровень заработной платы специалистов с высшим или средним образованием были практически сопоставимы – в среднем от 50 до 75 тыс. рублей, но работодатели, как правило, предпочитают нанимать людей с высшим образованием.

Заключение. В среднем по регионам спрос на специалистов с высшим образованием ниже, чем со средним. По заработной плате ожидания фармацевтов совпадают с предложением работодателя, в то время как зарплатные ожидания специалистов с высшим образованием оказываются выше рынка. Специалистов со средним образованием значительно больше поскольку их подготовку осуществляет значительно большее количество учебных заведений. Для фармацевта аптека часто является единственным местом работы по специальности, тогда как для провизора это как правило место для старта.

Ключевые слова: фармацевтический рынок; фармацевтические специалисты; рынок труда; провизор; фармацевт; вакансии

Список сокращений: МО – Москва и Московская область; СПб – Санкт-Петербург; ЛО – Ленинградская область; ЦФО – Центральный аедеральный округ (без Московской области); СЗФО – Северо-Западный федеральный округ (без Ленинградской области); ПФО – Приволжский федеральный округ; УФО – Уральский федеральный округ; СФО – Сибирский федеральный округ; ДФО – Дальневосточный федеральный округ; ЮФО+СКФО – Южный и Северо-Кавказский федеральные округа.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-131-149

INTRODUCTION

From many positions, pharmaceutical industry is of strategic importance to ensure the functioning and development of various state institutions. Maintaining a progressive trend towards increasing life expectancy and quality of life, effectiveness of preventive measures and treatment of diseases is impossible without the development of pharmaceutical industry [1, 2]. As in most socio-economic spheres, human resources are a key element of a progressive development. Personnel training, including a higher qualification, is the most important factor in the development of the society [3]. A highly qualified pharmacy specialist should be able to give competent advice on the issues related to the regimen, peculiarities of the action, side effects, interactions and contraindications of medicines. The population often seeks information from pharmacists and pharmacy technicians at the pharmacy, so they can influence the adherence to treatment. In case of prescription drugs, a pharmacist in counseling should be able, without going beyond their competence, to give full information about the drug, facilitating its safe and correct administration. Therefore, a high level of knowledge among pharmacists and pharmacy technicians is an essential tool in providing competent and effective counseling to the population [4, 5].

Higher pharmaceutical education traditionally occupies high positions in the ranking of specialties in terms of demand among both applicants and employers [6–8].

Meeting the requirements of the modern labor market and the demands of the society[1], including the ones in the field of educational services, the system of higher education is in the state of a constant transformation. An increasing competition between higher education institutions encourages their development; herewith, the quality is the key advantage [9–11]. An obligatory element of the pharmaceutical education development is the expansion of ideas about its labor functions [12], opportunities and directions of a specialist's the self-realization [13], a potential of the digital and technological development of all the areas of pharmaceutical industry [14, 15]. Thus, modern pharmaceutical industry tends to a more and more detailed segmentation [16–18], which forms a demand for training personnel with not only the competencies "classic" for a pharmacist, but also a more universal set of them, which will make them possible to be realized in any direction of labor activities (science, production, analytics, advertising, marketing, supervision, management, pedagogy) [19–21].

The educational process on specialty 33.05.01 "Pharmacy" takes 5 years, after a successful completion of training a graduate is awarded a "Pharmacist" qualification. After passing the initial accreditation, a specialist can work as a pharmacist with the right to manufacture medicines according to individual prescriptions in the form of intra-pharmacy preparations, as well as concentrates, semi-finished products, and perform a quality control of dosage forms. The "Pharmacist" qualification allows to occupy managerial positions in pharmacies and pharmacy networks, manage a pharmacy and conduct independent pharmaceutical activities. In addition to higher professional education, there is specialty 33.02.01 "Pharmacy" of the secondary professional education with a "Pharmacy technician" qualification [22]. The period of training is from 1 year and 10 months (on the basis of secondary general education) to 2 years and 10 months (on the basis of basic general education). The graduates who have mastered the educational program should be ready to perform the main types of activities: wholesale and retail kinds of trade in medicines and dispensing of medicines; manufacturing of medicines in the conditions of pharmacy organizations. In 2024, 77 educational organizations in 56 cities of Russia are recruiting applicants[2]. Given a high prevalence of educational institutions and the number of graduates, the question arises about a real demand for specialists of these profiles and qualifications because employment opportunities, career and salary expectations are important motivation aspects when choosing a specialty [23–25]. Accordingly, a market research of the vacancy market of pharmacists and pharmacy technicians in Russia in the period from 2019 to 2022 was planned and conducted. The aspect of pharmacy in the veterinary field has not been considered in this article because it is a separate area of a specialty on the scale of science and technology.

THE AIM of the work was to conduct a comparative analysis of supply and demand indicators in the labor market of pharmaceutical specialists in nine Russian regions in 2019–2022.

MATERIALS AND METHODS

As a data source, the posted offers (vacancies) and CVs were analyzed on the portal SuperJob.ru (a large online service of CVs and vacancies from direct

[1] Register of Professional Standards. Pharmacist. Available from: https://profstandart.rosmintrud.ru/obshchiy-informatsionnyy-blok/natsionalnyy-reestr-professionalnykh-standartov/reestr-professionalnykh-standartov/index.php?ELEMENT_ID=47709. Russian

[2] Pharmacy (cipher 33.05.01): Universities in Russia by specialty, which universities in Russia have Pharmacy. Specialty. Available from: https://vuzopedia.ru/spec/263/vuzy?page=4. Russian

employers, agencies, employment centers) for 2019–2022 in nine regions and federal districts of Russia – Moscow and the Moscow region, St. Petersburg and the Leningrad region, the Central Federal District (without the Moscow region), the Northwestern Federal District (without the Leningrad Region), the Volga Federal District, the Urals Federal District, the Siberian Federal District, the Far Eastern Federal District, the Southern and North Caucasus Federal Districts. Absolute values or their shares have been presented. The values for the analysis were taken from the resources of the recruitment agency. The statistical analysis was not performed due to the declarative nature of the data. To assess the dynamics similarity of the annual demand-to-supply ratio of vacancies for pharmacists and pharmacy technicians in different regions of Russia for the period 2019–2022, the Pearson correlation coefficient was calculated using Prism 6 (GraphPad Software Inc., USA). The data visualization was performed using MS Excel 365 (Microsoft, USA). The similar data for 2023 have not been considered in this paper as they do not reflect the trends observed. The main task of this manuscript is to draw attention to the labor market for professionals with pharmacy education and to reflect the absence or presence of staff shortages, as well as some career opportunities for those with higher or specialized secondary education.

The research methodology included the collection, quantitative and qualitative analyses of the data on SuperJob.ru. The data from nine Russian regions have been compared with each other and with the data from other public sources in order to confirm the reliability of the results obtained.

The data obtained in the study were grouped according to supply and demand indicators, each of which has been subsequently analyzed in a region-by-region comparison:

1. A number of vacancies and CVs posted, and a competition for vacancies – a quantitative ratio of open vacancies and CVs posted for pharmacists and pharmacy technicians – compared across the regions, was assessed as an indicator of demand for pharmaceutical specialists.
2. The top of 10 areas popular with candidates – the popularity of pharmacy among other areas of employment – was assessed in a region-by-region comparison.
3. Top cities were assessed by the number of candidates: the popularity of certain cities in the regions when potential employees choose a place of work.
4. The salaries were assessed in Russian rubles in a region-by-region comparison;
5. Additional working conditions offered by employers to pharmaceutical specialists in different regions, were assessed in vacancies.
6. Educational requirements for pharmaceutical specialists – employers' preferences regarding the availability of higher education for potential employees, were also assessed.

RESULTS AND DISCUSSION

The study provided results for nine regions of Russia. They were grouped by supply and demand indicators. Each indicator was analyzed separately; the comparison of the obtained data was also carried out individually for each indicator.

Number of published vacancies and CVs

The quantitative ratio of open vacancies and published CVs of pharmacists and pharmacy technicians is an important indicator of the demand for specialists with secondary and higher pharmaceutical education. The COVID-19 pandemic and world events have changed the balance of power in the pharmacy labor market.

In the period from 2019 to 2021, the pharmaceutical labor market was stable in Moscow and the Moscow region. The number of published vacancies / posted CVs for pharmacy technicians pharmacists were in 6704 / 4609 in 2019, 6305 / 4260 in 2020, 6959 / 4003 in 2021 (Fig. 1). During this period, the demand also exceeded the supply for pharmacists – the number of published vacancies was on average 2.4 times the number of published CVs: 6099 / 2710 in 2019; 5681 / 2487 in 2020; 6159 / 2409 in 2021 (Fig. 2). This significant staff shortage could be caused by various reasons: low salaries; complicated working conditions associated with the common phenomenon of "saving" on staff in pharmacy chains; and the reluctance of Moscow job seekers to work in the profession in general. In 2022, against the backdrop of the world events, many foreign companies left Russia, resulting in an increase in supply, but the number of pharmacist vacancies decreased. The continuation of this trend in the future may presumably entail a number of negative consequences, primarily such as an increase in unemployment among specialists with secondary education. Among pharmacists, the demand for specialists and supply were almost equal, which confirms a high relevance of higher pharmaceutical education and employers' interest in qualified specialists. Overall, in 2022, the number of vacancies for the position of pharmacists decreased to 2453 vacancies and to 3278 vacancies for the position of

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-131-149

pharmacy technicians. Such a drastic reduction in jobs may lead to the unemployment in this niche. In 2021/22, people from other regions were still moving to Moscow and the Moscow region and finding vacancies easily.

In St. Petersburg and the Leningrad Region, in 2021, the need for specialists with secondary education increased compared to 2019 and 2020, from 895 and 716 to 1720, respectively. The active interest of employers in personnel contributed to the reduction of the unemployment and an increase in the labor salaries in the pharmaceutical market. In 2021, the number of vacancies for pharmacists (1587 vacancies) was almost twice as high as the number of submitted CVs (819 CVs). Such a significant shortage of specialists could lead to significant losses for companies up to their bankruptcy. Due to a significant decrease in the share of foreign companies in the Russian business and the introduction of a number of sanctions, the number of submitted CVs again exceeded the number of available vacancies, in particular, the need for pharmacists decreased. But by 2022, the demand for specialists had become equal to the supply, and the employers needed pharmacists to a greater extent (in 2022 vacancies / CVs were 998 / 1341) than pharmacy technicians (there were 689 / 864 vacancies / CVs in 2022). Thus, the situation with human resources in the region can be assessed as complicated with a high level of instability, but it was not critical.

A similar situation with a significant shortage of jobs in 2019-2020 was observed in the Central Federal District: on average, there were two CVs for one pharmacy technician's vacancy (596 / 1335 vacancies / CVs in 2019, 540/1022 – in 2020). The number of CVs posted by pharmacists also exceeded the number of vacancies (552 / 763 vacancies / CVs in 2019, and 464 / 607 in 2020), but by 2021, the situation had changed significantly to an excess of vacancies over the number of professionals needing employment. In 2022, the number of vacancies and CVs were almost equal, and every specialist could find a job. Thus, the demand returned to the framework of the previous values. In terms of the quantity, in 2022, there were 697 posted vacancies for pharmacy technicians' jobs in the Central Federal District, 214 more than for pharmacists.

The demand for pharmaceutical specialists in the Northwestern Federal District was higher than the number of job seekers in all years of the study period. Even the departure of many foreign companies from the Russian labor market did not eliminate the shortage of personnel. By 2022, the number of pharmacy technicians' vacancies had amounted to 409, while the number of published CVs was 224. A similar situation was observed in terms of vacancies of pharmacists (in 2022, there were 321 / 166 vacancies / CVs). Despite a gradually increasing number of vacancies, there were still almost half as many specialists as vacancies in 2022. A comparative analysis of the number of pharmacists' and pharmacy technicians' vacancies showed only a slight predominance of the need for pharmacists, which was likely due to the overall staff shortage in this region and, as a result, a less "selectivity" of employers when looking for employees. Thus, there is a significant shortage of personnel in the Northwestern Federal District, presumably related to the "leakage" of personnel to the capitals.

In the Volga Federal District during the study period, there was a significant shortage of jobs. The first place in terms of the number of published CVs belonged to 2019: for every vacancy there were 3.3 people willing to fill it (there were 680 / 2233 vacancies / CVs). The resulting lack of open vacancies in this region led to a lot of pharmacy technicians moving into other "outside the profession" jobs. As a result, in 2022, there were already 1.4 CVs per a pharmacy technician's vacancy (there were 726 / 1018 vacancies / CVs). At the same time, the market for pharmacists was characterized by its instability: a significant shortage of jobs in 2021 was replaced by a staff shortage, and the departure of many foreign companies from Russia in 2022 brought back an intense competition and difficulties with the employment. As a result, in 2022, there were 1.3 CVs per open vacancy of a pharmacist (there were 513 / 688 vacancies / CVs). At the same time, the number of vacancies for pharmacy technicians only slightly exceeded the number of vacancies for pharmacists (213 more vacancies), which indicates the demand for both categories of specialists in this region.

The pharmaceutical labor market in the Urals Federal District is characterized by its instability. In 2019, there was a significant shortage of jobs, as the number of published CVs almost doubled the number of open pharmacy technicians' jobs (there were 339 / 566 vacancies / CVs). However, the number of CVs declined annually due to the demographic pitfall, a political migration and professionals leaving for other industries (mainly a digital technology), while the number of vacancies, on the contrary, increased annually. This has led to an increased competition among the employers for their staff. A similar situation took place in the market of pharmacists (there were 170 / 275 vacancies / CVs). By the beginning of 2022, the number of open vacancies had become twice as high as the number of published CVs among pharmacists (there were 280 / 172 vacancies / CVs). The number of new pharmacy technicians' CVs annually declined, eventually, by the end of the year the number of open positions had exceeded the number of CVs submitted by 108 publications. In the region as a whole, for many years,

the number of open pharmacy technicians' vacancies only slightly exceeded the demand for pharmacists. But in 2022 the demand for pharmacy technicians doubled (there were 425 / 244 vacancies / CVs). The current situation may cause many professionals with higher education to move to other fields of activities, which can be considered an unfavorable trend and means that the role of higher education in the field of pharmacy is decreasing.

Since 2019, the pharmacy labor market in the Siberian Federal District has suffered significant changes. The number of published CVs was annually decreasing more and more, resulting in a shortage of pharmacy specialists with both secondary (there were 204 / 534 vacancies / CVs in 2019 *vs*. 200 / 380 vacancies / CVs in 2020) and higher education (there were 194 / 403 vacancies / CVs in 2019 *vs.* 173 / 263 vacancies / CVs in 2020). Based on the 2022 data, the number of pharmacy technicians' vacancies exceeded the number of pharmacists looking for work, by 144 vacancies. Overall, the demand for pharmacy technicians in the Siberian Federal District always exceeded the need for pharmacists, but the preference gap only widened over time. For example, in 2022, there were 385 vacancies and 275 CVs for pharmacy technicians published, while for pharmacists, they were 214 and 217, respectively.

From 2019 to 2020, the pharmaceutical market in the Far Eastern Federal District experienced a shortage of jobs (in 2019, there were 98 / 198 vacancies / CVs for pharmacy technicians and 97 / 132 for pharmacists; in 2020, there were 80 / 136 vacancies / CVs for pharmacy technicians and 67 / 93 for pharmacists). However, in 2021, the situation changed significantly towards an excess of vacancies over the number of specialists in search of jobs (in 2021, there were 309 / 202 vacancies / CVs for pharmacy technicians and 248 / 129 – for pharmacists). This trend did not last long, and in 2022, the number of submitted CVs exceeded the number of existing vacancies (in 2022, there were 82 / 111vacancies / CVs for pharmacy technicians and 68 / 77 – for pharmacists). It is characteristic for the region that the share of pharmacy specialists with higher education is extremely low. This is probably due to a small number of pharmaceutical companies and an almost complete absence of production pharmacies in the region, and, accordingly, to the initially lower demand for specialists with higher education. Thus, the demand for pharmacists in the Far Eastern Federal District has always exceeded the need for pharmacists.

In the Southern Federal District and the North Caucasus Federal District, the number of submitted CVs exceeded the number of open vacancies throughout the study period. Despite the fact that the number of specialists in search of the employment decreased more than twice between 2019 and 2022, the demand for pharmacists and pharmacy technicians did not increase (in 2019, 510 / 1217 vacancies / CVs for pharmacy technicians and pharmacists, and in 2020 – 412 / 929 for pharmacy technicians and 386 / 616 for pharmacists; in 2021 – 840 / 904 for pharmacy technicians and 771 / 535 for pharmacists). Based on this, it can be concluded that there is a shortage of jobs for candidates with both higher and secondary pharmacy education in these regions. The demand for pharmacists in the Southern and North Caucasus Federal Districts slightly exceeded the need for pharmacists throughout the study period. Thus, in 2022, 276 vacancies for pharmacists were published which was 86 vacancies fewer than pharmacists.

Thus, in most regions there is a slight predominance of demand for specialists with secondary education and a drop in demand for specialists with pharmacist education. This may lead to a decrease in the role of the latter in recruitment, to a decrease in motivation for higher education among young specialists and, as a result, to a decrease in the quality of pharmaceutical care in general.

When calculating the Pearson correlation coefficient, it was noted that the demand/supply ratio of vacancies for the period 2019–2022 (Fig. 1, 2) changed in a similar way (a high positive correlation, $r > 0.94$ at $p < 0.05$) for both pharmacists and pharmacy technicians in the following regions. They were: St. Petersburg and the Leningrad region, the Central Federal District (without the Moscow region), the Northwestern Federal District (without the Leningrad Region), the Volga Federal District, the Far Eastern Federal District and the Southern and North Caucasus Federal Districts. In these regions, a high degree of correlation can be noted. It indicates similar trends in changes in the labor market over a given period for specialists with both secondary and higher education, which allows the use of general strategies for managing supply and demand. It should be noted that the demand/supply ratio of vacancies in Moscow and the Moscow region for pharmacy technicians and pharmacists shows a relatively low correlation with other regions. This is likely due to a high population density, economic differences and a large number of vacancies, which makes the conditions for the formation of supply and demand in the labor market of the capital region unique. Compared to the other regions, there is also a low correlation coefficient between demand and supply of vacancies in the Ural and the Siberian Federal Districts. This may be due to regional economic and demographic characteristics, the specifics of the pharmaceutical market development in these regions, which requires a further study.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-131-149

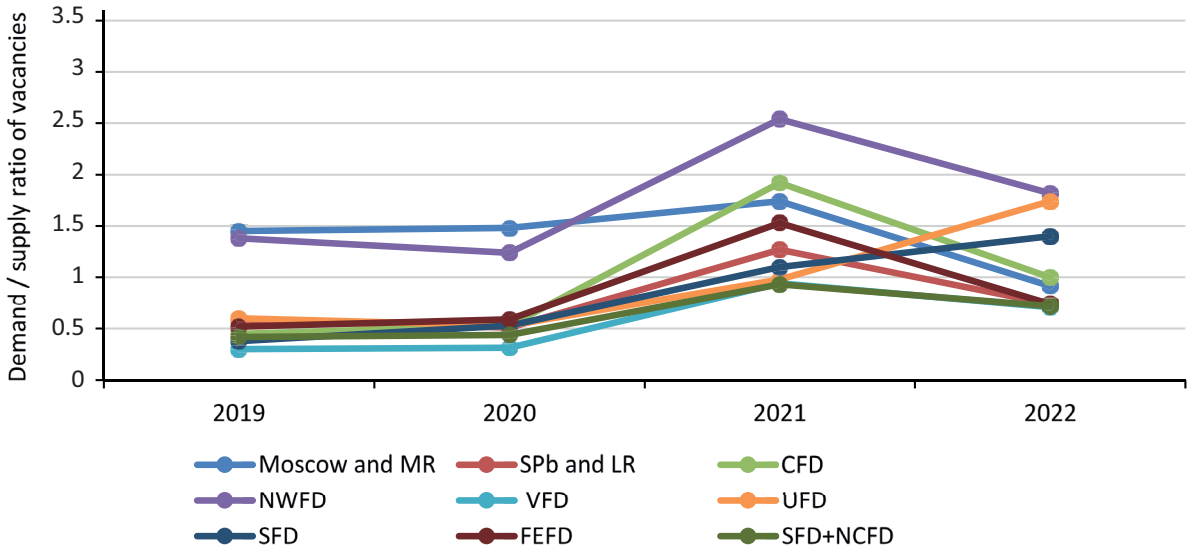


Figure 1 – Demand / supply ratio of pharmacy technicians vacancies in the Russian Federation regions for 2019–2022

Note: Moscow and MR – Moscow the Moscow region; SPb and LR – St. Petersburg and the Leningrad region; CFD – the Central Federal District (without the Moscow region); NWFD – the Northwestern Federal District (without the Leningrad Region); VFD – the Volga Federal District; UFD – the Urals Federal District; SFD – the Siberian Federal District; FEFD – the Far Eastern Federal District; SFD+NCFD – the Southern and North Caucasus Federal Districts.

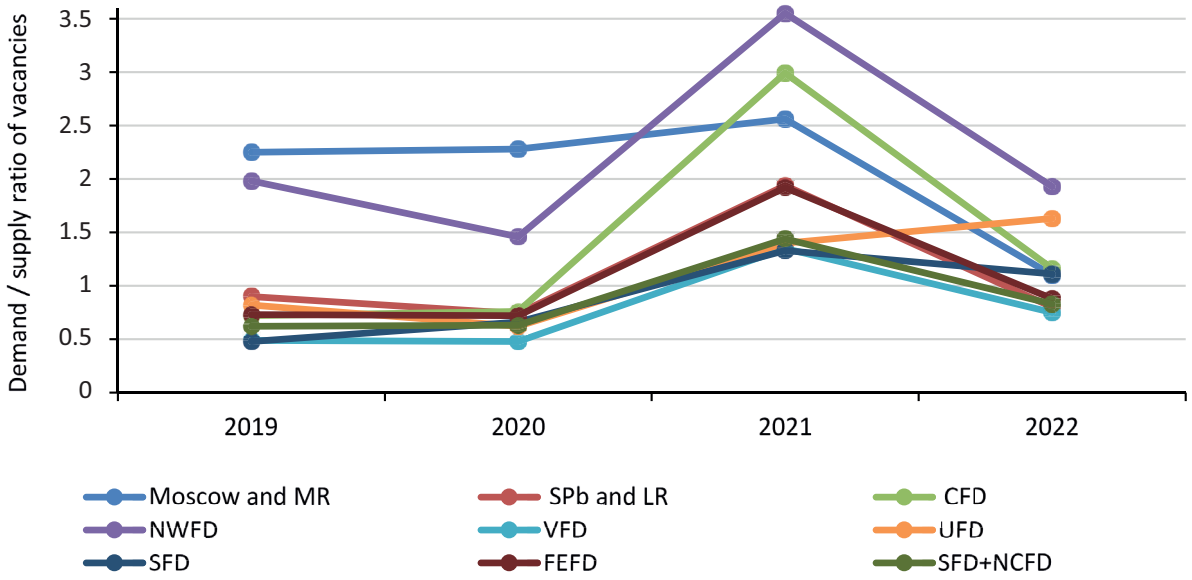


Figure 2 – Demand / supply ratio of pharmacists' vacancies in the Russian Federation regions for 2019–2022

Note: Moscow and MR – Moscow the Moscow region; SPb and LR – St. Petersburg and the Leningrad region; CFD – the Central Federal District (without the Moscow region); NWFD – the Northwestern Federal District (without the Leningrad Region); VFD – the Volga Federal District; UFD – the Urals Federal District; SFD – the Siberian Federal District; FEFD – the Far Eastern Federal District; SFD+NCFD – the Southern and North Caucasus Federal Districts.

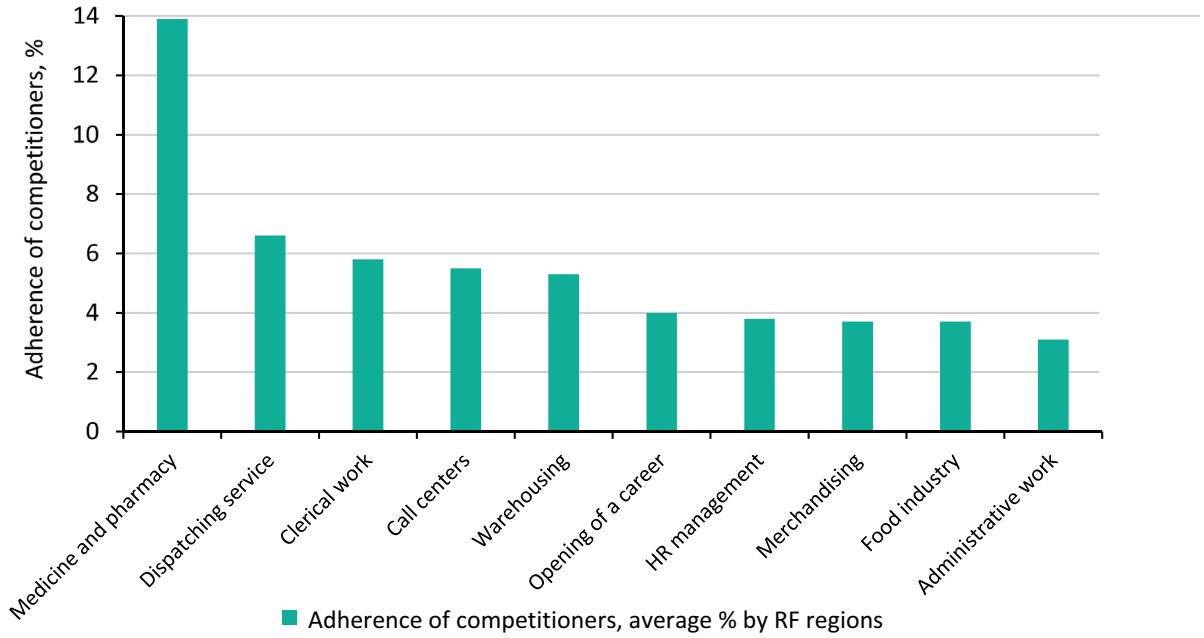


Figure 3 – Top popular pharmacy occupation areas among job seekers in 2019–2022 (on average across analyzed regions and districts)

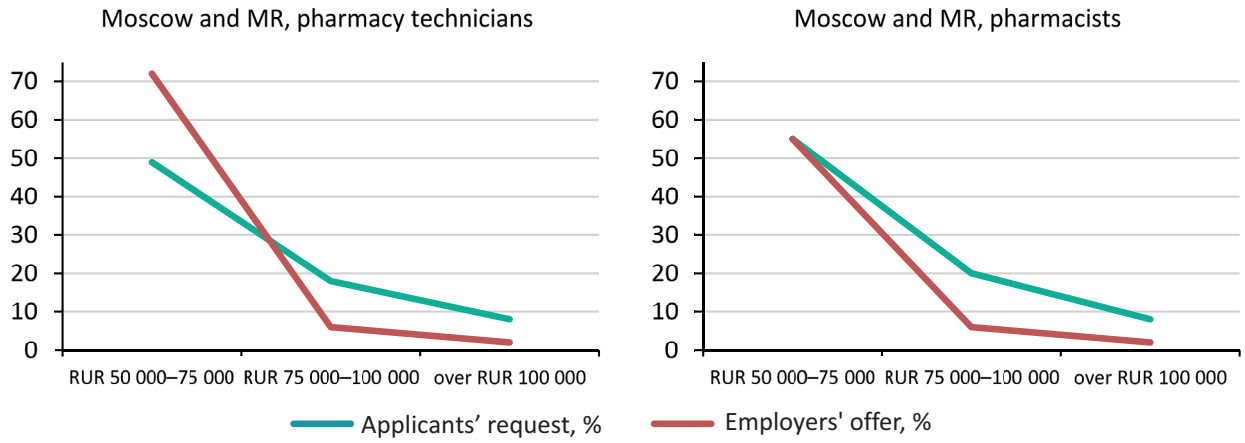


Figure 4 – Candidates' salary expectations / employers' offers ratio in Moscow and Moscow region (MR)

Top 10 popular areas among candidates

At this stage of the study, the popularity and demand for the profession of pharmacists and pharmacy technicians among other areas of work in the field of pharmacy were assessed in a region-by-region comparison. Professions related to health have been in demand at all times, so this industry, compared to others, is always in demand and can guarantee a stable employment. It seemed interesting to assess what other areas of work in the field of pharmacy are of interest to pharmaceutical specialists besides working in a pharmacy. The Top 10 areas popular with candidates included medicine and pharmacy (meaning working in pharmacies and medical institutions); warehousing (pharmaceutical warehouses); clerical work (working with documentation in pharmaceutical organizations); administrative work (secretariat, reception and office management in pharmaceutical and medical organizations); a dispatching service; food industry; work in call centers and a number of others. The data are presented from the total number of professions in demand at the request of "a pharmacists" and "a pharmacy technician" for the entire period as a percentage of the total number of vacancies offered (Fig. 3).

In terms of demand for candidates with secondary pharmaceutical education in Moscow and the Moscow

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-131-149

region, medicine and pharmacy (working in pharmacies, medical organizations) occupy the first place in terms of popularity among other areas of occupation committed to 12% of respondents from the total number of vacancies. Warehousing is 7% and the office management is 6%; they are also popular because they can provide a stable job with a guaranteed salary and a career advancement. Thus, pharmacy technicians in the region can develop in different directions: in addition to a career in medical institutions and pharmacies, working in pharmaceutical warehouses is popular, where a specialist can grow to the position of a director, work with documents, carry out administrative work in medical centers. In addition to specialty work in medicine and pharmacy, among 19% of pharmacists in the region, 7% of them adhere to working in warehousing, 5% to the clerical work, 4% adhere to the dispatching services and 3% – to the administrative support of business.

In St. Petersburg and the Leningrad region, such areas of occupation as medicine and pharmacy are demanded by pharmacy technicians first of all, which amounts to 8% of respondents, because the absence of employment problems in this region allows candidates to work directly in their specialty. The area of the clerical work, which accounts for 8%, is also in demand due to the high level of employment and a wide range of applications in this region. Warehousing is chosen by 7% of specialists; it is in demand due to the favorable geographical location of the Leningrad region (the proximity to the European borders); it is also of great interest to candidates, as it guarantees stable earnings and career advancement opportunities. Thus, in this region, it is quite easy for pharmacists to find employment in their specialty due to the lack of a job shortage and a small competition in the pharmaceutical market of the region. However, the situation is aggravated by the unwillingness of pharmacy technicians to work directly in their specialty, and their preference for related industries are administrators in medical centers (5%) of respondents, procurement specialists (4%). Pharmacists in the region, in addition to working in their specialty, which is 11%, often consider working as clerks (7%), in warehousing (5%), dispatching services (2%), government procurement (2%), administrative departments and food and consumer goods trade – 3% of professionals.

In the Central Federal District, applicants are traditionally more progressive and flexible, so they have a more pronounced interest in other industries. Nevertheless, the majority of candidates with secondary pharmaceutical education want to develop primarily in the field of pharmacy, and they are 10%. Many specialists (especially young people), are interested in other areas of activities, involving quick and high earnings, such as work in call centers (9%), dispatching services (7%), clerical work (4%), courier delivery (2%). Specialists with higher education are most in demand for working in their specialty and make up 14%, because they already have certain skills that allow them to receive a stable and guaranteed income. In addition to working in their specialty, candidates are interested in call centers consulting (10)%, working in the dispatching services (7%), as well as in the area of warehousing – 6% of specialists.

In the Northwestern Federal District, despite a high demand for pharmacy technicians, candidates with high school education primarily consider the area occupations not directly related to their primary occupation, such as: human resources (10%), dispatching services (10%), and food industry (8%). There are not so many companies hiring pharmacists in this district as in other regions, so, there is a wide range of candidate preferences across industries. In the region, pharmacists also primarily favor more modern digital areas that guarantee stable earnings and a career advancement. These include working in call centers (11%), food services (11%), human resources (11%) and dispatching services (9%). Only 6% of candidates with higher education and 3% with secondary education are interested in the areas of medicine and pharmacy, which indicates its low relevance among specialists in the region. This trend indicates the need to improve labor conditions in the pharmaceutical market of the region, otherwise the outflow of specialists from the areas of medicine and pharmacy will be even higher.

In the Volga Federal District, due to the difficulty of employment in their specialty, candidates with secondary education consider the area of medicine and pharmacy only in the third place (6% of specialists), preferring a variety of different areas that guarantee both the job itself and its stability to the main type of activity. These include dispatching services (8%), working in call centers (8%), clerical work (5%), warehousing (4%) and a number of other areas of activities. Herewith, medical and pharmacy areas are most in demand among pharmacists (11%). Due to the difficulties with employment in their main specialty in the Volga Federal District, pharmacists also consider looking for work in other areas, such as dispatching services (8%), working in call centers (6%) and clerical work (4%).

Given a wide range of employment opportunities

in the Urals Federal District, 11% of candidates with secondary education consider medicine and pharmacy as their first choice, but many specialists prefer other industries such as dispatching services (7%), clerical work (6%), working in call centers (5%), and warehousing (4%) as their main areas of activities. The shortage of specialists with higher education in the region allows a vast majority of pharmacists to develop in their core business. There are few pharmacists in the region, and the majority work in their specialty (75% of specialists with higher education). Only 8% of candidates consider sales, which guarantees a less stable but higher income.

The data for the Siberian Federal District show pharmacy technicians' interest in other spheres of activities, because specialists first of all consider vacancies in the following areas: dispatching services and clerical work, 9 and 8%, respectively. The areas of medicine and pharmacy are on the same level with agriculture – 5%. Pharmacists in the region are also more interested in other areas of work, and primarily consider vacancies in the fields of dispatching services (12%) and clerical work (10%). The areas of medicine and pharmacy only rank third (8%). This trend may lead to an increase in the shortage of personnel in the region due to the transition of qualified specialists to other areas of activities.

A peculiarity of the Far Eastern Federal District is that candidates with secondary and higher education are interested in the development in the military and contract service (5% among pharmacy technicians and 7% among pharmacists). Otherwise, specialists are looking for work in very popular areas such as medicine and pharmacy (7%), warehousing (6%) and dispatching services (6%). A low level of interest in the area directly related to the core business indicates the availability of alternative, more convenient and better-paid options. In contrast, pharmacists are looking for jobs in highly popular areas such as medicine, pharmacy (18%) and warehousing (9%).

In the Southern Federal District and the North Caucasus Federal District, the areas of medicine and pharmacy are in the highest demand among 9% of candidates due to a direct correlation with their main occupations. However, employment difficulties force specialists with secondary education to consider other areas of activities that imply the availability of jobs and stable earnings, for example, 8% of pharmacy technicians choose to work in call centers and dispatching services. Among pharmacists, the areas of medicine and pharmacy are also in the highest demand, 17% of specialists choose to work in their specialty. Due to difficulties in finding employment in their main specialty, pharmacists also consider looking for work in other areas, such as clerical work (6%), dispatching services (5%), and working in call centers (5%).

Thus, in a number of regions, the majority of specialists choose related professions, and where it is difficult to work in pharmacy due to the shortage of vacancies, there is a tendency to the so-called "washout" of pharmaceutical personnel from the profession, which consists in the employment of specialists in related fields of activities. This leads to non-use of previously mastered professional competencies by pharmacists and pharmacy technicians in their daily work activities. In the future, such a specialist will not be able to provide a high quality pharmaceutical care when working in specialty. It is characteristic that pharmaceutical specialists with secondary education are more inclined to migrate to other spheres of activities. At the same time, specialists with higher education more often choose to work in their specialty as an opportunity to realize their skills and abilities to the fullest extent possible.

Top cities by the number of candidates

In Moscow and the Moscow Region, traditionally leading in terms of the population density and average earnings, 73% of job seekers with secondary education and 74% with higher education are looking for work. The cities of the Moscow Region also have vacancies, but many candidates (especially young people) counting primarily not on personal comfort, but on high earnings and career advancement opportunities, prefer to work in the capital, while living in the Moscow Region.

In St. Petersburg and the Leningrad Region, St. Petersburg is the undisputed leader in terms of the number of candidates (93% of pharmacy technicians and 94% of pharmacists). Being a center of federal importance, the city offers great opportunities for employment and development. Only 6–7% of specialists are interested in working in the city of residence due to its convenient location and minimal competition. A significant shortage of personnel in smaller cities indicates the reluctance of specialists to work there due to low salaries and lack of career prospects.

In the Central Federal District, a number of applicants has a direct correlation with the city's population and proximity to the capital. Voronezh, due to its high

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-131-149

population and proximity to Moscow, is the absolute leader in terms of the number of candidates (15% of pharmacy technicians and 18% of pharmacists); here the deficit of specialists is the least. Yaroslavl, Ryazan and Kursk are regional centers, so they collectively possess 23% of all candidates for the position of a pharmacist and 17% of pharmacy technicians. In the remaining cities (Orel, Tambov, Vladimir, Ivanovo) there is an acute shortage of personnel.

Big cities of the Northwestern Federal District do not have an obvious leader in terms of the number of candidates with pharmacist education. This indicates their equal distribution across the cities of the district (Kaliningrad – 13%, Veliky Novgorod – 11% and Arkhangelsk – 9%). In terms of the number of specialists with higher education, Veliky Novgorod is the leader (18% of all candidates), largely due to Yaroslav the Wise Novgorod University which turns out young diploma holders every year. Kaliningrad ranks second in this indicator due to its favorable geographical location (2% of pharmacists), while the biggest shortage of personnel is recorded in Vorkuta, Kotlas and Severomorsk (a total of only 3%).

In the Volga Federal District, the cities of Ufa (10%), Nizhny Novgorod (9.7%) and Saratov (9%) are leaders in the number of candidates for the position of pharmacy technicians and pharmacists due to their large population and developed infrastructure. The biggest shortage of pharmacy technicians and pharmacists is in Saransk, Kirov and Izhevsk.

In the Urals Federal District, the greatest number of candidates for the position of pharmacist is concentrated in Yekaterinburg, Tyumen, and Chelyabinsk. A significant shortage of specialists is observed in Khanty-Mansiysk, Kamensk-Uralsky, and Neftyugansk. The capital city of Yekaterinburg concentrates 24% of all candidates. Despite the harsh climate, the large population and abundance of jobs make it possible for 15% of candidates to look for vacancies in Tyumen, 12% in Chelyabinsk and 7% in Surgut. Yekaterinburg and Tyumen, thanks to their high standard of living, concentrate 41% of all candidates for the position of a pharmacist, but smaller cities such as Miass, Kamensk-Uralski and Noyabrsk have a significant shortage of staff, as no more than 1% of the total number of specialists with higher education live there.

Pharmacy technicians in the Siberian Federal District are equally distributed across major cities. Novosibirsk is home to 18% of candidates, Omsk – 13%, Irkutsk and Krasnoyarsk – to 11% each. This even distribution is due to the vital needs for specialists throughout the district. The biggest shortage of pharmacy technicians takes place in the cities of Abakan, Usolie-Sibirskoye, Anzhero-Sudzhensk, Angarsk and Bratsk. Pharmacists in the district are evenly distributed in the five largest cities: Novosibirsk (14%), Irkutsk (14%), Barnaul (12%), Omsk (11%), Krasnoyarsk (10%). The biggest shortage of pharmacists is observed in the cities of Usolye-Sibirskoye, Prokopyevsk, Abakan, Bratsk, Gorno-Altaisk.

In the Far Eastern Federal District, Khabarovsk has the highest concentration of specialists (47% of pharmacy technicians and 51% of pharmacists) due to its favorable geographical location, developed infrastructure and significant population. Ulan-Ude is in the second place (11%). There is an acute shortage of pharmacy technicians and pharmacists in Amursk, Birobidzhan, Belogorsk, Nakhodka, Magadan (in total, no more than 5% of specialists work in these cities. Thus, there is an acute shortage of pharmacy technicians and pharmacists in most cities in the district.

In the Southern Federal District and the North Caucasus Federal District, more than half of all candidates (54% of pharmacy technicians and 55% of pharmacists) are concentrated in the largest cities (Krasnodar, Rostov-on-Don, and Volgograd). In the remaining cities of the regions, there is an acute shortage of specialists.

Thus, according to the indicator of the number of candidates for the positions of pharmacist and pharmacy technician in most regions, there is a tendency for applicants (especially young professionals) to prefer working in capitals and large densely populated cities with a developed infrastructure. In this regard, there is an acute shortage of staff in small towns everywhere.

Salaries

Salaries were assessed in Russian rubles in a region-by-region comparison. The highest salaries for specialists are traditionally offered by Moscow and the Moscow region. The vast majority of employers (72%) are ready to offer pharmacists a salary of 50 to 75 thousand rubles, which is within the average salary range in Moscow and the Moscow region (Fig. 4). Half of the candidates (49%) expect to receive this amount. However, when it comes to higher salaries, candidates' expectations are much higher than employers' offers: 18% of candidates want to receive from 75 to 100 thousand rubles for their work, but there are 3 times fewer vacancies, and there are 4 times more candidates who want to receive a salary

of 100 thousand rubles. As for pharmacists, 55% of employers are ready to offer a salary of 50 to 75 thousand rubles, and this coincides with the expectations of half of the candidates. However, when it comes to higher salaries, the expectations of candidates are much higher than the employers' offers: 20% of candidates want to receive from 75 to 100 thousand rubles for their work. In the region as a whole, pharmacy technicians have a 17% higher salary than pharmacists do. Thus, the salary requests of candidates have grown significantly over the last couple of years and exceeded the salary offer. It is expected that in the next couple of years employers will increase salaries in order to attract specialists.

In St. Petersburg and the Leningrad Region, most employers are ready to offer specialists with secondary education a salary of 35 to 50 thousand rubles, while candidates expect a monthly salary of 50 to 75 thousand rubles. Problems with the employment, which have arisen in the pharmaceutical market due to the excess of supply over demand, limit not only the amount of salary, but also the opportunities for a career advancement. Employers are ready to pay specialists the amount of 75 thousand rubles and above only in 6% of cases, and the salary expectation of candidates for the same amount is 22%. The pharmacist market in St. Petersburg and the Leningrad Region is very similar to the pharmacy market. In most cases, specialists wish to receive from 50 to 75 thousand rubles for their work, but in more than half of cases, employers offer a salary of 35 to 50 thousand rubles. However, 37% of companies are ready to offer specialists a larger salary. The average salary of a pharmacy technician is 18% higher than that of a pharmacist. At the same time, 18% of pharmacists have to work for 20–35 thousand rubles a month, and pharmacy technicians who agree to work for the same amount of money, are only 5%. That suggests that specialists with secondary education have higher salaries. Thus, in the Leningrad Region, there is a serious imbalance between the salary expectations of candidates and the salary offer of companies. The candidates compare salaries with other industries and go there. Against the background of a tightening competition for personnel, it is expected that in the next couple of years the salaries of pharmaceutical specialists will become higher.

A large number of pharmaceutical plants and factories are localized in the Central Federal District, which gives opportunities for a career advancement and earn high wages to candidates with secondary education. Only 11% of companies are ready to offer salaries from 100 thousand rubles, but 40% of small firms offer candidates a fairly high monthly salary from 50 to 75 thousand rubles, and 34% – from 35 to 50 thousand rubles. In general, salary offers are close to the expectations; in addition, there are new attractive conditions to keep specialists in the profession: bonuses, a flexible schedule, opportunities for training and development. When hiring pharmacists, 55% of employers are interested in an employee who agrees to work for 50–75 thousand rubles per month, but these conditions are considered by only 44% of candidates. At the same time, 30% of specialists are looking for a job with a monthly salary from 75 thousand rubles. Only 8% of companies are ready to offer such high earnings. This trend may lead to the departure of many candidates to other, more highly paid, spheres of labor. In general, pharmacists' and pharmacy technicians' salaries differ insignificantly, but pharmacy technicians have a higher share of those who are willing to pay them a salary of more than 100 thousand rubles. That may be a consequence of the migration of high-potential specialists with higher education to other regions; as a result, high-paying vacancies are occupied by specialists with secondary professional education.

In the Northwestern Federal District, 42% of specialists with secondary education aim to find a job with a monthly salary of 50 to 75 thousand rubles, but only 36% of salary offers correspond to this request. 55% of companies in the district are also ready to provide pharmacists with a salary of 50 to 75 thousand rubles, 18% of companies offer a salary of 20 to 35 thousand rubles, while only 6% of candidates are willing to consider such a salary. However, every twelfth vacancy indicates a salary over 75 thousand rubles. It is often easier for candidates to immediately find a better-paid job in other areas, than find opportunities for a career advancement. In this region, the salaries of pharmacy technicians and pharmacists differ significantly. More than half of pharmacists (55%) can claim an average salary of 50 to 75 thousand rubles per month, while only 36% of pharmacy technicians can claim for the same amount of salary.

Despite the obvious shortage of vacancies, in the Volga Federal District, the salary of pharmacy technicians exceeds the salary expectations of candidates. The shortage of staff has led to the fact that since 2020, when employers sought to find specialists quickly, the salary offers have increased significantly, and now employers are willing to pay even more than candidates expect. This

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-131-149

is especially evident in the salary offer of 100 thousand rubles per month, which only 8% of candidates aim for, but 21% of companies can offer. Among pharmacists, the salary expectations of beginners are similar to the offers, but experienced candidates have higher salary expectations than the offered salary. 27% of candidates aim for a monthly salary of 75 thousand rubles or more, which is more than one fourth of all specialists, but only 8% of companies can offer such a high salary. 26% of pharmacy technicians and only 8% of pharmacists can claim a salary of 75 thousand rubles or more. The salary expectations of novice specialists are similar to the offers, but experienced candidates have higher salary expectations than the companies offer them.

In the Urals Federal District, employers offer candidates a salary between 35 and 75 thousand rubles. 27% of candidates expect a salary over 75 thousand rubles, but only 10% of employers offer such a salary. Higher earnings require higher education, as well as mastering certain skills and knowledge. In general, there is no significant gap in salary expectations and offers in the district. 55% of employers offer candidates earnings from 50 to 75 thousand rubles monthly, and 45% of specialists are satisfied with this. Pharmacists rarely consider higher salaries, from 75 thousand rubles and above, so the expected earnings with the proposed one diverge only by 10%. In general, the salaries of pharmacy technicians and pharmacists in the district differ insignificantly. This is explained by the shortage of specialized and experienced candidates and employers' focus on newcomers, but the average salary of pharmacists is between 50 and 75 thousand rubles, which is 17% higher than that of pharmacy technicians.

The Siberian Federal District is characterized by similar salary expectations and offers in terms of average salaries: 42% of pharmacy technicians are looking for a job that implies monthly earnings of 50 to 75 thousand rubles, and 43% of companies offer the same conditions. However, it is difficult for specialists to claim for higher earnings; 26% of candidates are aiming for a salary of 75 thousand rubles and above, but only 7% of employers are ready to offer such amounts. A similar situation is observed in the pharmacists' market: 47% of specialists are looking for a job with a monthly salary from 50 to 75 thousand rubles, and 55% of companies offer the same conditions; 26% of candidates aim for a salary from 75 thousand rubles and above, but only 8% of employers are ready to offer such amounts. A significant shortage of pharmacists is observed in low-paying jobs. Thus, for earnings from 25 to 35 thousand rubles, only 5% of candidates claim, and there are 18% of organizations offering these conditions (there is an abundance of low paying positions). In general, in the region, the salaries are lower than in other regions. Earnings of pharmacy technicians and pharmacists are almost the same. Most specialists are able to reach average earnings, but only a few are able to find opportunities for a career advancement.

In the Far Eastern Federal District, the possibility of high earnings for pharmacy technicians, from 75 thousand rubles per month, is extremely small – only 4% of all offers. Although 93% of employers are ready to pay from 30 to 75 thousand rubles, only 59% of candidates consider these conditions. In general, the size of pharmacy technicians' salaries in the region starts from 35 thousand rubles, while almost half of the employers offer payment to pharmacists below this amount. The lack of sufficient number of high-paying vacancies may lead to a loss of personnel with higher education to other areas of labor. For pharmacists in the region, there is a significant resonance in salary expectations and offers, especially in the segment involving a salary of 75 thousand rubles or more per month, which is the target for 40% of candidates. Employers are ready to offer this size of salary only in 8% of cases. Candidates compare salary with other industries, where offers are often higher. Due to that, companies are forced to raise salaries to attract specialists with specialized education.

The Southern Federal District and the North Caucasus Federal District are characterized by a significant imbalance between the salary offers of companies and the salary expectations of candidates. Mostly, employers are ready to offer specialists with secondary education a salary of 25 to 50 thousand rubles, while candidates, in turn, expect a monthly salary of 50 to 75 thousand rubles. The employment problems that have arisen in the pharmaceutical market of the region due to the excess of supply over demand, limit not only the size of salaries of employees, but also the opportunities for a career advancement. Employers are ready to pay specialists the amount of 75 thousand rubles and above only in 6% of cases and the salary expectations of candidates for the same amount is 23%. Such dissonance risks entailing a shift of candidates to other higher-paying labor markets. Among pharmacists in these regions, salary expectations converge with employers' salary offers for salaries up to 75 thousand

rubles. In the region, there is a wide variation of salaries depending on specialist qualifications. In vacancies where higher education is required, salaries start from 50 thousand rubles.

Thus, in most regions, salary expectations of pharmacy technicians are more in line with the offered salary than those of pharmacists. It is characteristic that in some regions employers pay pharmacy technicians and pharmacists equally, although candidates with higher education quite reasonably expect to be paid more (which corresponds to a broader set of skills mix inherent in a pharmacist). As a result, this reduces the value of higher pharmacy education as such and, in particular, leads to a low motivation among young people to obtain it. Such a "devaluation" may lead to the loss of importance of the profession of a pharmacist in the future, to a complete shift of a pharmaceutical specialist's functions towards a salesperson's ones, and, consequently, to the deterioration of the quality of pharmaceutical care in general.

Additional labor conditions offered in vacancies

At this stage of the study, working conditions (in addition to salaries) offered to pharmaceutical specialist by employers in different regions, were analyzed. These conditions included the possibility of free training at the company's expense; opportunities for career advancement within the company; the possibility of receiving bonuses; health insurance and meals at the employer's expense; flexible working hours.

In Moscow and the Moscow region, free training at the company's expense is the most widely offered condition of employment (39% for pharmacy technicians and 25% for pharmacists) due to the specifics of pharmaceutical activities. Career development opportunities (25% for pharmacy technicians and 19% for pharmacists) and bonuses (13 and 16%, respectively) are a significant motivation for specialists. Health insurance (8 and 5%, respectively) and free meals (8 and 7%, respectively) are rarely included in the terms and conditions of employment as they incur additional costs for the employer. Due to some peculiarities of a pharmacy technician's work, a flexible schedule is almost impossible (4%). Flexible working hours are more common among pharmacists due to a wider range of positions (14%).

In St. Petersburg and the Leningrad region, in 43% cases, employers are ready to offer pharmacists a flexible schedule (shifts). A high prevalence of this benefit is due to the regional peculiarities of the Leningrad region, which includes a small number of 24-hour pharmacies. Free training (21% for pharmacy technicians and 26% for pharmacists) is provided in order to ensure that candidates have all the necessary skills by the time they start working at their main workplace. Employers also often provide career development opportunities for specialists (16% for pharmacy technicians and 20% for pharmacists). Additional health insurance for pharmacy technicians (5%) and meals at the expense of the employer (3%) are quite rare.

In the Central Federal District, employers offer fairly standard but favorable working conditions for specialists. The most frequently offered conditions are opportunities for a career advancement (28% for pharmacy technicians and 25% for pharmacists) and free training (25% and 30%, respectively). Many companies are targeted not only at attracting candidates, but also at retaining them. Training of specialists by the employer and their financial incentives significantly inhibit their departure to other spheres. A number of organizations offer various cash bonuses and bonuses as an additional incentive (15% for pharmacy technicians and 17% for pharmacists).

In the Northwestern Federal District, organizations provide employees with opportunities for a career development in 24% of cases for pharmacy technicians and 23% for pharmacists, which is due to the need to retain highly qualified specialists in companies for many years. A number of enterprises are ready to train new specialists in the required skills free of charge (18 and 16% respectively). Bonuses and other monetary incentives along with flexible schedules are found in 13% of cases. Flexible working hours that allow specialists to work in conditions that are comfortable for them, are also offered quite often (13% for pharmacy technicians and 20% for pharmacists). Few employers are willing to provide meals at the employer's expense (3%) and additional health insurance (4%) due to additional costs.

In the Volga Federal District, employers use widespread ways to attract specialists with favorable working conditions in order to retain them. In general, conditions in the region are oriented towards young specialists. These include free training (27% for pharmacy technicians and 25% for pharmacists), opportunities for a career and professional advancement (25 and 20%, respectively), and bonuses (17 and 14%, respectively). Quite often, flexible working hours are offered, allowing to combine study and work (15%).

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-131-149

The organizations interested in finding candidates in the Urals Federal District, use widespread ways of attracting specialists in order to retain them in the company. The region's labor market is also oriented towards young specialists. The conditions offered include free training opportunities (23% for pharmacy technicians and 21% for pharmacists), opportunities for a career and professional advancement within the company (19 and 22% respectively), bonuses (14 and 11% respectively). Flexible schedules that allow combining studies with work are also offered quite often (20% of vacancies).

In the Siberian Federal District, free training for new employees is a fairly widespread offer in vacancies (22% for pharmacy technicians and 23% for pharmacists). Employers also often provide specialists with great opportunities to develop their career potential (23 and 22%, respectively), as well as offer a system of bonuses (12 and 11%, respectively). Flexible working hours are also often offered in the region (16% for pharmacy technicians and 17% for pharmacists), allowing specialists to combine work with other areas of their lives.

The most common labor conditions offered to specialists in the Far Eastern Federal District are opportunities for a career advancement (21% for pharmacy technicians and 20% for pharmacists) and free training (21 and 22%, respectively). Many companies target not only at attracting candidates, but also at retaining them, so they also offer bonuses (12 and 10%, respectively). In this region, free meals (10%) and flexible hours (11% for pharmacy technicians and 18% for pharmacists) are offered to employees more often than in other regions. This may be due to the peculiarities of the geographical location and climate of the region, and may also indicate the desire of companies to attract employees in additional non-standard ways.

Organizations of the Southern and the North Caucasus Federal Districts most often offer vacancies with the possibility of free training (26% for pharmacy technicians and 24% for pharmacists); opportunities for a career advancement (22 and 23%, respectively). Flexible working hours (18 and 19%, respectively), as well as various bonuses (17 and 15%, respectively) are widespread in vacancies. This significantly improves the working conditions of the region's employees.

Thus, all the surveyed regions, to a greater or lesser extent, provide specialists with free training at the expense of the companies, as well as the opportunity for a career development within the organization. In addition, most employers are interested in attracting and retaining young specialists in the profession by providing them with flexible work schedules to enable them to combine their university studies with employment. This allows companies to ease the staff turnover and maintain a high quality of care and pharmaceutical aid in general.

Education requirements for pharmaceutical specialists

The final stage of the study assessed the education requirements for pharmaceutical workers, i.e. employers' preferences regarding the presence or absence of higher education in potential employees.

In Moscow and the Moscow region, in 39% of vacancies, employers are interested in candidates with higher education, 44% – in specialists with secondary education, and in 27% of vacancies, the requirements for education are not specified.

In St. Petersburg and the Leningrad Region, in almost half of cases (47%), employers do not specify the need for higher education in job vacancies, which increases the number of specialists willing to take up a vacant position and creates some confusion at interviews. In 17% of cases, companies are looking for specialists with secondary vocational education, and in 36%, they are interested in personnel with relevant higher education.

In the Central Federal District, many organizations are targeted at attracting specialists with certain skills and competencies. In 41% of cases, employers are looking for specialists with higher education, while 34% of companies need candidates with secondary vocational education.

In the Northwestern Federal District, employers are focused on finding highly qualified personnel; therefore, companies are very interested in specialists with higher education (42%). Pharmacy technicians are of less interest to employers (32%).

In the Volga Federal District, there is an almost equal demand for specialists with secondary and higher education: 36% of organizations need pharmacist, 30% of employers are interested in hiring pharmacy technicians, and 34% do not specify educational requirements in published vacancies, focusing solely on the candidates' personal qualities, knowledge and experience.

In the Urals Federal District, organizations need candidates with higher education (51%). Pharmacy technicians are less in demand (9%). This is probably due to the specifics of the pharmaceutical industry in

the region and its geography. In large cities of the region, organizations are interested in employees with higher education, who are ready to constantly improve their skills. In general, there is an extreme shortage of both pharmacists and pharmacy technicians in the region.

In the Siberian Federal District, organizations also need candidates with higher education to a greater extent (51%). Pharmacy technicians are required to a lesser extent (25%).

In the Far Eastern Federal District, many organizations are targeted at attracting specialists with higher education and certain knowledge and skills (41% of employers). At the same time, 21% of companies need pharmacists, 38% of vacancies do not contain information about the preferred higher or secondary education.

In the Southern and North Caucasus Federal Districts, employers are most often focused on finding highly qualified staff, so in 53% of cases, they are interested in candidates with higher education. In case of vacancies requiring secondary education (29%), it can be assumed that such employers offer very low salaries but uncomplicated and monotonous work.

Thus, in most regions, organizations are interested in finding a variety of employees. Higher education is not in the least demand among candidates (43% of the regional average), but it provides for higher salaries and broader career opportunities. In general, the larger the city, the more pharmacists live there, which is most likely due to the localization of regional medical and pharmaceutical universities. Many organizations that do not impose strict educational requirements on specialists and offer free training at the start of a career, are aimed at finding young specialists who do not expect high earnings.

Study limitations

This study has a number of limitations that should be taken into account when interpreting the results:

1. The research covered only 9 regions of Russia, which does not allow extrapolating the findings to the whole country without an additional analysis;
2. The analysis was based on the data from one recruiting agency (superjob.ru), which may not fully reflect the situation on all job search platforms;
3. The study did not cover veterinary pharmacy, which is a separate and evolving segment of the pharmaceutical market with its own particularities in terms of training and employment;
4. The time period of the study (2019–2022) includes the period of the COVID-19 pandemic and related economic turmoil, which may have affected the dynamics of supply and demand in the labor market;
5. The data for 2023 were not included in the sample because the full data for 2023 were not yet available at the time of the study, making it impossible to include them in the analysis without risking distorting the overall picture.

CONCLUSION

On average, in the regions, the demand for specialists with higher education is lower than that for specialists with secondary education, and in terms of a salary level, the expectations of pharmacy technicians coincide with the employers' offers, while the salary expectations of specialists with higher education are higher than the market. The selection of vacancies / CVs includes mainly the data from pharmacy chains, which have established certain work standards and salary systems to be taken into account. Specialists with secondary education are much more numerous, as they are trained by a much larger number of educational institutions, both specialized, subordinate to the Ministry of Health of the Russian Federation, and not. It is important to take into account that for a pharmacy technician, pharmacy is often the only place of work in the specialty, while for a pharmacist; it is usually a place to start. The potential and opportunities of specialists with higher education are usually greater and broader than those of the people who have completed only a secondary vocational education program. If we consider employment opportunities in the field of production or promotion of medicines, then for specialists with secondary education, only starting positions are available; their functionality is rather monotonous and does not imply career building. For pharmacists, practically all directions of labor activities are open, both horizontally (changes of positions within a similar functionality) and vertically (changes of positions with changes of functionality).

Under the current circumstances, it is important to conduct educational and career guidance work at higher education institutions, which often graduate not only pharmacists, but also pharmacy technicians, since it is becoming more and more difficult for a specialist with secondary education to obtain higher education. On the other hand, specialists experienced as pharmacists, tend to be better prepared, more motivated and disciplined after entering higher education, and their learning

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-131-149

outcomes are higher than those who enter immediately after general education.

Despite an impressive number of graduates of secondary and higher education institutions, the expansion of the range of labor functions and the rapid expansion of all segments of the pharmaceutical market, the number of required specialists and the competencies they must possess, continue to increase, which indicates the advisability of opening new pharmaceutical institutes and updating training programs.

FUNDING

This study had no financial support from outside organizations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Denis V. Kurkin – idea and planning of the structure of the work, design of graphic material, editing the final version of the manuscript; Yulia S. Knyazeva, Olga V. Ivanova, Yuri A. Kolosov, Yulia V. Gorbunova, Dmitry A. Bakulin, Ivan S. Krysanov, Darya L. Klabukova, Marina A. Javakhyan, Valentina I. Zvereva, Natalia A. Lycheva, Victoria N. Shoich, Veronika V. Neyolova, Evgeniy I. Morkovin, Elizaveta V. Pavlova, Elena A. Kalashnikova, Alexander B. Bosenko, Djamilya Z. Zyazikova, Ruslan E. Musaev, Abakar G. Gadzhiev, Boris M. Gabrielyan, Anna A. Agamirova – collection of material and writing the draft manuscript, editing the final version of the manuscript; Vladimir I. Petrov – consultations on highly specialized issues, editing and approval of the final version of the article.

REFERENCES

1. Golikova NS, Prisyazhnaya NV. Recommendations for conducting medical and sociological monitoring of the personnel needs of medical organizations on the example of studying the needs of regional pharmaceutical enterprises in pharmacists' training (part 1). Chief Medical Officer. 2022;2:13–39. DOI: 10.33920/MED-03-2202-01
2. Golikova NS, Prisyazhnaya NV. Recommendations for conducting medical and sociological monitoring of the personnel needs of medical organizations on the example of studying the needs of regional pharmaceutical enterprises in pharmacists' training (part 2*). Chief Medical Officer. 2022;3:11–32. DOI: 10.33920/med-03-2203-02
3. Bat NM, Siyukhova FSh, Kadakoeva GV. Upgrading higher pharmaceutical education by actualizing a competence approach. Vestnik Majkopskogo Gosudarstvennogo Tehnologicheskogo Universiteta. 2020;2(45):50–58. DOI: 10.24411/2078-1024-2020-12005
4. Knyazeva YS. Pharmaceutist awareness of hypolipidemic drugs in the Volgograd region. Journal of VolgSMU. 2016;2(58):36–40.
5. Knyazeva Y.S., Kurkin D.V., Atapina N.V., Bakulin D.A., Sokolova A.A. The effectiveness of the implementation of the algorithm for advising customers when dispensing prescription drugs in a pharmacy. Farmaciya (Pharmacy). 2023;72(2):34–42. DOI: 10.29296/25419218-2023-02-06
6. Klishchenko MY, Kuznetsov DA. Results of the study of threats to the personnel security of pharmaceutical organizations. Current Drug Supply Management. 2022;9(1):28–35. DOI: 10.30809/solo.1.2022.3
7. Oganesyan E.T. About pharmaceutical education. Vestnik Bashkir State Medical University. 2022;(S9):136–140.
8. Meliksetyan AA. Global trends in pharmaceutical education. Innovations in the health of the nation: Proceedings of VI All-Russian Scientific and Practical Conference with international participation; St. Petersburg State Chemical and Pharmaceutical University. St. Petersburg: St. Petersburg State University of Chemistry and Pharmacy; 2018. P. 507–510.
9. Golikova NS. Approaches to training pharmacists in the Russian Federation. Chief Medical Officer. 2021;11:27–45. DOI: 10.33920/med-03-2111-02
10. Cisse DS, Ilinova YuG, Narkevich IA, Umarov SZ Assessment of employment preferences of students mastering pharmaceutical education programs. Current problems of health care and medical statistics. 2024;(1):128–137. DOI: 10.24412/2312-2935-2024-1-128-137
11. Shvedov GI, Berezhnova TA, Selutin OA, Shvedova VG, Pluzhnikov DYu, Mukovnina MD, Kuzmenko NYu, Zanina IA, Bredikhina TA. Current issues of higher phamaceutical education. Journal of New Medical Technologies. 2019;(1):187–192. DOI: 10.24411/2075-4094-2019-16226
12. Lobanova EE, Artman AS. Tools of the education and labor market in shaping the competencies of pharmaceutical industry workers. Human resource management is the basis for the development of an innovative economy: Proceedings of the VIII International Scientific and Practical Conference; Krasnoyarsk, April 25–27, 2019. Krasnoyarsk: Reshetnev Siberian State University of Science and Technology; 2019. P. 99–105.
13. Katkova YaE, Menshikh AS. Labour mobility in the pharmaceutical market. Politics, Economics and Innovation. 2018;1(18):12.
14. Golikova NS, Prisyazhnaya NV. Features of the modern human resources component of the pharmaceutical industry (based on the materials of a survey of employees of pharmaceutical and biotechnological companies

and enterprises in Moscow). Sociology of Medicine. 2022;21(1):117–126. DOI: 10.17816/socm110877

15. Sidullina SA. Modern pharmaceutical education must be different. Fundamental and Applied Science: new challenges and breakthroughs: Proceedings of the International Scientific and Practical Conference, Petrozavodsk, January 26, 2020. Volume Part 2; Petrozavodsk: International Center for Scientific Partnership "New Science"; 2020. P. 280–284.
16. Gladkaya YV, Losenkova SO, Pantyukhina KI. Actual perspectives opening with the reforming of pharmaceutical education. Smolensk Medical Almanac. 2018;(3):56–58.
17. Fedina EA. Rationalization and optimization of pharmaceutical education in modern conditions. Innovative science. 2020;(8):64–65.
18. Sergeeva MS. The main trends in the development of domestic pharmaceutical education at the beginning of the XX century. Russian Academy of Medical Sciences. Bulletin of the National Research Institute of Public Health. 2012;(S1):157–159.
19. Ilkevich TG, Teige SV. Contents of vocational motiving training in pharmaceutical education. Vestnik GSU. 2019;(3):30–36.
20. Yudina LYu, Beregovykh VV, Aladysheva ZhI, Pyatigorskaya NV. On trends of modern pharmaceutical professional education. Medical education and university science. 2013;1(3):61–67.
21. Kolesnik NV. Pharmaceutical education in Russia: local issues in the context of globalization. Innovations in the health of the nation: Proceedings of the IV All-Russian scientific and practical conference with international participation; St. Petersburg, November 09-10, 2016. St. Petersburg: St. Petersburg State Chemical and Pharmaceutical Academy; 2016. P. 20–23.
22. Sokolova OV, Lavrentyeva LI, Alekseyeva KS. The study for regional retail pharmaceutical market. Pharmacy & Pharmacology. 2015;3(5(12)):64–68. DOI: 10.19163/2307-9266-2015-3-5(12)-64-68
23. Gritsanenko DS, Narkevich IA, Ilyinova YG, Umarov SZ. Quantitative characteristics of the labor potential of students enrolled in pharmaceutical education programs. Farmaciya (Pharmacy). 2023;72(8):50–56. DOI: 10.29296/25419218-2023-08-08
24. Shalenkova EV, Bonyushko NA. Pharmaceutical labor market local problems that reduce the quality of retail pharmacy chain social results on the example of Nizhny Novgorod region. Economics and Management: Problems, Solutions. 2023;6(11):171–178. DOI: 10.36871/ek.up.p.r.2023.11.06.018
25. Bandura AF, Kulik VV, Kovaleva TG, Yemanova AM, Bandura VYu. Analysis of employers' offers for pharmaceutical workers on the regional labor market. Belikov readings: Proceedings of the VII All-Russian Scientific and practical conference, Pyatigorsk, December 04-05, 2018; Pyatigorsk: Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University; 2019. P. 514–519.

AUTHORS

Denis V. Kurkin – Doctor of Sciences (Pharmacy), Associate Professor, Director of the Scientific and Educational Institute of Pharmacy n.a. K.M. Lakin, Russian University of Medicine. ORCID ID: 0000-0002-1116-3425. E-mail: strannik986@mail.ru

Yulia S. Knyazeva – Candidate of Sciences (Pharmacy), Associate Professor, Department of Clinical Pharmacology and Intensive Care of Volgograd State Medical University. ORCID ID: 0000-0002-9571-2793. E-mail: knjazeva1978@mail.ru

Olga V. Ivanova – Candidate of Sciences (Pharmacy), Senior Researcher, Scientific and Educational Institute of Pharmacy of Volgograd State Medical University; Scientific and Educational Institute of Pharmacy n.a. K.M. Lakin, Russian University of Medicine. ORCID ID: 0000-0003-4333-322X. E-mail: ovivanova134@mail.ru

Yuri A. Kolosov – Candidate of Sciences (Medicine), Associate Professor, Deputy Director for Educational Work, Scientific and Educational Institute of Pharmacy n.a. K.M. Lakin, Russian University of Medicine. ORCID ID: 0000-0003-1506-2565. E-mail: tronk79@gmail.com

Yulia V. Gorbunova – Candidate of Sciences (Pharmacy), Head of the laboratory, Scientific and Educational Institute of Pharmacy n.a. K.M. Lakin, Russian University of Medicine. ORCID ID: 0000-0002-1116-3425. E-mail: yvgorbunova@yandex.ru

Dmitry A. Bakulin – Candidate of Sciences (Medicine), Head of the Interdepartmental Scientific and Educational Center of Pharmacy, Scientific and Educational Institute of Pharmacy n.a. K.M. Lakin, Russian University of Medicine. ORCID ID: 0000-0003-4694-3066. E-mail: mbfdoc@gmail.com

Ivan S. Krysanov – Candidate of Sciences (Pharmacy), Associate Professor, Head of the Laboratory of Health Technology Assessment and Clinical and Economic Expertise, Scientific and Educational Institute of Pharmacy n.a. K.M. Lakin, Russian University of Medicine. ORCID ID: 0000-0002-3541-1120. E-mail: krysanov-ivan@mail.ru

Darya L. Klabukova – Candidate of Sciences (Biology), senior staff scientist of the laboratory "Evaluation of health care technologies and clinical and economic expertise", Scientific and Educational Institute of Pharmacy n.a. K.M. Lakin, Russian University of Medicine. ORCID ID: 0000-0002-5621-5700. E-mail: daria.klabukova@gmail.com

Marina A. Javakhyan – Doctor of Sciences (Pharmacy), Associate Professor, Deputy Director for Implementation and Development, Scientific and Educational Institute of Pharmacy n.a. K.M. Lakin, Russian University of Medicine. ORCID ID: 0000-0003-2673-6203. E-mail: akopovamarina13@mail.ru

Valentina I. Zvereva – Candidate of Sciences (Pharmacy), Head of the Laboratory of Development

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2024-12-2-131-149

and Implementation of Innovative Medicines, Scientific and Educational Institute of Pharmacy n.a. K.M. Lakin, Russian University of Medicine. ORCID ID: 0000-0001-5274-3736. E-mail: valentinca1988@mail.ru

Natalia A. Lycheva – Candidate of Sciences (Biology), Research Associate, Scientific and Educational Institute of Pharmacy n.a. K.M. Lakin, Russian University of Medicine. ORCID ID: 0000-0002-5842-5728. E-mail: Natalia.lycheva@yandex.ru

Victoria N. Shoich – Head of the Department of support of automation of the educational process of Russian University of Medicine. E-mail: SHOICH-VN@msmsu.ru

Veronika V. Neyolova – information technology specialist, Russian University of Medicine. ORCID ID: 0009-0005-0326-599X. E-mail: nika080601@gmail.com

Elizaveta V. Pavlova – junior researcher, Scientific and Educational Institute of Pharmacy n.a. K.M. Lakin, Russian University of Medicine. ORCID ID: 0000-0003-0651-3205. E-mail: lizapavlova1609@yandex.ru

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

Elena A. Kalashnikova – 6th year student of the Medicine Faculty of Volgograd State Medical University. ORCID ID: 0000-0002-4219-7820. E-mail: elenakalashnikova@yandex.ru

Alexander B. Bosenko – 4th year student of the Faculty of Medicine of Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University. ORCID ID: 0009-0008-8845-4053. E-mail: bosenko_a00@mail.ru

Djamilya Z. Zyazikova – 5th year student of the Faculty of Pharmacy of Volgograd State Medical University. ORCID ID: 0009-0000-4037-1234. E-mail: djamilazyazikova@yandex.ru

Ruslan E. Musaev – 5th year student of the Faculty of Dentistry of Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University. ORCID ID: 0009-0006-7200-140X. E-mail: jhonsonrobbin@yandex.ru

Abakar G. Gadzhiev – 5th year student of the Faculty of Dentistry of Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University. ORCID ID: 0009-0008-9044-7370. E-mail: gadjievabakar@yandex.ru

Boris M. Gabrielyan – 5th year student of the Faculty of Dentistry of Russian-Armenian University. ORCID ID: 0009-0000-8444-3535. E-mail: borisgabrielian@mail.ru

Anna A. Agamirova – 5th year student of the Faculty of Dentistry of Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University. ORCID ID: 0009-0001-8120-654X. E-mail: Anya.agam@mail.ru

Petrov Vladimir Ivanovich – Doctor of Sciences (Medicine), Professor, Academician of the Russian Academy of Sciences, Head of the Department of Clinical Pharmacology and Intensive Therapy, Volgograd State Medical University; Chief Outside Specialist – Clinical Pharmacologist, Ministry of Health 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

REVIEWS

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Spiramycin: The past and future of an antibiotic with pleiotropic effects in the therapy of community-acquired infections

O.I. Butranova[1], S.K. Zyryanov[1,2], A.A. Abramova[1]

[1] Peoples' Friendship University of Russia named after Patrice Lumumba, 6 Miklukho-Maklay Str., Moscow, Russia, 117198
[2] Municipal Clinical Hospital No. 24 of the Moscow City Health Department, 10 Pistsovaya Str., Moscow, Russia, 127015

E-mail: butranova-oi@rudn.ru

Received 03 May 2024 After peer review 28 Sep 2024 Accepted 06 Oct 2024

The aim of the work was to search and analyze works on pharmacokinetic (PK) and pharmacodynamic (PD) parameters of spiramycin, allowing to evaluate the potential of this macrolide in the therapy of community-acquired infections.

Materials and methods. The abstract databases of PubMed, Google Scholar, EMBASE, the ResearchGate scientific information network and elibrary.ru were used to search for the materials. The following key queries were used in the work: "pharmacokinetics of spiramycin", "pharmacokinetic parameters of spiramycin", "pharmacodynamics of spiramycin", "mechanism of action of spiramycin", "targets for spiramycin", "pharmacodynamic effects of spiramycin". The search depth – 69 years (1955–2024), the total number of publications included in the literature review in the areas of "pharmacokinetics" and "pharmacodynamics" was 72. The total number of the sources used in the article amounted is 152.

Results. With the spread of the antibiotic resistance (AR) among the pathogens of both nosocomial and community-acquired infections, it is important for physician to search for strategies to preserve the possibility of using first-line antibacterial drugs (ABDs) in patients with infectious diseases. Spiramycin has been characterized by a minimal consumption by the population in the last decades, thus, it has a potential for the therapy of infectious diseases. The analysis of the PK spiramycin parameters indicates the ability to form effective concentrations in various tissues and organs, as well as a minimal risk of drug interactions that can alter the therapeutic response. The evaluation of its antibacterial activity *in vitro* and *in vivo* yields different results, indicating the ability of the drug to exhibit significantly greater efficacy *in vivo*. This paradox may be based on pleiotropic effects of spiramycin involving both host cells (immunomodulatory and anti-inflammatory effects, the ability to favorably affect the tissue regeneration, the antitumor activity, the inhibition of adipogenesis) and pathogen targets (the ability to reduce the virulence of *P. aerugenosa*, the antiviral effect, the reduction of the adhesion ability of cocci).

Conclusion. The PK and PD parameters and the properties of spiramycin along with the results of the published clinical studies evaluating its efficacy indicate that, despite its lower *in vitro* activity, the presence of additional pleiotropic effects may be the key to its superiority over the traditional macrolides in *in vivo* methods.

Keywords: spiramycin; macrolides; community-acquired respiratory tract infections; toxoplasmosis; pleiotropic effects

Abbreviations: ABDs – antibacterial drugs; AR – antibiotic resistance; CI – confidence interval; DNA – deoxyribonucleic acid; GIT – gastrointestinal tract; IL – interleukin; CFU – colony-forming unit; IUs – international units; MIC – minimum inhibitory concentration; AR – adverse reaction; RR – relative risk; OR – odds ratio; PAE – post-antibiotic effect; PMNs polymorphonuclear leukocytes; RNA – ribonucleic acid; PD – pharmacodynamics; PK – pharmacokinetic; aP2 – activating protein 2; AUC – area under the curve; C/EBPα – CCAAT/enhancer-binding protein alpha; clogp – logarithm of the octanol-water distribution coefficient; C_{max} – maximum concentration; CYP3A4 – cytochrome P450 3A4; ERK – extracellular signal-regulated kinase; ERM – erythromycin ribosomal methylase; GLUT4 – glucose transporter type 4; I(Kr) – K+-delayed rectification current; IC_{50} – half maximal inhibitory concentration; iNOS – inducible form of nitric oxide synthase; JNK – Jun N-terminal kinases; MAPK – mitogen-activated protein kinase; NF-κB – nuclear factor kB; Pgp – P-glycoprotein; PPARγ – peroxisome proliferator-activated receptor gamma; Ro5 – Lipinski's rule of five; SASP – senescence-associated secretory phenotype; SREBP1c – sterol regulatory element-binding protein;$T_{1/2}$ – half-life period.

For citation: O.I. Butranova, S.K. Zyryanov, A.A. Abramova. Spiramycin: The past and future of an antibiotic with pleiotropic effects in the therapy of community-acquired infections. *Pharmacy & Pharmacology.* 2024;12(2): 150-171. **DOI:** 10.19163/2307-9266-2024-12-2-150-171

Для цитирования: О.И. Бутранова, С.К. Зырянов, А.А. Абрамова. Спирамицин: прошлое и будущее антибиотика с плейотропными эффектами в терапии внебольничных инфекций. *Фармация и фармакология.* 2024;12(2): 150-171. **DOI:** 10.19163/2307-9266-2024-12-2-150-171

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОБЗОРЫ

DOI: 10.19163/2307-9266-2024-12-2-150-171

Спирамицин: прошлое и будущее антибиотика с плейотропными эффектами в терапии внебольничных инфекций

О.И. Бутранова[1], С.К. Зырянов[1,2], А.А. Абрамова[1]

[1] Федеральное государственное автономное образовательное учреждение высшего образования «Российский университет дружбы народов имени Патриса Лумумбы», 117198, Россия, г. Москва, ул. Миклухо-Маклая, д. 6

[2] Государственное бюджетное учреждение города Москвы «Городская клиническая больница № 24 Департамента здравоохранения города Москвы», 127015, Россия, г. Москва, ул. Писцовая, д. 10

E-mail: butranova-oi@rudn.ru

Получена 03.05.2024 После рецензирования 28.09.2024 Принята к печати 06.10.2024

Цель. Поиск и анализ работ, посвященных фармакокинетическим (ФК) и фармакодинамическим (ФД) параметрам спирамицина, позволяющим оценить потенциал данного макролида в терапии внебольничных инфекций.

Материалы и методы. Для поиска материалов были использованы реферативные базы данных: PubMed, Google Scholar, EMBASE, научно-информационная сеть ResearchGate и elibrary.ru. В работе использовали следующие ключевые запросы: «фармакокинетика спирамицина», «фармакокинетические параметры спирамицина», «pharmacokinetics of spiramycin», «pharmacokinetic parameters of spiramycin», «фармакодинамика спирамицина», «механизм действия спирамицина», «мишени для спирамицина», «фармакодинамические эффекты спирамицина», «pharmacodynamics of spiramycin», «mechanism of action of spiramycin», «targets for spiramycin», «pharmacodynamic effects of spiramycin». Глубина поиска – 69 лет (1955–2024 гг.), общее число публикаций, включённых в литературный обзор по направлениям «фармакокинетика» и «фармакодинамика», – 72. Общее число использованных в статье источников составило 152.

Результаты. В условиях распространения феномена антибиотикорезистентности (АБР) среди возбудителей как нозокомиальных, так и внебольничных инфекций, актуальным для врача является поиск стратегий, позволяющих сохранить возможность использования антибактериальных препаратов (АБП) первой линии в ведении пациентов с инфекционными заболеваниями. Спирамицин в последние десятилетия характеризовался минимальным уровнем потребления среди населения, в связи с чем имеет потенциал для терапии инфекционных заболеваний. Анализ ФК параметров спирамицина свидетельствует о способности формировать эффективные концентрации в различных тканях и органах, а также о минимальном риске лекарственных взаимодействий, способных изменять терапевтический ответ. Оценка его антибактериальной активности *in vitro* и *in vivo* даёт различные результаты, свидетельствующие о способности препарата проявлять значительно большую эффективность в условиях живого организма. В основе этого парадокса могут лежать плейотропные эффекты спирамицина, вовлекающие как клетки организма хозяина (иммуномодулирующее и противовоспалительное действие, способность благотворно воздействовать на процессы регенерации тканей, противоопухолевая активность, угнетение адипогенеза), так и мишени возбудителей (способность снижать вирулентность *P. aerugenosa*, противовирусное действие, снижение способности кокков к адгезии).

Заключение. ФК и ФД параметры и свойства спирамицина наряду с результатами опубликованных клинических исследований, оценивавших его эффективность, указывают на то, что, несмотря на меньшую активность *in vitro*, наличие дополнительных плейотропных эффектов может быть залогом его превосходства над традиционными макролидами в методах *in vivo*.

Ключевые слова: спирамицин; макролиды; внебольничные инфекции дыхательных путей; токсоплазмоз; плейотропные эффекты

Список сокращений: АБП — антибактериальные препараты; АБР — антибиотикорезистентность; ДИ — доверительный интервал; ДНК — дезоксирибонуклеиновая кислота; ЖКТ — желудочно-кишечный тракт; ИЛ — интерлейкин; КОЕ — колониеобразующие единицы; МЕ — международные единицы; МПК — минимальная подавляющая концентрация; НР — нежелательная реакция; ОР — отношение рисков; ОШ — отношение шансов; ПАЭ — постантибиотический эффект; ПМЯЛ — полиморфоядерные лейкоциты; РНК — рибонуклеиновая кислота; ФД — фармакодинамика; ФК — фармакокинетика; aP2 — активирующий белок; AUC — площадь под фармакокинетической кривой; C/EBPα — цитозин-цитозин-аденозин-аденозин-тимидин/альфа-белок, связывающий энхансер; clogp — логарифм коэффициента распределения октанол-вода; C_{max} — максимальная концентрация; CYP3A4 — цитохром P450 3A4; ERK — внеклеточная сигнальная регулируемая киназа; ERM —эритромицин-рибосомальная метилаза; GLUT4 — глюкозный транспортёр тип 4; I(Kr) — блокада калиевого тока задержанного выпрямления; IC_{50} — среднеингибирующая концентрация; iNOS — индуцируемая форма синтазы оксида азота; JNK — Jun N-концевые киназа; MAPK — митоген-активируемая протеинкиназа; NF-κB — ядерный фактор κB; Pgp — P-гликопротеин; PPARγ — рецептор, активируемый пероксисомным пролифератором гамма; Ro5 — правило пяти Липински; SASP — секреторный фенотип, связанный со старением; SREBP1c — белок, связывающий регуляторный элемент стерола-1; $T_{1/2}$ — период полувыведения.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

INTRODUCTION

In the history of the antibacterial drugs (ABDs) use, the 21st century represents a separate milestone, indicating that mankind has not won the victory over bacterial infections. The analysis of more than 100 years of this pharmacological group use (if we take 1910, when the first ABD, salvarsan [1], was first used as a starting point, signifies the fact that the more actively ABDs are used, the more intensively antibiotic resistance (ABR) mechanisms of bacteria develop. Modern clinical practice reveals dramatic trends in the spread of multidrug-resistant bacteria among infectious agents [2]. It is important to note that the problem of AR is currently relevant not only for pathogens of nosocomial infections, but also for those that cause community-acquired infections. Thus, the world practice demonstrates that about 40% of *Streptococcus pneumoniae (S. pneumoniae)* strains, the main causative agent of community-acquired pneumonia (as well as a frequent causative agent of bacteremia, meningitis, otitis media and sinusitis), are characterized by resistance to such ABDs of choice as beta-lactams and macrolides [3]. A national assessment of S. pneumoniae resistance to macrolides in the United States revealed greater values in outpatients (45.3% vs 37.8% in hospitalized patients). At the same time, the proportion of resistant isolates from the respiratory tract was 47.3%, and the proportion of the isolated ones from the blood was significantly lower — 29.6% [4]. In Europe, the resistance of *S. pneumoniae* to erythromycin is maximum in Bulgaria (58.5%), in Asian countries this phenomenon is almost absolute: in China and Japan the level is more than 94% [5–8]. There are published studies indicating that more than 99% of *S. pneumoniae* strains isolated in China (northern regions) have simultaneous resistance to macrolides, lincosamides and streptogramine [9]. According to the study by Mohammadi Gharibani K. et al. conducted in Iran, the resistance of pneumococci to both erythromycin and azithromycin exceeds 70% [10].

In the Russian Federation, the situation with *S. pneumoniae* resistance can be illustrated by the results of the multicenter epidemiological study "PeGAS 2014–2017". It was found out that 24.3% of the strains showed the resistance to erythromycin, 28.5% to clarithromycin, and 31% to azithromycin [11].

Another pathogen of community-acquired infections, *S. pyogenes,* is characterized by a relatively low level of resistance to macrolides (from 0 to 25%) in Europe and the USA, but extremely high in China (about 75 to 100%) [12]. According to the data obtained in the Russian Federation (2013–2018, 601 strains isolated in the infections of the respiratory tract and ENT-organs in 14 cities of the Russian Federation), *S. pyogenes* resistance to erythromycin was noted in 10.5%, to clarithromycin – in almost 12%, and to azithromycin – in 16.5% [13].

Mycoplasma pneumonia, a pathogen characterized by an intracellular localization and a natural lack of sensitivity to beta-lactam ABDs, is one of the common causative agents of community-acquired respiratory tract infections. Macrolides are the drugs of choice in the management of patients with mycoplasma infections, but the possibility of their effective use is also rapidly decreasing in recent years due to the active spread of resistant strains. Thus, according to a multicenter study conducted in China (11 clinics in Beijing, 822 children with community-acquired respiratory tract infections, including 341 ones with confirmed *M. pneumoniae* infection), 87.69% of the strains were resistant to macrolides (erythromycin, azithromycin, josamycin) [14]. In European countries, the prevalence of macrolide-resistant mycoplasmas is an order of magnitude lower, which follows from the results of a systematic review and meta-analysis of 22 studies; however, most of the studies included in the analysis were published in 2011–2015, which limits the value of this meta-analysis for understanding the current picture of *M. pneumonia* AR dynamics in the European region [15].

Toxoplasma gondii is another agent of widespread community-acquired infections in the human population. It is believed that this pathogen can infect up to one third of the human population (the prevalence is highest in developing countries, where it can reach 90%) [16–18]. Among the isolated strains, the level of resistance is maximally high to pyrimethamine and sulfadiazine, and the spread of resistance genes to macrolides and lincosamides is also noted [19].

Analyzing the spectrum of macrolides typically used for the therapy of outpatient infections, it can be noted that erythromycin, clarithromycin and azithromycin are among the most used ABDs of choice both in the world and in the Russian Federation [20–22]. The increasing proportion of resistant bacterial strains in the outpatient practice triggers a cascade of events that aggravates the ABR phenomenon in general. In particular, the inability to use first-line ABDs in the therapy of community-acquired infections forces the physician to prescribe a reserve drug, which in the long term contributes to a significant limitation of antibiotic therapy tools.

Spiramycin is a representative of 16-member macrolides, the use of which in clinical practice in recent decades has been minimal. It was originally discovered in 1952 in the products of *Streptomyces ambofaciens*, and now methods have been developed to intensify its production, in particular, under the action of 0.5% methyl-β-cyclodextrin [23]. In the form for the oral administration, it began to be used since 1955, and since 1987, parenteral spiramycin has become available [24]. The structure features of the macrolactone spiramycin ring, underlie its improved pharmacodynamic characteristics. Spiramycin molecules are able to penetrate deeper into the protein exit tunnel of ribosomes, blocking the maximum number of domains of the peptidyl transferase center for the assembly of pathogen proteins. This action contributes to the

DOI: 10.19163/2307-9266-2024-12-2-150-171

reduction of virulence of prokaryotes, as well as several clinical benefits, including the improved tolerability (e.g., less gastrointestinal adverse reactions — ARs) and lower risk of drug interactions [25, 26]. The lack of a history of a widespread use of this drug may suggest its success as an alternative to other macrolides characterized by a marked increase in ABR [27].

In order to understand the place and role of spiramycin in the management of patients with various infectious diseases, it is necessary to understand both the individual characteristics of the patient, the causative agent and the localization of the infection, as well as knowledge of the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of the drug. Regarding the latter, it should be noted that PK / PD indices of ABDs determine the choice and ways to optimize the dosing regimen, as they describe the quantitative relationship between PK and PD parameters. There are three main variants of PK / PD indices describing the ABDs efficacy [28, 29]:

– the ratio of the area under the concentration-time curve (AUC) from zero to 24 h (AUC_{0-24}) to the minimum inhibitory concentration (MIC) — f_{AUC} / MIC;

– the ratio of the maximum plasma concentration (C_{max}) to MIC — fC_{max} / MIC;

– the time at which the free plasma concentration exceeds the MIC — $\%f_T$ >MIC.

Although macrolides exhibit a bacteriostatic activity, under certain circumstances they also exhibit a bactericidal activity. Accordingly, macrolides differ from other ABDs classes in the following: they do not rely on a single PK / PD index, but on both ones — $\%f_T$ >MPK and f_{AUC} / MPK [30, 31]. Significant PD factors determining the efficacy of macrolides, are also a post-antibiotic effect (PAE) [32–34] and the effects unrelated to the antibacterial action (e.g., immunomodulation and anti-inflammatory effects) [27, 35–38].

THE AIM of the work was to analyze the published works on pharmacokinetic (PK) and pharmacodynamic (PD) parameters of spiramycin, making it possible to evaluate the potential of this macrolide in the therapy of community-acquired infections.

MATERIALS AND METHODS

To write this literature review, the search for materials included the following abstract databases: PubMed, Google Scholar, EMBASE, ResearchGate scientific information network and a scientific electronic library (elibrary.ru). Each author performed an independent search for publications to exclude the errors in two areas: “pharmacokinetics of spiramycin” and ‘pharmacodynamics of spiramycin’. The keywords for the first direction of search included “pharmacokinetics of spiramycin, pharmacokinetic parameters of spiramycin”; for the second – “pharmacodynamics of spiramycin, mechanism of action of spiramycin, targets for spiramycin, pharmacodynamic effects of spiramycin”. The keywords for the second search direction were as follows: “pharmacodynamics of spiramycin”, “mechanism of action of spiramycin”, “targets for spiramycin”, “pharmacodynamic effects of spiramycin”. The depth of the search was 69 years (publications in the specified abstract databases from 1955 to 2024), a total of 5 720 publications were found out after excluding duplicates, invalid papers, publications with no free access to the full text; the total number of papers included in this literature review on PK and PD was 72. The total number of sources in the article is 152.

RESULTS AND DISCUSSION

Pharmacokinetics of spiramycin

Spiramycin consists of a 16-membered lactone ring with 2 amino sugars and 1 neutral sugar. The clinical efficacy of spiramycin is believed to be limited by the mean values of a half-life ($T_{1/2}$) and an oral bioavailability [39]. With regard to the half-life, it should be noted that it is compensated to some extent by the presence of a number of additional PD spiramycin effects, besides an antibacterial effect, discussed below (e.g., PAE, anti-inflammatory and immunomodulatory effects). The bioavailability of spiramycin, erythromycin and azithromycin is about 40% when taken orally (Table 1). The bioavailability of drugs is influenced by a membrane permeability, solubility and dissolution rate of the drug, the latter two being the main factors. Spiramycin is evaluated as a substance with a low solubility and a slow dissolution rate in water; among the methods to increase its solubility there is synthesis in a micronized form [40]. However, current evidence suggests that the dissolution behavior of spiramycin is more complex than previously thought. Historically, low molecular weight drugs have been developed with the Rule of Five (Lipinski C.A. (Ro5) [41]). According to Ro5, the oral absorption of a drug is higher if its molecular weight is ≤500 Da, the number of hydrogen bond donors ≤5, acceptors ≤10 and the calculated lipophilicity (cLogP) ≤5. There are hypotheses suggesting that the molecules of drugs beyond Ro5 have a certain flexibility that allows them to hide polar fragments when passing through the cell membrane and reveal them again when entering the aqueous medium [42]. Such substances are called molecular chameleons [43–45], they show a satisfactory solubility, an ability to overcome cellular barriers and form satisfactory absorption rates as a result. Among macrolides, roxithromycin and spiramycin are labeled as “complete molecular chameleons” [43, 45]. The “chameleon” effect is important both from the point of view of the PK analysis (an absorption and bioavailability formation) and from the point of view of PD, because it makes possible the interaction of drug molecules with targets that are difficult to reach (e.g., planar ones without distinct stereochemical configurations).

Another factor affecting the bioavailability of macrolides is the saturation of the intestinal efflux when they are administered in high doses [46, 47]. Macrolides

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

are substrates for a number of transporter proteins including, first of all, P-glycoprotein (Pgp), among them azithromycin, roxithromycin and erythromycin show quite pronounced properties of P-glycoprotein (Pgp) inhibitors. Despite a relatively low membrane permeability for macrolides in the apical-basolateral direction, they move much more actively in the reverse basolateral-apical direction. When transporter proteins (Pgp) saturate more molecules of azithromycin, roxithromycin and erythromycin with an increasing oral bioavailability [47], there may be an increased risk of toxic reactions of the above macrolides when used at high doses. Spiramycin is also a substrate of Pgp, but does not have inhibitor or inducer properties, therefore, the value of its bioavailability is stable, which provides a stable therapeutic response.

The dose of spiramycin is standardly calculated in international units (IUs), which is, due to the natural origin of the drug – 1 mg of spiramycin, corresponds to 3 000 IUs.

All macrolides, including spiramycin, have a high value of the volume of distribution. In blood, macrolides are predominantly bound to alpha-1-acid glycoprotein, most to a significant extent (Table 1), which may be accompanied by variations in the proportion of the pharmacologically active fraction due to drug interactions or changes in the concentration of plasma proteins. Alpha1-acid glycoprotein is one of the proteins of the acute phase, its concentration increases in inflammation, especially in septicemia, sepsis, complicated surgical interventions, malignant neoplasms. Severe infection is the main factor of a significant increase in the concentrations of this protein, which can lead to a decrease in a pharmacologically active fraction of those macrolides that have a significant degree of binding (see Table 1), and therefore, to a decrease in their therapeutic efficacy.

Spiramycin is characterized by a minimal binding to plasma proteins regardless of the dose taken. This distinguishes it from azithromycin, for which the binding value depends on the concentration: at a level equal to 0.02 μg/mL, it is 51%, and when increased by 100 times, it decreases to 7% [48, 49]. Considering that the pharmacologically active (unbound) fraction of spiramycin is 75%, it penetrates into organs and tissues in high concentrations, which suggests its continued effectiveness in conditions accompanied by severe disturbances in plasma protein concentration.

Due to the ability to form high concentrations in lysosomes, macrolides are characterized by an accumulation in the lung fluid and in phagocytes. In the studies on healthy volunteers it was shown that 3 h after a single oral administration of 3 g (9 000 000 IUs) of spiramycin, the concentration in serum reached 2.8 mg/L [50], after an intravenous administration of 500 mg (1 500 000 IUs) within one hour – 3.10 mg/L [51]. Comparing the maximum blood concentrations of macrolides, it can be noted that the value formed during the oral administration of spiramycin is superior to that of most macrolides (see Table 1). The studies have demonstrated that the plasma concentration achieved with spiramycin exceeds the MIC for most sensitive pathogens [52–55].

With regard to the distribution of spiramycin in other liquids and tissues, the following can be noted. The content of spiramycin in saliva is 1.3–4.8 times higher than in serum [56, 57]. Maximum high concentrations of spiramycin are formed in respiratory tract tissues: in the lung tissue – 30-45 mg/kg, in bronchial mucosa – 6.5–36 mg/kg, in bronchial secretion and sputum – from 1.5 to 7.3 mg/l, in sinus mucosa – from 8 to 13 mg/kg, in tonsil and adenoid tissues – from 15 to 29.5 mg/kg (a multiple dose regimen) [50]. It can be also noted that spiramycin is characterized by a slow release from the intracellular space [58].

An important feature of such macrolides as clarithromycin and erythromycin is a rather high potential for drug interactions. The explanation is their ability to inhibit the CYP3A4 isoenzyme [59, 60], as well as the formation of metabolites with properties of cytochrome inhibitors (anhydroerythromycin [61], 14(R)-hydroxyclarithromycin and N-desmethylclarithromycin [62]). The advantageous difference of spiramycin is its inability to undergo significant metabolic transformations and the absence of inhibitory or inducing effects on the enzymes involved in the biotransformation of xenobiotics. Due to this, the risk of drug interactions of spiramycin is minimal, which limits the factors contributing to therapeutic failures during its administration.

The excretion of all macrolides is carried out mainly with bile. Transporter proteins, including Pgp, are involved in the process. Macrolides are characterized by binding to different bile acids, which is most pronounced in the cases of azithromycin and clarithromycin, showing a maximum affinity to cholate and deoxycholate micelles [63].

From the clinical point of view, a decreased binding of macrolides to bile acids (observed in the representatives with a lower lipophilicity) leads to a decrease in their excretion rate and an increase in the half-life, respectively; the exposure time increases. On the contrary, the use of more lipophilic macrolides characterized by a better binding to bile acids is accompanied by their faster elimination from the organism [63, 64]. The parameters of lipophilicity of spiramycin allow to refer it to moderately lipophilic preparations, which are not characterized by an accelerated excretion from the organism. An additional factor in the stable excretion of spiramycin from the body is the absence of its inducing or inhibitory effect on Pgp, which favorably distinguishes it from a number of other macrolides (see Table 1) [65, 66].

DOI: 10.19163/2307-9266-2024-12-2-150-171

Pharmacodynamics

Macrolides are inhibitors of bacterial protein synthesis, interacting with 50S subunit of 70S ribosome. For the realization of the action, it is necessary to bind macrolide molecules to the regions of the peptide exit tunnel of the forming peptide from the bacterial ribosome (NPET), located near the peptidyltransferase center. The traditional hypothesis explaining the mechanism of the macrolides action is to stop the translation process by closing NPET [72]. The studies revealing the absence of a complete arrest of protein synthesis in some pathogens, the studies devoted to the structural features of macrolides, as well as the peculiarities of the bacterial genome and changes in the bacterial protein synthesis during a prolonged exposure to ABDs, allow the authors to speak about the second theory that illuminates the details of the mechanism of the macrocyclic ABDs action [73, 74]. It consists in the fact that macrolides can act as context-specific inhibitors of a peptide bond formation, and both the features of the ABDs molecule's structure and the features of the proteins synthesized by bacteria determine the boundaries and degree of the protein synthesis arrest [72, 74].

The structure of macrolide molecules largely determines the type of an antibacterial action, whether it will be bacteriostatic or bactericidal [72, 75]. Macrolides with a bacteriostatic action only and macrolides also capable of a bactericidal effect differ from each other, first of all, by such parameters as the kinetics of binding and dissociation from the ribosome, not just the affinity degree [76]. Representatives with a bactericidal action demonstrate a pronounced slowdown in the rate of dissociation from the ribosome, which is associated with the presence of an elongated side chain in their molecules. The longer the macrolide is bound to the ribosome, the longer the translation of the pathogen protein stops; as a result, the factors critical for the resumption of a gene expression may be completely depleted in the bacterial cell, and the cell dies [76]. If the macrolide molecule dissociates rapidly from the ribosome, a predominantly bacteriostatic action is manifested. Spiramycin, binding three domains of the peptidyl-transferase center on the prokaryote ribosome, provides a prolonged translation arrest, which determines its bactericidal effect [25, 26].

Comparative estimation of bactericidal effect of different macrolides (erythromycin, azithromycin, clarithromycin, dirithromycin, roxithromycin and spiramycin) against 10 strains of pneumococci with different levels of susceptibility to penicillin was carried out in the work by Fuursted K., et al. As a result, a bactericidal effect (a decrease in the number of colony-forming units (CFUs) per milliliter ≥3 log10 after 4 h of the exposure to a concentration 10 times higher than the MIC) was established for all drugs except azithromycin. A comparative analysis of the severity of this effect found out that spiramycin and clarithromycin had significantly higher bacterial killing rates after 1 h of the exposure compared with azithromycin, dirithromycin, and erythromycin. Among all macrolides, spiramycin showed the most pronounced bactericidal effect comparable to penicillin G after 4 hours of the exposure, while azithromycin showed a minimal bactericidal activity. The development rate of the bactericidal macrolides effect did not depend on the sensitivity of the pathogen to penicillin [32].

The structural spiramycin features (the presence of a disaccharide consisting of a mycaminose and a micarose in the C5 carbon atom position) ensure a pronounced binding to the peptidyl transferase center in the NPET region. From the molecular point of view, the features of the macrocyclic spiramycin ring allow it to penetrate deeper into the protein exit tunnel and form stronger bonds when interacting with the ribosome, which may indicate its increased activity against resistant bacterial strains and its ability to counteract the AR formation in general [25, 26]. This fact favorably distinguishes spiramycin from 14-membered macrolides, the use of which can trigger AR genes in relation not only to macrolides, but also to lincosamides and streptogramins (e.g., *ERM*) [26, 77]. This thesis is supported by the fact that 16-member macrolides show a clinically significant activity against erythromycin-resistant *Staphylococcus aureus* strains [26].

Spiramycin is characterized by both bacteriostatic and bactericidal actions. The latter is characteristic when ABDs are used in a dosage form containing 3 000 000 IUs in one tablet. This form is represented by the preparation "Doramytsin VM".

Analyzing the antibacterial activity of spiramycin, it is necessary to dwell on its ability to produce a PAE. Having a high affinity for tissues, spiramycin is slowly released from cells and provides both a direct antibacterial action and an indirect one, through the realization of PAE [78, 79]. In the studies of *Staphylococcus aureus* strains, the spiramycin PAE was found to be more pronounced and prolonged compared to erythromycin [78]. The PAE spiramycin value against mutant *E. coli* strains (ΔacrAB strains with the presence of an efflux pump) was found to be 1.7±0.26 h, which was higher than that obtained for erythromycin (0.2±0.1 h) and azithromycin (1.3±0.28) [80]. With respect to *Streptococcus pneumonia,* the mean duration of PAE was significantly higher for macrolides. The maximum value was found for spiramycin (3.88 h), smaller values were found for clarithromycin (3.60 h), erythromycin (3.50 h), roxithromycin (3.13 h) and azithromycin (2.83 h) [32].

The spectrum of the antimicrobial macrolides activity includes predominantly Gram-positive pathogens and only some Gram-negative ones. Spiramycin has demonstrated a high activity against *Staphylococcus*

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

aureus [30, 71], coagulase-negative staphylococci [30], β-hemolytic streptococci [30], pneumococci [80, 81], *Haemophilus influenzae* [30, 71], some pathogenic *Neisseria* species [30, 71, 82], *Bordetella* [30, 82], *Corynebacterium* [30], *E. Coli* [83], *Rickettsia* [30], *Legionella* [30, 71], *Chlamydia* [30, 82, 84], *Mycoplasma* [30, 71, 82, 84], *Toxoplasma (Toxoplasma gondii)* [82, 84, 85], some *Cryptosporidium* species [84] and *Moraxella* [71].

Among Gram-negative microorganisms sensitive to spiramycin, the main ones are intracellular pathogens. All the more interesting are the results of the studies that investigated the spiramycin effect on the pathogens included in the group of the microorganisms especially dangerous for humans, ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp.*). Calcagnile M. et al. (2022) demonstrated the results of a spiramycin action on the blue bacillus, *Pseudomonas aeruginosa,* which has a natural resistance to this ABDs as well as to any other macrolides. The authors found a decrease in the virulent properties of this pathogen, in particular, a decrease in the formation of pyocyanin, pyoverdine and rhamnolipids [86]. The studies in this direction were further developed by the authors; in their later work, they confirmed the ability of spiramycin to suppress the expression of virulence determinants in *P. aeruginosa*: in its presence, the production of pyoverdine and pyocyanin was significantly reduced, the concentrations causing the reduction were 15.6 and 7.8 μg/mL, respectively. Spiramycin also found important effects concerning the inhibition of a biofilm formation (almost twofold at spiramycin concentrations of 30 μg/mL or more), the reduction of a bacterial swarming motility and a rhamnolipid production. The treatment of *P. aeruginosa* with spiramycin (at concentrations ranging from 60 to 180 μg/ml) resulted in the sensitization of the bacterial cell to the damaging effect of hydrogen peroxide. The results were obtained both *in vitro* and *in vivo*; the latter revealed a decrease in the mortality rate by approximately 50% when spiramycin was administered against the background of the *P. aeruginosa* infection (the model with *Galleria mellonella*), as well as the presence of an immunomodulatory activity of this macrolide [87].

When analyzing the spiramycin PD, it is necessary to recall such a concept as the “spiramycin paradox” used for the first time in 1988 [88]. The paradox consisted in the fact that the antibacterial effect of this ABD was inferior to that of erythromycin *in vitro*, but superior in *in vivo* studies. The explanation is the complex mechanism of the spiramycin action, which includes both a direct antibacterial action on bacterial cells (and, as it was found, on some other pathogens) and the effect on host cells, the study of which allowed to reveal pleiotropic effects of the macrolide in question.

Additional pharmacodynamic effects of spiramycin

Antiviral activity

Macrolides are a group of drugs that have discovered the ability not only to antibacterial, but also to antiviral actions [89]. This feature is based on the “chameleon” effect of the macrolides discussed above. It is macrocyclic chameleon compounds that exhibit a unique ability to interact with hard-to-reach targets, which are characteristic of viruses [43–45].

Among macrolides, the antiviral activity has been reported in a number of representatives, including spiramycin. The early studies revealed the effect of clarithromycin against H1N1 influenza virus, but a comparative study of different macrolides (josamycin, spiramycin, erythromycin, clarithromycin) conducted in 2014 demonstrated that it had the lowest activity measured *in vivo* and assessed by the survival rate of laboratory animals and their weight dynamics after the infection with a lethal dose of H1N1. The 16-member macrolides josamycin and spiramycin were recognized as the most effective [90].

The use of macrolides has shown definite prospects in the treatment of enteroviral vesicular stomatitis (the causative agents are enterovirus A71 (EV-A71) and coxsackie virus A16 (CV-A16)). Zeng S. et. al. (2019) showed the comparative study results of the effects of 8 macrolides (erythromycin, clarithromycin, dirithromycin, roxithromycin, azithromycin, midecamycin, jozamycin and spiramycin) on EV-A71 and CV-A16 *in vitro*, finding the presence of anti-enteroviral activity in spiramycin and azithromycin. Spiramycin most significantly reduced EV-A71 RNA and protein levels by likely disrupting a viral RNA replication. The average inhibitory concentration (IC_{50}) of spiramycin against EV-A71 and CV-A16 was 15 and 75 μM, respectively. The values for azithromycin were 26 and 50 μM, respectively. The authors noted that the inhibition of the viral replication by spiramycin and azithromycin was not accompanied by cytotoxic reactions, and both drugs showed an anti-enteroviral activity, more pronounced against EV-A71 and less for CV-A16. With respect to EV-A71, spiramycin proved to be a stronger replication inhibitor than azithromycin [91].

Effect on human cells and tissues

The ability of spiramycin to have not only an antibacterial effect, but also to affect the host cells, causing a comprehensive improvement in their function, has been shown in a number of studies involving patients with infections of various etiologies. In particular, this applies to such a typical indication for spiramycin as toxoplasmosis, where this ABD contributes to the improvement of tissues of the internal organs. The

DOI: 10.19163/2307-9266-2024-12-2-150-171

phenomenon may be based on the improvement of tissue regeneration rates in the presence of spiramycin, which has been shown for liver, spleen and brain cells [92]. Regular spiramycin passes through the blood-brain barrier poorly, but its modified form based on chitosan nanoparticles provides an effective crossing of histohematic barriers with the formation of sufficient concentrations in the brain tissue [93]. The nanoparticles loaded with spiramycin have shown a maximum efficacy in the treatment of acute toxoplasmosis, including those with a central nervous system damage [94]. Clinical data indicate a marked reduction in the number of brain cysts (by 88.7%) and an improvement of the brain tissue in patients with chronic toxoplasmosis [95]. A complex improvement of the organs and tissues state was also observed with the use of a combination of probiotics *(Lactobacilli acidophilus)* and spiramycin in the patients with toxoplasmosis against the background of diabetes mellitus [96].

Potential of spiramycin in the therapy of obesity

The ability to inhibit adipogenesis and reduce the severity of obesity caused by a high-fat diet, has been demonstrated for spiramycin. A spiramycin-induced suppression of a preadipocyte differentiation against the background of the attenuation of an intracellular lipid accumulation underlies a weight loss. Spiramycin has been shown to inhibit the expression of key adipocyte regulators (PPARγ, C/EBPα and SREBP1c) and their target genes (FAS, aP2 and GLUT4), as well as to activate the phosphorylation of an adenosine monophosphate-activated protein kinase, AMPK, in 3T3-L1 cells during their early differentiation. In *in vivo* models, spiramycin led to a significant weight loss by reducing the adipose tissue mass and also contributed to minimizing a lipid accumulation in hepatocytes. In the liver, a decrease in the severity of steatohepatosis was found against the background of a spiramycin administration [97]. The ability of spiramycin to counteract adipogenesis distinguishes it from other macrolides, for which (primarily azithromycin), on the contrary, the studies have revealed a certain relationship with the development of obesity. It was found out that both in the USA and in Europe, macrolide uses at the population level had a positive association with a subsequent childhood obesity that persisted regardless of the period between the ABDs use and the formation of overweight [98]. Another large-scale study analyzed the relationship between the consumption of the most common macrolides and the development of obesity in adults, revealing the ability of this group of ABDs to induce processes leading to the weight gain [99].

Anti-inflammatory and immunomodulatory effects of spiramycin

Macrolides have quite pronounced immunomodulatory properties that have the potential to correct an immune dysregulation even in critical patients, but they do not inhibit endogenous antimicrobial defense mechanisms [100]. The anti-inflammatory potential of macrolides has been demonstrated in modeling septic processes and tissue damages of various localizations caused by the infectious process [101].

The anti-inflammatory and immunomodulatory actions of spiramycin are based on their effect on several cell types, primarily macrophages. The results of a study evaluating the anti-inflammatory potential of a topical application of spiramycin in humans (an application to the skin) have been published. They found the ability of this ABD to reduce the secretion of interleukins, ILs (IL-6 and IL-1β) by macrophages, reduce the NO synthesis by affecting the expression of the inducible form of nitric oxide synthase (iNOS) by inhibiting the nuclear factor κB (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways [102]. In general, an effective attenuation of the macrophage activation in the presence of spiramycin and its significant potential for use as a topical anti-inflammatory agent have been observed [102].

Another study of the topical spiramycin effect (applied to the nasal mucosa) showed its actions on fibroblasts. The structure analysis of the cells exposed to the ABDs revealed the absence of damage to the cytoskeleton and nuclei, and a preservation of the spindle shape. Spiramycin increased a viability of fibroblasts and had no damaging effect on them at a short-term application for 24 and 48 h, but decreased a viability at doses of 50 and 100 μM at a long-term application for 72 h. As a result, the authors recommended using spiramycin in the form for a topical application during septorhinoplasty procedures [57].

It was shown that macrolides can change the differentiation and maturation of mononuclear phagocytes, endothelial and epithelial cells and fibroblasts [103]. Thus, the inhibition of the activation of inflammatory phenotype M1 macrophages and the enhancement of the formation of phenotype M2 macrophages, contributing to the arrest of inflammatory processes and a subsequent healing, were found out [104].

Macrolides also act as inhibitors of the adhesion molecules expression on neutrophils and endothelial cells. This effect leads to the inability of leukocytes to adhere to the endothelium at the very beginning of the diapedesis process (the transition of cells from blood to tissues through the vascular wall, which is typical for

ISSN 2307-9266 e-ISSN 2413-2241
Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

an inflammation) [105]. Macrolides have demonstrated the ability to induce phagocytosis and enhance the formation of certain lines of macrophages, and both in relation to macrophages and neutrophils ABDs of this group, have demonstrated the ability to inhibit the so-called "oxidative burst" [100].

The results of the spiramycin treatment of *Staphylococcus aureus* and various species of streptococci *(S. pyogenes, S. mutans, S. sanguis,* and *S. faecalis)* with a subsequent evaluation of the degree of their adhesion to buccal cells and the efficiency of phagocytosis by polymorphonuclear leukocytes are of interest. A pretreatment of cocci with a serial twofold spiramycin dilutions (1/2 to 1/1024 MPC) resulted in an increase in the diameter of bacterial cells and a decrease in their adhesion to buccal cells. The exposure of streptococci to spiramycin led to an increase in the phagocytic ability of polymorphonuclear leukocytes, and the effect was observed both when using the therapeutic concentration (2 mg/L) and when using 1/4 MIC [53].

Another aspect of a macrolides action is their effect on the mucus secretion in the respiratory tract. The basis is the inhibition of a cytokine induction of a mucin 5AC (MUC5AC) gene expression [106, 107].

In addition to their anti-inflammatory and immunomodulatory actions, macrolides have important effects such as slowing down the aging process. Aging cells can produce and secrete pro-inflammatory cytokines, they are called "senescence-associated secretory phenotype (SASP)". SASP is a major factor in the chronic inflammation and tissue damage [108] and has a close relationship with inflammasomes.

There have been published works demonstrating the senolytic activity (an ability to inhibit aging processes in the body) of a number of macrolides manifested in the form of death of lung fibroblasts damaged by aging processes, against the background of the effect absence on healthy normal fibroblasts [109]. The specific effect of spiramycin on a number of proinflammatory cytokines and cell signaling pathways allowed the authors to identify it as a drug with a potential of the antitumor action. This potential was found, in particular, in experiments with a MCF-7 cell line, representing a human breast cancer cell line with estrogen, progesterone and glucocorticoid receptors [110]. The mechanism underlying the beneficial effects included the ability to bind topoisomerase II and inhibit a complex formation between this enzyme and DNA. Antiproliferative properties of spiramycin were manifested against the background of its rather low concentration (IC_{50}=0.67±0.43 μM), very close to the concentration of the standard antitumor drug from the ABDs group, doxorubicin. A comparison of the antitumor potential of spiramycin, roxithromycin, clarithromycin and azithromycin performed on the MCF-7 cell line revealed the unambiguous superiority of spiramycin [110].

The combined PD effects of spiramycin, complementary to its antibacterial action, are summarized in Figure 1.

Clinical efficacy of spiramycin

A high intracellular concentration of spiramycin, formed in cells of various organs and tissues, along with its antimicrobial spectrum, underlie the possibility of a fairly wide use of this ABD in the therapy of community-acquired infections.

One of the most topical issues of a spiramycin application is its use in patients with community-acquired bacterial infections of the upper and lower respiratory tract. Clinical studies indicate the effectiveness of this macrolide in patients with pharyngitis and tonsillitis, which is due, in particular, to its high concentrations in tonsil and pharyngeal tissues, and a high content in saliva [56]. An efficacy comparison of spiramycin (3 000 000 IU (1 g) 2 twice a day, *n*=49) and amoxicillin (500 mg capsules 3 times a day, *n*=50) in the therapy of patients with acute community-acquired upper respiratory tract infections revealed comparable results (success of antibiotic therapy in 89% in the spiramycin group and 83.3% in the amoxicillin group) [111].

In patients with lower respiratory tract infections, the efficacy of spiramycin is demonstrated by the results of an open multicenter study (Brazil and Colombia, *n*=125, patients with a radiologically confirmed pneumonia, acute bronchitis or exacerbation of chronic bronchitis). Clarithromycin was administered at a dose of 500 mg every 12 h and spiramycin at a dose of 3 000 000 IUs (1 g) every 12 h in the courses of 5–10 days. The clinical efficacy scores of the drugs did not show significant differences, nor did the safety scores (*p*=0.768 and 0.236, respectively) [112]. Another open randomized multicenter study (*n*=55, patients with pneumonia and/or bronchitis) demonstrated a similar comparable efficacy of spiramycin and clarithromycin. the antibiotic therapy success was observed in 96.15% of patients in the spiramycin group and 96.43% in the clarithromycin group. Side effects were slightly less frequent in the spiramycin group (7.69 vs. 10.71% for clarithromycin) [113].

The use of spiramycin (1 500 000 IUs 3 times a day) in 30 patients (adults aged 16–65 years) with community-acquired pneumonia revealed a high clinical efficacy – approximately half of the patients showed positive clinical dynamics on the 3rd day after starting ABDs. At the end of the course of the antibiotic therapy the clinical efficacy was 90.0%. ARs were observed in 20.0% [114]. A 100% efficacy of spiramycin in patients

DOI: 10.19163/2307-9266-2024-12-2-150-171

with pneumonia was demonstrated in another domestic study (*n*=30, a moderately severe course, a mean age 35±12 years) [115].

A high efficacy of spiramycin was also found in the outpatient practice (dose 3 000 000 IUs, the duration of treatment – up to 14 days, *n*=21). The treatment efficacy was 95.2%, and no side effects were registered [116]. A comparison of the efficacy of oral spiramycin and ampicillin in the management of children with infectious-inflammatory diseases of the respiratory tract, tonsils and middle ear revealed a number of advantages of macrolide. The study found pronounced positive dynamics: a rapid reduction of fever, a sore throat on swallowing and some symptoms of intoxication [117].

The above studies were predominantly published in the twentieth century, attracting an increased interest in the contemporary data that could illustrate the activity of spiramycin against the strains of streptococci circulating today. In 2024, the Interregional Association for Clinical Microbiology and Antimicrobial Chemotherapy (Russia) performed an *in vitro* activity testing of the spiramycin substance from the manufacturer World Medicine, Turkey (350 strains of *S. pneumonia* and *S. pyogenes* from patients with community-acquired respiratory tract infections from sixteen cities in the Russian Federation). The results demonstrated the sensitivity of 70.5% of *S. pneumonia* and 94.7% of *S. pyogenes* strains to spiramycin. Among the streptococci resistant to 14- and/or 15-member macrolides (erythromycin, clarithromycin, roxithromycin, azithromycin), 25–36% of *S. pneumonia* strains and 69.6-97.3% of *S. pyogenes* isolates showed the sensitivity to spiramycin[1].

The oral cavity is a close anatomical region in relation to the upper respiratory tract. A high content of spiramycin in saliva, gums and bones makes it a promising drug for use in dental practice, which has been confirmed by a number of studies [118–120].

Helicobacter pylori, a major etiologic factor in the peptic ulcer disease, is a pathogen with rapidly increasing ARs. The macrolide traditionally used in *H. pylori* eradication is clarithromycin, and the resistance growth of the pathogen against this ABD is dramatic [121–123]. Spiramycin has no history of use as a traditional component of eradication regimens despite the fact that several papers were published in the 1990s indicating its efficacy in *H. pylori* eradication comparable to tetracycline [124], oxytetracycline [125], and amoxicillin [126]. A modern evaluation of the efficacy and safety of spiramycin when used as a part of the triple therapy of the first-line *H. pylori* eradication was performed in the study including 122 patients with a confirmed *H. pylori* infection and no history of the eradication therapy (70 people — a study group, received pantoprazole 40 mg twice a day, spiramycin 1 500 000 IUs and metronidazole 250 mg – 3 times a day for 10 days; 52 people – a control group, received pantoprazole, clarithromycin and amoxicillin for 14 days). A month after the therapy completion, the status of *H. pylori* was evaluated. In the study group, the eradication was noted in 74.3%, in the control group – in 86.58%. No significant differences between the groups were found (*p*=0.097). Side effects were observed in 54.5% in the study group and in 45.5% in the control one (nausea, abdominal pain and diarrhea), there were no statistically significant differences between the groups (*p*=0.266). The authors concluded that the comparable efficacy of macrolides against the background of a satisfactory safety profile suggests the potential of spiramycin in the eradication of *H. pylori* in conditions of the increasing resistance to clarithromycin [127].

A high activity of spiramycin against intracellular pathogens includes chlamydia. The risk of chlamydiosis in the world and in the Russian Federation population shows increasing trends [128]. The published studies indicate the effectiveness of spiramycin in respiratory tract infections caused by *Chlamydia pneumonia* [129], genital infections caused by *C. trachomatis* [130]. An interesting publication describes the use of spiramycin in a 30-year-old patient with acute pleuro-myocarditis developed against the background of the community-acquired pneumonia caused by *C. psittaci* (as a result of a professional contact with birds). The patient received intravenously 1 g of amoxicillin and 1 500 000 IUs of spiramycin 3 times a day in combination with 0.5 mg of colchicine 4 times a day and 1 g of acetylsalicylic acid 3 times a day. Positive dynamics was noted starting from the third day of the ABDs administration (the absence of fever and chest pain), laboratory tests revealed a normalization of blood leukocyte and lymphocyte counts, as well as a reduction of C-reactive protein to 23 mg/L on the fifth day. Amoxicillin was discontinued, the patient continued taking spiramycin for 14 days, no side effects were reported, a further follow-up for 3months revealed normal ECG values, and no cardiac abnormalities were detected for 2 years [131].

For the diseases listed above, the use of spiramycin is not currently widespread and most published studies date back to the twentieth century. The situation is different for an infectious disease such as toxoplasmosis. Spiramycin is the main drug of choice for the treatment of toxoplasmosis in various categories of patients [132–135], including pregnant women [136, 137]. It has no teratogenic potential and is safe for use in the first trimester of pregnancy, which distinguishes it from pyrimethamine and sulfadiazine, which should not be used until the second trimester of pregnancy because of significant adverse effects on the fetus [134].

[1] The in vitro activity of macrolides against S. pneumoniae and S. pyogenes was studied // Pharmaceutical Bulletin. Available from: https://pharmvestnik.ru/ content/news/Izuchena-in-vitro-aktivnost-makrolidov-v-otnoshenii-S-pneumoniae-i-S-pyogenes.html. Russian

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Table 1 – Comparative pharmacokinetic parameters of macrolides

Macrolide	Bioavailability, %	C_{max}, mg/L proteins	Volume of distribution Binding to plasma proteins	Binding to plasma proteins	$T_{1/2}$	Clearance	Metabolism	Excretion	Effect on transporter proteins	Source
Erythromycin	18–45%	1,5±0,6	2,34±1,76 l/kg	80–90%	2,4–3,1 h	Healthy individuals: In the presence of cirrhosis: Increased 3–fold or more	Substrate CYP3A4 inhibitor	95% in bile and 5% in urine	Pgp inhibitor	[59–61, 65, 66]
Roxithromycin	50–60%	2,3±0,39	0,43–0,44 l/kg	96%	12 h	Reduced in the elderly	Virtually unmetabolized Weak CYP3A4 inhibitor	53% by feces; 10% in urine	Pgp inhibitor	[67,68]
Clarithromycin	50%	2,1±0,7	3–4 l/kg	70%	3–4 h	29,2–58,1 l/h	Substrate for CYP3A4 CYP3A4 inhibitor	70–80% in bile; 20–30% in urine	Weak Pgp inhibitor	[59, 60, 63, 65]
Azithromycin	37%	0,43±0,2	31,1 l/kg	51% – at a concentration of 0.02 µg/mL in plasma; 7% – at a concentration of 2 µg/mL in plasma	68 h	Healthy individuals: 630 ml/min.	Minimal metabolism No effect on cytochromes	94% – in bile; 6% – in urine	No significant effect on Pgp	[47–49, 63, 65, 66, 69]
Josamycin	51%	1.64+0.67 mg/mL (intravenously); 0.05 to 0.71 mg/mL (orally)	300 L	15%	1–2 h	4,71 l/kg/h	Substrate for a number of cytochromes in liver. Probable inhibitor of a number of CYP450 isoenzymes.	75–80% – in bile; 15–20% – in urine	No significant effect on Pgp	[68, 70]
Spiramycin	30–39%	2,8 mg/mL (orally) 3,1 mg/mL (intravenously);	300 L (about 5 l/kg)	10–25%	On average: 6,2–7,7 h Intravenous: young adults (18 to 32 years of age): 4.5 to 6.2 hours; the elderly (73 to 85 years of age): 9.8 to 13.5 hours. Orally: 5.5 to 8.0 hours	1.42 l/min (renal clearance – 144 ml/min, extrarenal clearance – 887 ml/min	Minimal metabolism. No effect on cytochromes	More than 80% – in bile; up to 14% in urine	No significant effect on Pgp	[51, 71]

Note: C_{max} — maximum concentration; $T_{1/2}$ — half-life time period.

DOI: 10.19163/2307-9266-2024-12-2-150-171

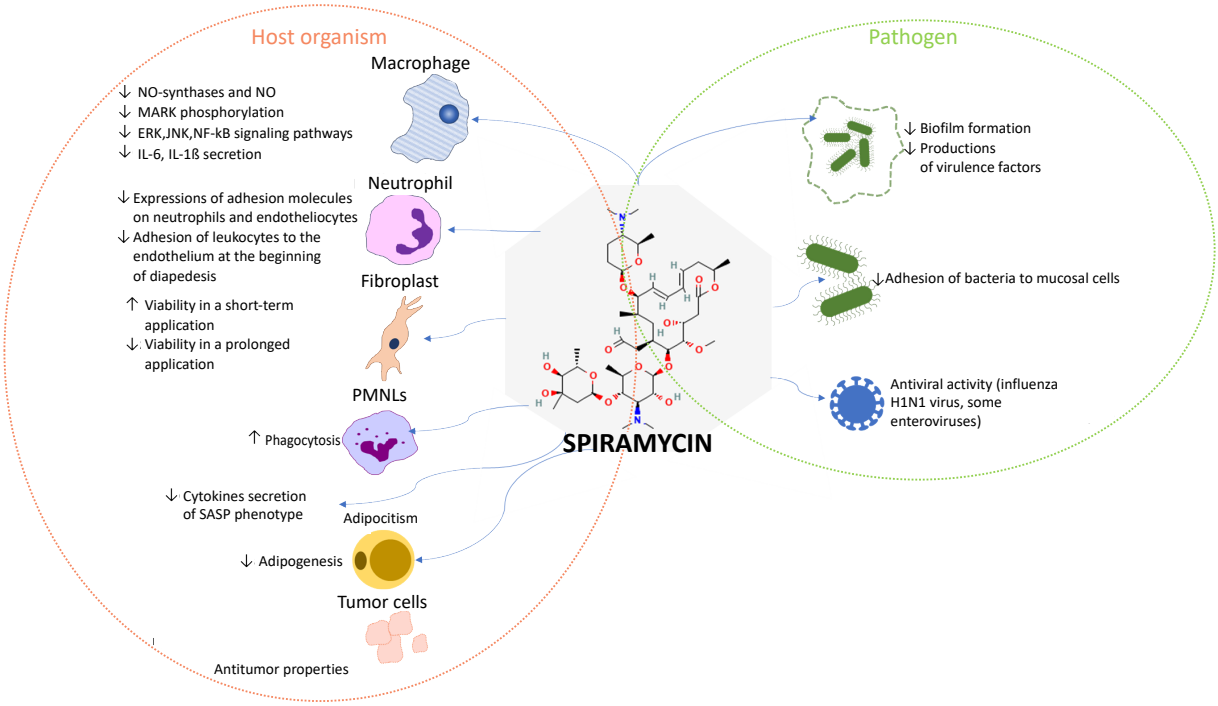


Figure 1 – Pharmacodynamic effects underlying the pleiotropic action of spiramycin
Note: MARK – mitogen-activated protein kinase; ERK – extracellular signal-regulated kinase; JNK – Jun N-terminal kinase; NF-κB – nuclear factor κB; IL – interleukin; PMNL – polymorphonuclear leukocytes.

According to a 2015 year meta-analysis, spiramycin was recognized as a drug with a high efficacy against the infections caused by *T. gondii.* The pooled negative conversion rate values (a positive test before the treatment, a negative test after it) for spiramycin, azithromycin and trimethoprim-sulfamethoxazole were 83.4% (95% confidence interval (CI): 72.1 to 90.8%); 82.5% (95% CI: 75.9 to 87.6%) and 85.5% (95% CI: 71.3 to 93.3%). The analysis of the incidence of a vertical mother-to-child transmission after a course of spiramycin in pregnant women with a primary infection included 11 publications (*n*=3596) and found a minimum value of 9.9% (95% CI: 5.9 to 16.2%). The evaluation of a spiramycin efficacy in toxoplasmosis encephalitis was based on the data from 14 publications (*n*=727), with a pooled cure rate of 49.4% (95% CI: 37.9 to 60.9%) [138].

In 2021, another meta-analysis was published to evaluate the efficacy of spiramycin in pregnant women followed by pyrimethamine-sulfonamide-folic acid or without it, compared with the absence of treatment. The evaluation was based on the incidence of a mother-to-child transmission of *T. gondii* and the incidence / severity of the sequelae in children. The meta-analysis pooled 33 studies (32 cohort and 1 cross-sectional study, number of mothers was *n*=15 406, newborns was *n*=15250). The incidence of the vertical transmission was significantly lower in the patients receiving the spiramycin monotherapy, 17.6% (95% CI: 9.9 to 26.8%) compared with the group of the therapy absence, 50.7% (95% CI: 31.2 to 70%; p <0.001), indicating an unequivocal efficacy of spiramycin [139]. A high efficacy in curing pregnant patients from toxoplasmosis and preventing a vertical transmission was found for the combination of spiramycin with co-trimoxazole (the data from a retrospective study including 120 pregnant women and 123 newborns, the period from 1992 to 2011) [140].

The data analysis of 685 pregnant patients with toxoplasmosis treated with spiramycin in Germany (spiramycin is the standard of care in the country's clinical guidelines) confirmed the efficacy of its use (from the diagnosis time until the 16th week of pregnancy, followed by pyrimethamine, sulfadiazine, and folinic acid for at least 4 weeks) in combination with a standardized follow-up program to reducea transplacental transmission and the disease burden in the newborn [141].

According to the Russian draft clinical guidelines on diagnosis, treatment and prevention of congenital toxoplasmosis, macrolides belong to the second-line etiotropic therapy of newborns, among which spiramycin is the drug of choice (the first line – pyrimethamine+sulfadimezine for 4–6 weeks).[2]

[2] Clinical recommendations [project] for the diagnosis, treatment and prevention of congenital toxoplasmosis. Available from: https://www.raspm.ru/files/toksoplazmoz.pdf. Russian

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

The dosing regimen of spiramycin in pregnant women with toxoplasmosis according to the clinical guidelines 2023, Germany, is as follows [135]: Up to 14+6 weeks of gestation – spiramycin (3.0 g or 9 000 000 IU/day); from 15+0 weeks of gestation – for at least 4 weeks the combination of pyrimethamine (50 mg on day 1 and 25 mg from day 2)+sulfadiazine (50 mg/kg/day; 3 g/day for a body weight up to 80 kg; 4 g/day for a body weight of 80 kg or more)+folinic acid (10–15 mg/day, a folic acid intake should be discontinued).

If sulfadiazine intolerance/inability: 15+0 weeks gestation: Spiramycin (3.0 g or 9 IUs/day)+co-trimoxazole (2×960 mg/day)+folinic acid (10–15 mg/day, a folic acid intake should be discontinued) or co-trimoxazole (2×960 mg/day)+folinic acid (10–15 mg/day, folic acid should be discontinued) or pyrimethamine (50 mg/day)+ clindamycin (3×600 mg/day)+folinic acid (10 to 15 mg/day, folic acid should be discontinued).

Summarizing the reviewed results of a spiramycin clinical use, it can be noted that its use at a dose of 3 000 000 IUs twice/day provides an effect superior or comparable to that of other 14- or 15-member macrolides and aminopenicillins. Comparing the dosing regimen of spiramycin given in the studies with that of the second 16-member macrolide, josamycin (1–2 g/day, divided into 2 or 3 doses), a similar result based on the data of PK and PD characteristics analysis of the drugs, can be assumed.

Side effects

Macrolides have a very high level of safety and are generally well tolerated by patients of various ages. Nevertheless, a real clinical practice has revealed a number of ARs characteristic of this group, the spectrum of which depends on the specific macrolide. A Cochrane review including 183 studies and nearly a quarter of a million patients treated with macrolides (*n*=252 886) [142] found the highest risk gastrointestinal tract (GIT) ARs (odds ratio, OR=2.16 [95% CI: 1.56 to 3.00]). Among ARs on the GI side, an abdominal pain (OR=1.66 [95% CI: 1.22 to 2.26]), diarrhea (OR=1.70 [95% CI: 1.34 to 2.16]), and nausea (OR=1.61 [95% CI: 1.37 to 1.90]) were leading. In most cases, mac rolide-induced diarrhea is not a consequence of impaired gut microflora, but is associated with an activation of motilin receptors. It was found that 14-membered macrolides erythromycin and oleandomycin have a pronounced affinity for them, comparable, in fact, with motilin itself, and exhibit the properties of their agonists, which leads to a pronounced stimulatory effect on the intestinal motility [143]. This distinguishes them from 16-membered macrolides, which do not cause such a reaction due to their structural features, which do not allow them to bind to this type of receptors. Thus, a connection between the structure and function of macrolide ABDs and their GIT side effects can be noted [64, 144].

Taste disturbances are another typical side effect of macrolides, compared to placebo, with an OR of 4.95 (95% CI: 1.64 to 14.93) [142]. The data are based on the results of the most common representatives of the group (azithromycin, erythromycin, clarithromycin, roxithromycin).

Hearing impairment is a rare AR occurring in some patients taking macrolides. According to the Cochrane review, hearing loss was slightly more common in patients taking the most common ABDs in this group (OR=1.30; 95% CI: 1.00 to 1.70) compared to placebo [142]. The association between macrolides and hearing impairment is also supported by the results of a systematic review and meta-analysis from 2024 (13 studies included, 1 142 021 patients, 267 546 of whom received macrolides, 875 089 were controls), which demonstrated a pooled OR of 1.25 (95% CI: 1.07 to 1.47) [145]. A similar OR of hearing impairment, 1.25 (95% CI: 1.07 to 1.46), was also found in the results of the analysis carried out as a part of the population-based Rotterdam Study (started in Rotterdam, the Netherlands, in 1989, a cross-sectional analysis – 4 286 patients, a longitudinal analysis – 636) [146].

Negative effects of macrolides on the heart have been a matter of debate for a long time. The Cochrane review found no evidence of a significant increase in the risk of cardiac pathology associated with macrolides (OR=0.87; 95% CI: 0.54 to 1.40) compared to placebo [142]. A meta-analysis of 80 studies involving almost 40 million patients demonstrates different results. Compared to the group of patients who had not taken macrolides, those who had used them had a significant risk of ventricular arrhythmia or a sudden cardiac death: for azithromycin the hazard ratio, OR, was – 1.53 (95% CI: 1.19 to 1.97), for clarithromycin – 1.52 (95% CI: 1.07 to 2.16). The authors [147] also found an association between an azithromycin intake and a higher risk of a cardiovascular death (OR=1.63; 95% CI: 1.17 to 2.27) and an increased risk of myocardial infarction (OR=1.08; 95% CI: 1.02 to 1.15).

It is assumed that the basis of macrolides cardiotoxicity is the ability of some of them to prolong the QT interval and cause ventricular tachycardia of a pirouette type (Torsades de pointes, TdP). This fact is associated with the third phase prolongation of the action potential, a disruption of depolarization and repolarization processes. In turn, these processes are a consequence of blocking special potassium channels

DOI: 10.19163/2307-9266-2024-12-2-150-171

in the membrane of cardiomyocytes (blockade of a potassium current of a delayed rectification – I(Kg)) [148]. It is known that the severity of I(Kr) blockade under the action of macrolides is presented as follows: clarithromycin≈roxythromycin>erythromycin [147, 149]. The level of evidence regarding the risk of TdP for clarithromycin and erythromycin is B, for roxithromycin it is C [148]. Similar mechanisms of cardiotoxicity have also fluoroquinolones, the ABDs group, the representatives of which are often used as an alternative to macrolides.

Azithromycin has long been considered one of the safest macrolides, but a population-based analysis found a significant association between its use and an increased risk of death from a cardiovascular disease [147]. An increase not only in the risk of myocardial infarction, but also in ventricular arrhythmias and a sudden cardiac death was noted. The cardiotoxicity of azithromycin is based on slightly different mechanisms that distinguish it from clarithromycin, roxithromycin, erythromycin and fluoroquinolones. High concentrations of this macrolide have been found to cause an increased heart rate and acute shortening of the QT interval. At the subcellular level, a change in the activity of lysosomes was observed; it was accompanied by an excessive formation of autophagosomes, leading to the formation of vacuoles, damage to sarcomeres, and cardiomyocyte death [150].

In the studies reviewed above that evaluated the profile of the ARs characteristic of macrolide ABDs, the most common drugs were azithromycin, clarithromycin, roxithromycin, and erythromycin. There are no meta-analyses on spiramycin, which is due to its rare use in modern practice. Nevertheless, in the light of a potential influence on the cardiovascular system, the results of a spiramycin long-term use in a patient with acute myocarditis, indicating its safety and absence of cardiotoxic properties, are interesting [131]. The data on the wide use of spiramycin in pregnant women, which revealed no cases of cardiac disorders in newborns, are also indicative [151]. In general, the evidence analysis of a spiramycin safety use in humans indicates its favorable profile, the absence of influence on the GI motility, hepatotoxicity and cardiotoxicity [152].

CONCLUSION

Macrolide ABDs have lost their leading position in recent years and are now alternative drugs in the treatment of community-acquired infections. It is interesting to note that for this group, an *in vitro* efficacy is not always identical to an *in vivo* efficacy. A vivid illustration of this thesis is spiramycin; its MIC against many pathogens is quite high, but, nevertheless, due to additional pleiotropic effects, the results of its clinical use may exceed those of many traditional macrolides. These effects include, first of all, anti-inflammatory and immunomodulatory ones, which allow spiramycin to effectively eliminate the symptoms of infectious diseases against the background of the improvement of tissues condition of various organs damaged as a result of the disease. The properties of spiramycin considered in the present literature review explain its greater effectiveness in *in vivo* studies compared to those conducted *in vitro* and make it possible to consider this ABD an effective tool for the antibiotic therapy of community-acquired infections. If josamycin cannot be used, a 16-membered macrolide such as spiramycin may be an alternative when choosing an ABD.[3]

[3] The specialist reported on the successful import substitution of the Japanese antibiotic. Bulletin of Pharmacy. Available from: https://pharmvestnik.ru/content/news/Specialist-zayavil-ob-uspeshnom-importozameshenii-yaponskogo-antibiotika.html. Russian).The appearance of a new drug “Doramycin VM” 3 000 000 IU on the Russian pharmaceutical market makes it possible to add to the arsenal of doctors a macrolide with pleiotropic favorable effects and a low ABR level, which favorably distinguishes it from the traditional representatives of this group.

FUNDING

The review was carried out with the support of Ornej LLC (Russia).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS’ CONTRIBUTION

Olga I. Butranova – search and analysis of literature sources, systematization of information, writing and editing the text of the manuscript; Sergey K. Zyryanov – search and analysis of literature sources, editing the text of the manuscript; Anna A. Abramova – search and analysis of literature sources, writing and editing the text of the manuscript. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made a significant contribution to the development of the concept, research and preparation of the article, read and approved the final version before publication).

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

REFERENCES

1. Hutchings MI, Truman AW, Wilkinson B. Antibiotics: past, present and future. Curr Opin Microbiol. 2019;51:72–80. DOI: 10.1016/j.mib.2019.10.008
2. Uddin TM, Chakraborty AJ, Khusro A, Zidan BRM, Mitra S, Emran TB, Dhama K, Ripon MKH, Gajdács M, Sahibzada MUK, Hossain MJ, Koirala N. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. J Infect Public Health. 2021;14(12):1750–1766. DOI: 10.1016/j.jiph.2021.10.020
3. Zahari NIN, Engku Abd Rahman ENS, Irekeola AA, Ahmed N, Rabaan AA, Alotaibi J, Alqahtani SA, Halawi MY, Alamri IA, Almogbel MS, Alfaraj AH, Ibrahim FA, Almaghaslah M, Alissa M, Yean CY. A Review of the Resistance Mechanisms for β-Lactams, Macrolides and Fluoroquinolones among Streptococcus pneumoniae. Medicina (Kaunas). 2023;59(11):1927. DOI: 10.3390/medicina59111927
4. Gupta V, Yu KC, Schranz J, Gelone SP. A Multicenter Evaluation of the US Prevalence and Regional Variation in Macrolide-Resistant S. pneumoniae in Ambulatory and Hospitalized Adult Patients in the United States. Open Forum Infect Dis. 2021;8(7):ofab063. DOI: 10.1093/ofid/ofab063
5. Gergova R, Boyanov V, Muhtarova A, Alexandrova A. A Review of the Impact of Streptococcal Infections and Antimicrobial Resistance on Human Health. Antibiotics (Basel). 2024;13(4):360. DOI: 10.3390/antibiotics13040360
6. Alexandrova A, Pencheva D, Setchanova L, Gergova R. Association of pili with widespread multidrug-resistant genetic lineages of non-invasive pediatric Streptococcus pneumoniae isolates. Acta Microbiol Immunol. Hung. 2022;69:177–184. DOI: 10.1556/030.2022.01816
7. Okada T, Sato Y, Toyonaga Y, Hanaki H, Sunakawa K. Nationwide survey of Streptococcus pneumoniae drug resistance in the pediatric field in Japan. Pediatr Int. 2016;58:192–201. DOI: 10.1111/ped.12781
8. Fu J, Yi R, Jiang Y, Xu S, Qin P, Liang Z, Chen J. Serotype distribution and antimicrobial resistance of Streptococcus pneumoniae causing invasive diseases in China: A meta-analysis. BMC Pediatr. 2019;19:424. DOI: 10.1186/s12887-019-1722-1
9. Zhou X, Liu J, Zhang Z, Cui B, Wang Y, Zhang Y, Xu H, Cheng G, Liu Y, Qin X. Characterization of Streptococcus pneumoniae Macrolide Resistance and Its Mechanism in Northeast China over a 20-Year Period. Microbiol Spectr. 2022;10(5):e0054622. DOI: 10.1128/spectrum.00546-22
10. Mohammadi Gharibani K, Azami A, Parvizi M, Khademi F, Mousavi SF, Arzanlou M. High Frequency of Macrolide-Resistant *Streptococcus pneumoniae* Colonization in Respiratory Tract of Healthy Children in Ardabil, Iran. Tanaffos. 2019;18(2):118–125.
11. Ivanchik NV, Chagaryan AN, Sukhorukova MV, Kozlov RS, Dekhnich AV, Krechikova OI, Vinogradova AG, Kuzmenkov AYu, Trushin IV, Sivaya OV, Muravyev AA, Strebkova VV, Kochneva NA, Amineva PG, Ishakova LM, Dik NG, Morozova OA, Lazareva AV, Chernyavskaya YuL, Kirillova GSh, Bekker GG, Popova LD, Elokhina EV, Zubareva NA, Moskvitina EN, Petrova TA, Zholobova AF, Gudkova LV, Khokhlyavin RL, Burasova EB, Kholodok GN, Panina OA, Ershova MG. Antimicrobial resistance of clinical Streptococcus pneumoniae isolates in Russia: the results of multicenter epidemiological study «PEHASus 2014–2017». Clinical Microbiology and Antimicrobial Chemotherapy. 2019;21(3):230–237. DOI: 10.36488/cmac.2019.3.230-237
12. Berbel D, González-Díaz A, López de Egea G, Càmara J, Ardanuy C. An Overview of Macrolide Resistance in Streptococci: Prevalence, Mobile Elements and Dynamics. Microorganisms. 2022;10(12):2316. DOI: 10.3390/microorganisms10122316
13. Stetsyuk OU, Andreeva IV, Egorova OA. Antibiotic resistance of the main ENT pathogens. RMJ Medical Review. 2019;9(II):78–83.
14. Guo DX, Hu WJ, Wei R, Wang H, Xu BP, Zhou W, Ma SJ, Huang H, Qin XG, Jiang Y, Dong XP, Fu XY, Shi DW, Wang LY, Shen AD, Xin DL. Epidemiology and mechanism of drug resistance of Mycoplasma pneumoniae in Beijing, China: A multicenter study. Bosn J Basic Med Sci. 2019;19(3):288–296. DOI: 10.17305/bjbms.2019.4053
15. Loconsole D, De Robertis AL, Sallustio A, Centrone F, Morcavallo C, Campanella S, Accogli M, Chironna M. Update on the Epidemiology of Macrolide-Resistant Mycoplasma pneumoniae in Europe: A Systematic Review. Infect Dis Rep. 2021;13(3):811–820. DOI: 10.3390/idr13030073
16. Molan A, Nosaka K, Hunter M, Wang W. Global status of Toxoplasma gondii infection: systematic review and prevalence snapshots. Trop Biomed. 2019;36(4):898–925.
17. Khabisi SA, Almasi SZ, Zadeh SL. Seroprevalence and Risk Factors Associated with Toxoplasma gondii Infection in the Population Referred to Rural and Urban Health Care Centers in Zahedan, Primary Referral Level, in Southeastern Iran. J Parasitol Res. 2022;2022:7311905. DOI: 10.1155/2022/7311905
18. Yu CP, Chen BC, Chou YC, Hsieh CJ, Lin FH. The epidemiology of patients with toxoplasmosis and its associated risk factors in Taiwan during the 2007-2020 period. PLoS One. 2023;18(8):e0290769. DOI: 10.1371/journal.pone.0290769
19. Montazeri M, Mehrzadi S, Sharif M, Sarvi S, Tanzifi A, Aghayan SA, Daryani A. Drug Resistance in Toxoplasma gondii. Front Microbiol. 2018;9:2587. DOI: 10.3389/fmicb.2018.02587
20. Adriaenssens N, Bruyndonckx R, Versporten A, Hens N, Monnet DL, Molenberghs G, Goossens H, Weist K, Coenen S; ESAC-Net study group. Consumption of macrolides, lincosamides and streptogramins in the community, European Union/European Economic Area, 1997–2017. J Antimicrob Chemother. 2021;76(12 Suppl 2):ii30–ii36. DOI: 10.1093/jac/dkab175
21. Karnoukh KI, Lazareva NB. Analysis of the antibiotic consumption on the backdrop of the COVID-19 pandemic:

DOI: 10.19163/2307-9266-2024-12-2-150-171

hospital level. Medical Council. 2021;(16):118–128. DOI: 10.21518/2079-701X-2021-16-118-128

22. Zakharenkov IA, Rachina SA, Kozlov RS, Belkova YuA. Consumption of systemic antibiotics in the Russian Federation in 2017–2021. Clinical Microbiology and Antimicrobial Chemotherapy. 2022;24(3):220–225. DOI: 10.36488/cmac.2022.3.220-225
23. Calcagnile M, Bettini S, Damiano F, Talà A, Tredici SM, Pagano R, Di Salvo M, Siculella L, Fico D, De Benedetto GE, Valli L, Alifano P. Stimulatory Effects of Methyl-β-cyclodextrin on Spiramycin Production and Physical-Chemical Characterization of Nonhost@Guest Complexes. ACS Omega. 2018;3(3):2470–2478. DOI: 10.1021/acsomega.7b01766
24. Vacek V. Spiramycin [Spiramycin]. Cas Lek Cesk. 1994;133(2):56–60. Czech
25. Arsic B, Barber J, Čikoš A, Mladenovic M, Stankovic N, Novak P. 16-membered macrolide antibiotics: a review. Int J Antimicrob Agents. 2018;51(3):283–298. DOI: 10.1016/j.ijantimicag.2017.05.020
26. Breiner-Goldstein E, Eyal Z, Matzov D, Halfon Y, Cimicata G, Baum M, Rokney A, Ezernitchi AV, Lowell AN, Schmidt JJ, Rozenberg H, Zimmerman E, Bashan A, Valinsky L, Anzai Y, Sherman DH, Yonath A. Ribosome-binding and anti-microbial studies of the mycinamicins, 16-membered macrolide antibiotics from Micromonospora griseorubida. Nucleic Acids Res. 2021;49(16):9560-9573. DOI: 10.1093/nar/gkab684
27. Yakovlev SV, Suvorova MP. The Renaissance of Spiramycin in Clinical Practice. Antibiotics and Chemotherapy. 2023;68(7–8):83–89. DOI: 10.37489/0235-2990-2023-68-7-8-83-89
28. Butranova OI, Ushkalova EA, Zyryanov SK, Chenkurov MS, Baybulatova EA. Pharmacokinetics of Antibacterial Agents in the Elderly: The Body of Evidence. Biomedicines. 2023;11(6):1633. DOI: 10.3390/biomedicines11061633
29. Butranova OI, Ushkalova EA, Zyryanov SK, Chenkurov MS. Developmental Pharmacokinetics of Antibiotics Used in Neonatal ICU: Focus on Preterm Infants. Biomedicines. 2023;11(3):940. DOI: 10.3390/biomedicines11030940
30. Baietto L, Corcione S, Pacini G, Perri GD, D'Avolio A, De Rosa FG. A 30-years review on pharmacokinetics of antibiotics: is the right time for pharmacogenetics? Curr Drug Metab. 2014;15(6):581–98. DOI: 10.2174/1389200215666140605130935
31. Nielsen EI, Cars O, Friberg LE. Pharmacokinetic/pharmacodynamic (PK/PD) indices of antibiotics predicted by a semimechanistic PKPD model: a step toward model-based dose optimization. Antimicrob Agents Chemother. 2011;55(10):4619–4630. DOI: 10.1128/AAC.00182-11
32. Fuursted K, Knudsen JD, Petersen MB, Poulsen RL, Rehm D. Comparative study of bactericidal activities, postantibiotic effects, and effects of bacterial virulence of penicillin G and six macrolides against Streptococcus pneumoniae. Antimicrob Agents Chemother. 1997;41(4):781–84. DOI: 10.1128/AAC.41.4.781
33. Wang L, Zhang Y. Postantibiotic effects and postantibiotic sub-MIC effects of tilmicosin, erythromycin and tiamulin on erythromycin-resistant Streptococcus suis. Braz J Microbiol. 2009;40(4):980–987. DOI: 10.1590/S1517-838220090004000033
34. Odenholt-Tornqvist I, Löwdin E, Cars O. Postantibiotic effects and postantibiotic sub-MIC effects of roxithromycin, clarithromycin, and azithromycin on respiratory tract pathogens. Antimicrob Agents Chemother. 1995;39(1):221–226. DOI: 10.1128/AAC.39.1.221
35. Kricker JA, Page CP, Gardarsson FR, Baldursson O, Gudjonsson T, Parnham MJ. Nonantimicrobial Actions of Macrolides: Overview and Perspectives for Future Development. Pharmacol Rev. 2021;73(4):233–262. DOI: 10.1124/pharmrev.121.000300
36. Pollock J, Chalmers JD. The immunomodulatory effects of macrolide antibiotics in respiratory disease. Pulm Pharmacol Ther. 2021;71:102095. DOI: 10.1016/j.pupt.2021.102095
37. Zarogoulidis P, Papanas N, Kioumis I, Chatzaki E, Maltezos E, Zarogoulidis K. Macrolides: from in vitro anti-inflammatory and immunomodulatory properties to clinical practice in respiratory diseases. Eur J Clin Pharmacol. 2012;68(5):479–503. DOI: 10.1007/s00228-011-1161-x
38. Culić O, Eraković V, Parnham MJ. Anti-inflammatory effects of macrolide antibiotics. Eur J Pharmacol. 2001;429(1–3):209–229. DOI: 10.1016/s0014-2999(01)01321-8
39. Cao X, Du X, Jiao H, An Q, Chen R, Fang P, Wang J, Yu B. Carbohydrate-based drugs launched during 2000-2021. Acta Pharm Sin B. 2022;12(10):3783–3821. DOI: 10.1016/j.apsb.2022.05.020
40. Zhang X, Wu X, Xie F, Wang Z, Zhang X, Jiang L. Physicochemical Properties and In Vitro Dissolution of Spiramycin Microparticles Using the Homogenate-Antisolvent Precipitation Process. Applied Sciences. 2017;7(1):10. DOI: 10.3390/app7010010
41. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001;46(1–3):3–26. DOI: 10.1016/s0169-409x(00)00129-0
42. Matsson P, Doak BC, Over B, Kihlberg J. Cell permeability beyond the rule of 5. Adv Drug Deliv Rev. 2016;101:42–61. DOI: 10.1016/j.addr.2016.03.013
43. Danelius E, Poongavanam V, Peintner S, Wieske LHE, Erdélyi M, Kihlberg J. Solution Conformations Explain the Chameleonic Behaviour of Macrocyclic Drugs. Chemistry. 2020;26(23):5231–5244. DOI: 10.1002/chem.201905599
44. Erckes V, Steuer C. A story of peptides, lipophilicity and chromatography – back and forth in time. RSC Med Chem. 2022;13(6):676–687. DOI: 10.1039/d2md00027j
45. Wieske LHE, Atilaw Y, Poongavanam V, Erdélyi M, Kihlberg J. Going Viral: An Investigation into the Chameleonic Behaviour of Antiviral Compounds. Chemistry. 2023;29(8):e202202798. DOI: 10.1002/chem.202202798
46. Padovan J, Ralić J, Letfus V, Milić A, Bencetić Mihaljević V. Investigating the barriers to bioavailability of macrolide

antibiotics in the rat. Eur J Drug Metab Pharmacokinet. 2012;37(3):163–171. DOI: 10.1007/s13318-011-0074-5

47. Doak BC, Over B, Giordanetto F, Kihlberg J. Oral druggable space beyond the rule of 5: insights from drugs and clinical candidates. Chem Biol. 2014;21(9):1115–1142. DOI: 10.1016/j.chembiol.2014.08.013
48. Peters DH, Friedel HA, McTavish D. Azithromycin. A review of its antimicrobial activity, pharmacokinetic properties and clinical efficacy. Drugs. 1992;44(5):750–799. DOI: 10.2165/00003495-199244050-00007
49. Foulds G, Shepard RM, Johnson RB. The pharmacokinetics of azithromycin in human serum and tissues. J Antimicrob Chemother. 1990;25 Suppl A:73–82. DOI: 10.1093/jac/25.suppl_a.73
50. Chabbert, Y. Etudes in vitro sur la spiramycine; activité, résistance, antibiogramme, concentrations humorales [In vitro studies on spiramycin; activity, resistance, antibiogram, humoral concentrations]. Ann Inst Pasteur (Paris). 1955;89(4):434–446. French
51. Frydman AM, Le Roux Y, Desnottes JF, Kaplan P, Djebbar F, Cournot A, Duchier J, Gaillot J. Pharmacokinetics of spiramycin in man. J Antimicrob Chemother. 1988;22 Suppl B:93–103. DOI: 10.1093/jac/22.supplement_b.93
52. Hamilton-Miller JM. In-vitro activities of 14-, 15- and 16-membered macrolides against gram-positive cocci. J Antimicrob Chemother. 1992;29(2):141–147. DOI: 10.1093/jac/29.2.141
53. Desnottes JF, Diallo N, Moret G. Effect of spiramycin on adhesiveness and phagocytosis of gram-positive cocci. J Antimicrob Chemother. 1988;22 Suppl B:25–32. DOI: 10.1093/jac/22.supplement_b.25
54. Ridgway GL, Mumtaz G, Fenelon L. The in-vitro activity of clarithromycin and other macrolides against the type strain of Chlamydia pneumoniae (TWAR). J Antimicrob Chemother. 1991;27 Suppl A:43–45. DOI: 10.1093/jac/27.suppl_a.43
55. Webster C, Ghazanfar K, Slack R. Sub-inhibitory and post-antibiotic effects of spiramycin and erythromycin on Staphylococcus aureus. J Antimicrob Chemother. 1988;22 Suppl B:33–39. DOI: 10.1093/jac/22.supplement_b.33
56. Chavanet P, Portier H. Traitement des angines aiguës [Treatment of acute pharyngitis]. Rev Prat. 1992;42(3):303–307.
57. Yagiz Aghayarov O, Bayar Muluk N, Vejselova Sezer C, Kutlu HM, Cingi C. Evaluation of spiramycin for topical applications: a cell culture study. Eur Rev Med Pharmacol Sci. 2023;27(2 Suppl):44–50. DOI: 10.26355/eurrev_202303_31701
58. Rubinstein E, Keller N. Spiramycin renaissance. J Antimicrob Chemother. 1998;42(5):572–576. DOI: 10.1093/jac/42.5.572
59. Chan TS, Scaringella YS, Raymond K, Taub ME. Evaluation of Erythromycin as a Tool to Assess CYP3A Contribution of Low Clearance Compounds in a Long-Term Hepatocyte Culture. Drug Metab Dispos. 2020;48(8):690–697. DOI: 10.1124/dmd.120.090951
60. Akiyoshi T, Ito M, Murase S, Miyazaki M, Guengerich FP, Nakamura K, Yamamoto K, Ohtani H. Mechanism-based inhibition profiles of erythromycin and clarithromycin with cytochrome P450 3A4 genetic variants. Drug Metab Pharmacokinet. 2013;28(5):411–415. DOI: 10.2133/dmpk.dmpk-12-rg-134
61. Krasniqi S, Matzneller P, Kinzig M, Sorgel F, Huttner S, Lackner E, Muller M, Zeitlinger M. Blood, tissue, and intracellular concentrations of erythromycin and its metabolite anhydroerythromycin during and after therapy. Antimicrob Agents Chemother. 2012;56(2):1059–1064. DOI: 10.1128/AAC.05490-11
62. Fohner AE, Sparreboom A, Altman RB, Klein TE. PharmGKB summary: Macrolide antibiotic pathway, pharmacokinetics/pharmacodynamics. Pharmacogenet Genomics. 2017;27(4):164–167. DOI: 10.1097/FPC.0000000000000270
63. Glanzer S, Pulido SA, Tutz S, Wagner GE, Kriechbaum M, Gubensäk N, Trifunovic J, Dorn M, Fabian WM, Novak P, Reidl J, Zangger K. Structural and functional implications of the interaction between macrolide antibiotics and bile acids. Chemistry. 2015;21(11):4350–4358. DOI: 10.1002/chem.201406413
64. Lenz KD, Klosterman KE, Mukundan H, Kubicek-Sutherland JZ. Macrolides: From Toxins to Therapeutics. Toxins (Basel). 2021;13(5):347. DOI: 10.3390/toxins13050347
65. Fassbender M, Lode H, Schiller C, Andro R, Goetschi B, Borner K, Koeppe P. Comparative pharmacokinetics of macrolide antibiotics and concentrations achieved in polymorphonuclear leukocytes and saliva. Clin Microbiol Infect. 1996;1(4):235–243. DOI: 10.1016/s1198-743x(15)60281-6
66. Eberl S, Renner B, Neubert A, Reisig M, Bachmakov I, König J, Dörje F, Mürdter TE, Ackermann A, Dormann H, Gassmann KG, Hahn EG, Zierhut S, Brune K, Fromm MF. Role of p-glycoprotein inhibition for drug interactions: evidence from in vitro and pharmacoepidemiological studies. Clin Pharmacokinet. 2007;46(12):1039–1049. DOI: 10.2165/00003088-200746120-00004
67. Puri SK, Lassman HB. Roxithromycin: a pharmacokinetic review of a macrolide. J Antimicrob Chemother. 1987;20 Suppl B:89–100. DOI: 10.1093/jac/20.suppl_b.89
68. Yamazaki H, Shimada T: Comparative studies of in vitro inhibition of cytochrome P450 3A4-dependent testosterone 6beta-hydroxylation by roxithromycin and its metabolites, troleandomycin, and erythromycin. Drug Metab Dispos. 1998;26(11):1053–1057.
69. Singlas E. [Clinical pharmacokinetics of azithromycin]. Pathol Biol (Paris). 1995;43(6):505–511.
70. Skinner M, Kanfer I. Comparative bioavailability of josamycin, a macrolide antibiotic, from a tablet and solution and the influence of dissolution on in vivo release. Biopharm Drug Dispos. 1998;19(1):21–29. DOI: 10.1002/(sici)1099-081x(199801)19:1<21::aid-bdd69>3.0.co;2-g
71. Brook I. Pharmacodynamics and pharmacokinetics of spiramycin and their clinical significance. Clin Pharmacokinet. 1998;34(4):303–310. DOI: 10.2165/00003088-199834040-00003

72. Vázquez-Laslop N, Mankin AS. How Macrolide Antibiotics Work. Trends Biochem Sci. 2018;43(9):668–684. DOI: 10.1016/j.tibs.2018.06.011
73. Kannan K, Vázquez-Laslop N, Mankin AS. Selective protein synthesis by ribosomes with a drug-obstructed exit tunnel. Cell. 2012;151(3):508–520. DOI: 10.1016/j.cell.2012.09.018
74. Aleksandrova EV, Ma CX, Klepacki D, Alizadeh F, Vázquez-Laslop N, Liang JH, Polikanov YS, Mankin AS. Macrolones target bacterial ribosomes and DNA gyrase and can evade resistance mechanisms. Nat Chem Biol. 2024. DOI: 10.1038/s41589-024-01685-3
75. Credito KL, Ednie LM, Jacobs MR, Appelbaum PC. Activity of telithromycin (HMR 3647) against anaerobic bacteria compared to those of eight other agents by time-kill methodology. Antimicrob Agents Chemother. 1999;43(8):2027–2031. DOI: 10.1128/AAC.43.8.2027
76. Svetlov MS, Vázquez-Laslop N, Mankin AS. Kinetics of drug-ribosome interactions defines the cidality of macrolide antibiotics. Proc Natl Acad Sci U S A. 2017;114(52):13673–13678. DOI: 10.1073/pnas.1717168115
77. Lewis JS 2nd, Jorgensen JH. Inducible clindamycin resistance in Staphylococci: should clinicians and microbiologists be concerned? Clin Infect Dis. 2005;40(2):280–285. DOI: 10.1086/426894
78. Pernodet JL, Alegre MT, Blondelet-Rouault MH, Guérineau M. Resistance to spiramycin in Streptomyces ambofaciens, the producer organism, involves at least two different mechanisms. J Gen Microbiol. 1993;139(5):1003–11. DOI: 10.1099/00221287-139-5-1003
79. Davoodi S, Daryaee F, Chang A, Walker SG, Tonge PJ. Correlating Drug-Target Residence Time and Post-antibiotic Effect: Insight into Target Vulnerability. ACS Infect Dis. 2020;6(4):629–636. DOI: 10.1021/acsinfecdis.9b00484
80. Kamme C, Kahlmeter G, Melander A. Evaluation of spiramycin as a therapeutic agent for elimination of nasopharyngeal pathogens. Possible use of spiramycin for middle ear infections and for gonococcal and meningococcal nasopharyngeal carriage. Scand J Infect Dis. 1978;10(2):135–142. DOI: 10.3109/inf.1978.10.issue-2.07
81. Kavi J, Webberley JM, Andrews JM, Wise R. A comparison of the pharmacokinetics and tissue penetration of spiramycin and erythromycin. J Antimicrob Chemother. 1988;22:105–10. DOI: 10.1093/jac/22.Supplement_B.105
82. Elazab ST, Elshater NS, Hashem YH, Al-Atfeehy NM, LeeEB,ParkSC,HsuWH.Pharmacokinetic/Pharmacodynamic Modeling of Spiramycin against *Mycoplasma synoviae* in Chickens. Pathogens. 2021;10(10):1238. DOI: 10.3390/pathogens10101238
83. Brisson-Noël A, Trieu-Cuot P, Courvalin P. Mechanism of action of spiramycin and other macrolides. J Antimicrob Chemother. 1988;22 Suppl B:13–23. DOI: 10.1093/jac/22.supplement_b.13
84. Pedra-Rezende Y, Macedo IS, Midlej V, Mariante RM, Menna-Barreto RFS. Different Drugs, Same End: Ultrastructural Hallmarks of Autophagy in Pathogenic Protozoa. Front Microbiol. 2022;13:856686. DOI: 10.3389/fmicb.2022.856686
85. Carbon C. Pharmacodynamics of macrolides, azalides, and streptogramins: effect on extracellular pathogens. Clin Infect Dis. 1998;27(1):28–32. DOI: 10.1086/514619
86. Calcagnile M, Alifano P. Off-Target Activity of Spiramycin Disarms Pseudomonas aeruginosa by Inhibition of Biofilm Formation, Pigment Production and Phenotypic Differentiation. Medical Sciences Forum. 2022;12(1):42. DOI: 10.3390/eca2022-12723
87. Calcagnile M, Jeguirim I, Tredici SM, Damiano F, Alifano P. Spiramycin Disarms Pseudomonas aeruginosa without Inhibiting Growth. Antibiotics (Basel). 2023;12(3):499. DOI: 10.3390/antibiotics12030499
88. Smith CR. The spiramycin paradox. J Antimicrob Chemother. 1988;22 Suppl B:141–144. DOI: 10.1093/jac/22.supplement_b.141
89. Poddighe D, Aljofan M. Clinical evidences on the antiviral properties of macrolide antibiotics in the COVID-19 era and beyond. Antivir Chem Chemother. 2020;28:2040206620961712. DOI: 10.1177/2040206620961712
90. Sugamata R, Sugawara A, Nagao T, Suzuki K, Hirose T, Yamamoto K, Oshima M, Kobayashi K, Sunazuka T, Akagawa KS, Ōmura S, Nakayama T, Suzuki K. Leucomycin A3, a 16-membered macrolide antibiotic, inhibits influenza A virus infection and disease progression. J Antibiot (Tokyo). 2014;67(3):213–222. DOI: 10.1038/ja.2013.132
91. Zeng S, Meng X, Huang Q, Lei N, Zeng L, Jiang X, Guo X. Spiramycin and azithromycin, safe for administration to children, exert antiviral activity against enterovirus A71 in vitro and in vivo. Int J Antimicrob Agents. 2019;53(4):362–369. DOI: 10.1016/j.ijantimicag.2018
92. Hagras NA, Mogahed NMFH, Sheta E, Darwish AA, El-Hawary MA, Hamed MT, Elwakil BH. The powerful synergistic effect of spiramycin/propolis loaded chitosan/alginate nanoparticles on acute murine toxoplasmosis. PLoS Negl Trop Dis. 2022;16(3):e0010268. DOI: 10.1371/journal.pntd.0010268
93. Allam AF, Hagras NA, Farag HF, Osman MM, Shalaby TI, Kazem AH, Shehab AY, Mogahed NMFH. Remarkable histopathological improvement of experimental toxoplasmosis after receiving spiramycin-chitosan nanoparticles formulation. J Parasit Dis. 2022;46(1):166–177. DOI: 10.1007/s12639-021-01431-9
94. Hagras NA, Allam AF, Farag HF, Osman MM, Shalaby TI, Fawzy Hussein Mogahed NM, Tolba MM, Shehab AY. Successful treatment of acute experimental toxoplasmosis by spiramycin-loaded chitosan nanoparticles. Exp Parasitol. 2019;204:107717. DOI: 10.1016/j.exppara.2019.107717
95. Abdel-Wahab AA, Shafey DA, Selim SM, Sharaf SA, Mohsen KK, Allam DM, Elkhadry SW, Gouda MA. Spiramycin-loaded maltodextrin nanoparticles as a promising treatment of toxoplasmosis on murine model. Parasitol Res. 2024;123(7):286. DOI: 10.1007/s00436-024-08280-4
96. El Saftawy EA, Turkistani SA, Alghabban HM, Albadawi EA, Ibrahim BE, Morsy S, Farag MF,

Al Hariry NS, Shash RY, Elkazaz A, Amin NM. Effects of *Lactobacilli acidophilus* and/or spiramycin as an adjunct in toxoplasmosis infection challenged with diabetes. Food Waterborne Parasitol. 2023;32:e00201. DOI: 10.1016/j.fawpar.2023.e00201

97. Kim MO, Ryu HW, Choi JH, Son TH, Oh SR, Lee HS, Yuk HJ, Cho S, Kang JS, Lee CW, Lee J, Lee CK, Hong ST, Lee SU. Anti-Obesity Effects of Spiramycin In Vitro and In Vivo. PLoS One. 2016;11(7):e0158632. DOI: 10.1371/journal.pone.0158632
98. Kenyon C, Laumen J, Manoharan-Basil SS, Buyze J. Strong association between adolescent obesity and consumption of macrolides in Europe and the USA: An ecological study. J Infect Public Health. 2020;13(10):1517–1521. DOI: 10.1016/j.jiph.2020.06.024
99. Ternák G, Németh M, Rozanovic M, Márovics G, Bogár L. "Growth-Promoting Effect" of Antibiotic Use Could Explain the Global Obesity Pandemic: A European Survey. Antibiotics (Basel). 2022;11(10):1321. DOI: 10.3390/antibiotics11101321
100. Reijnders TDY, Saris A, Schultz MJ, van der Poll T. Immunomodulation by macrolides: therapeutic potential for critical care. Lancet Respir Med. 2020;8(6):619–630. DOI: 10.1016/S2213-2600(20)30080-1
101. Pons S, Arrii E, Arnaud M, Loiselle M, Ferry J, Nouacer M, Lion J, Cohen S, Mooney N, Zafrani L. Immunomodulation of endothelial cells induced by macrolide therapy in a model of septic stimulation. Immun Inflamm Dis. 2021;9(4):1656–1669. DOI: 10.1002/iid3.518
102. Kang JK, Kang HK, Hyun CG. Anti-Inflammatory Effects of Spiramycin in LPS-Activated RAW 264.7 Macrophages. Molecules. 2022;27(10):3202. DOI: 10.3390/molecules27103202
103. Kanoh S, Rubin BK. Mechanisms of action and clinical application of macrolides as immunomodulatory medications. Clin Microbiol Rev. 2010;23(3):590–615. DOI: 10.1128/CMR.00078-09
104. Parnham MJ, Erakovic Haber V, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM, Vos R. Azithromycin: mechanisms of action and their relevance for clinical applications. Pharmacol Ther. 2014;143(2):225–245. DOI: 10.1016/j.pharmthera.2014.03.003
105. Pohl K, Grimm XA, Caceres SM, Poch KR, Rysavy N, Saavedra M, Nick JA, Malcolm KC. Mycobacterium abscessus Clearance by Neutrophils Is Independent of Autophagy. Infect Immun. 2020;88(8):e00024–20. DOI: 10.1128/IAI.00024-20
106. Kawamoto Y, Morinaga Y, Kaku N, Uno N, Kosai K, Sakamoto K, Hasegawa H, Yanagihara K. A novel macrolide, solithromycin suppresses mucin overexpression induced by Pseudomonas aeruginosa LPS in airway epithelial cells. J Infect Chemother. 2020;26(9):1008–1010. DOI: 10.1016/j.jiac.2020.06.014
107. Imamura Y, Yanagihara K, Mizuta Y, Seki M, Ohno H, Higashiyama Y, Miyazaki Y, Tsukamoto K, Hirakata Y, Tomono K, Kadota J, Kohno S. Azithromycin inhibits MUC5AC production induced by the Pseudomonas aeruginosa autoinducer N-(3-Oxododecanoyl) homoserine lactone in NCI-H292 Cells. Antimicrob Agents Chemother. 2004;48(9):3457–3461. DOI: 10.1128/AAC.48.9.3457-3461.2004
108. Tominaga K. The emerging role of senescent cells in tissue homeostasis and pathophysiology. Pathobiol Aging Age Relat Dis. 2015;5:27743. DOI: 10.3402/pba.v5.27743
109. Ozsvari B, Nuttall JR, Sotgia F, Lisanti MP. Azithromycin and Roxithromycin define a new family of "senolytic" drugs that target senescent human fibroblasts. Aging (Albany NY). 2018;10(11):3294–3307. DOI: 10.18632/aging.101633
110. Farouk F, Elmaaty AA, Elkamhawy A, Tawfik HO, Alnajjar R, Abourehab MAS, Saleh MA, Eldehna WM, Al-Karmalawy AA. Investigating the potential anticancer activities of antibiotics as topoisomerase II inhibitors and DNA intercalators: *in vitro*, molecular docking, molecular dynamics, and SAR studies. J Enzyme Inhib Med Chem. 2023;38(1):2171029. DOI: 10.1080/14756366.2023.2171029
111. Bunnag C, Jareoncharsri P, Voraprayoon S, Vitavasiri A, Supatchaipisit P, Kongpatanakul S. Efficacy of spiramycin as an alternative to amoxicillin in the treatment of acute upper respiratory tract infections. Clin Drug Investig. 1998;15(6):461–466. DOI: 10.2165/00044011-199815060-00001
112. Rocha RT, Awad CE, Ali A, Matyas R, Vital AC, Silva CO, Dainesi SM, Salazar MS, Nakatani J. Comparison of spiramycin and clarithromycin for community-acquired lower respiratory tract infections. Int J Clin Pract. 1999;53(6):433–436.
113. Bocheńska-Marciniak M, Kupryś I, Krzywiecki A, Sliwowski A, Kuna P. Clinical efficacy and safety of spiramycin and clarithromycin in the treatment of outpatients with lower respiratory tract infections. Pol Arch Med Wewn. 1998;100(3):222–235.
114. Strachunskiĭ LS, Sudilovskaia NN, Melikhov OG. Rovamitsin (spiramitsin)--makrolidnyĭ antibiotik dlia vnutrivennogo vvedeniia: opyt lecheniia pnevmonii [Rovamycin (spiramycin)--a macrolide antibiotic for intravenous administration: a trial in the treatment of pneumonia]. Ter Arkh. 1995;67(3):7–11. Russian
115. Gel'tser BI, Rubashek IA, Semisotova EF, Kramar AV. Makrolidnyĭ antibiotik rovamitsin pri lechenii pnevmoniĭ [The macrolide antibiotic rovamycin in the treatment of pneumonias]. Ter Arkh. 1996;68(12):22–5. Russian
116. Strachunskiĭ LS, Sudilovskaia NN, Shiriaeva NV, Nechaeva NB. Spiramitsin (rovamitsin)--makrolidnyĭ antibiotik dlia peroral'noĭ terapii vnebol'nichnykh pnevmoniĭ [Spiramycin (rovamycin), a macrolide antibiotic for oral treatment of outpatient pneumonia]. Klin Med (Mosk). 1995;73(2):45–8. Russian
117. Otsiians EN, Rziankina MF, D'iachenko VG, Suleĭmanov SSh, Zakharova EI, Bachaldina OM. Primenenie spiramitsina pri lechenii vospalitel'nykh zabolevaniĭ dykhatel'nykh puteĭ u deteĭ v ambulatornykh usloviiakh [Use of spiramycin in the treatment of inflammatory diseases ot the respiratory

DOI: 10.19163/2307-9266-2024-12-2-150-171

tract in children in ambulatory conditions]. Antibiot Khimioter. 1998;43(11):34–7. Russian

118. Rotzetter PA, Le Liboux A, Pichard E, Cimasoni G. Kinetics of spiramycin/metronidazole (Rodogyl) in human gingival crevicular fluid, saliva and blood. J Clin Periodontol. 1994;21(9):595–600. DOI: 10.1111/j.1600-051x.1994.tb00749.x

119. Rams TE, Dujardin S, Sautter JD, Degener JE, van Winkelhoff AJ. Spiramycin resistance in human periodontitis microbiota. Anaerobe. 2011;17(4):201–205. DOI: 10.1016/j.anaerobe.2011.03.017

120. Poulet PP, Duffaut D, Barthet P, Brumpt I. Concentrations and in vivo antibacterial activity of spiramycin and metronidazole in patients with periodontitis treated with high-dose metronidazole and the spiramycin/metronidazole combination. J Antimicrob Chemother. 2005;55(3):347–351. DOI: 10.1093/jac/dki013

121. Kocsmár É, Buzás GM, Szirtes I, Kocsmár I, Kramer Z, Szijártó A, Fadgyas-Freyler P, Szénás K, Rugge M, Fassan M, Kiss A, Schaff Z, Röst G, Lotz G. Primary and secondary clarithromycin resistance in Helicobacter pylori and mathematical modeling of the role of macrolides. Nat Commun. 2021;12(1):2255. DOI: 10.1038/s41467-021-22557-7

122. Mégraud F, Graham DY, Howden CW, Trevino E, Weissfeld A, Hunt B, Smith N, Leifke E, Chey WD. Rates of Antimicrobial Resistance in Helicobacter pylori Isolates From Clinical Trial Patients Across the US and Europe. Am J Gastroenterol. 2023;118(2):269–275. DOI: 10.14309/ajg.0000000000002045

123. Perfilova KM, Butina TYu, Neumoina NV, Shutova IV, Kuznetsova IA, Troshina TA, Shmakova TV, Levina SN. Macrolide resistance of H.pylori due to ermB gene during H. pylori infection in real practice. Opera Medica et Physiologica. 2024;11(2):129–138. DOI: 10.24412/2500-2295-2024-2-129-138

124. Berstad A, Berstad K, Wilhelmsen I, Hatlebakk JG, Nesje LB, Hausken T. Spiramycin in triple therapy of Helicobacter pylori-associated peptic ulcer disease. An open pilot study with 12-month follow-up. Aliment Pharmocol Ther. 1995;9(2):197–200. DOI: 10.1111/j.1365-2036.1995.tb00371.x

125. Olafsson S, Berstad A, Bang CJ, Nysaeter G, Coll P, Tefera S, Hatlebakk JG, Hausken T, Olafsson T. Spiramycin is comparable to oxytetracycline in eradicating H. pylori when given with ranitidine bismuth citrate and metronidazole. Aliment Pharmacol Ther. 1999;13(5):651–659. DOI: 10.1046/j.1365-2036.1999.00517

126. Kalach N, Raymond J, Benhamou PH, Bergeret M, Senouci L, Gendrel D, Dupont C. Spiramycin as an alternative to amoxicillin treatment associated with lansoprazole/metronidazole for Helicobacter pylori infection in children. Eur J Pediatr. 1998;157(7):607–608. DOI: 10.1007/s004310050891

127. Telaku S, Islamaj E, Veliu A, Bytyqi J, Telaku M, Fejza H, Alidema F. The Efficacy of Spiramycin-based Triple Therapy for First-Line Helicobacter Pylori Eradication. Pharmakeftiki. 2023;35(4):64–70. DOI: 10.60988/pj.v35i4.28

128. Mikhailova V.V., Lobova T.P., Shishkina M.S., Skvortsova A.N., Zyuzgina S.V., Zinovieva O.E. Review of the epizootic situation on chlamydia in animals and birds in the Russian Federation for the period from 2019 to 2021. Agrarian science. 2024;(3):57–61. DOI: 10.32634/0869-8155-2024-380-3-57-61

129. Fesolowicz S, Kwiatkowski A, Wszola M, Podsiadly E, Ostrowski K, Durlik M, Paczek L, Tylewska-Wierzbanowska S, Rowinski W, Chmura A. Chlamydia pneumoniae infection in patients after kidney transplantation treated with spiramycin. Transplant Proc. 2009;41(1):167–169. DOI: 10.1016/j.transproceed.2008.09.062

130. Dylewski J, Clecner B, Dubois J, St-Pierre C, Murray G, Bouchard C, Phillips R. Comparison of spiramycin and doxycycline for treatment of Chlamydia trachomatis genital infections. Antimicrob Agents Chemother. 1993;37(6):1373–1374. DOI: 10.1128/AAC.37.6.1373.

131. Sreiri N, Ben Abdallah Y, Belfeki N, Klopfenstein T, Zayet S. Chlamydia psittaci-related pleuro-myocarditis. Braz J Infect Dis. 2024;28(2):103739. DOI: 10.1016/j.bjid.2024.103739

132. Gomes Ferrari Strang AG, Ferrar RG, Falavigna-Guilherme AL. Gestational toxoplasmosis treatment changes the child's prognosis: A cohort study in southern Brazil. PLoS Negl Trop Dis. 2023;17(9):e0011544. DOI: 10.1371/journal.pntd.0011544

133. Briciu V, Ionică AM, Flonta M, Almaș A, Muntean M, Topan A, Horvat M, Ungureanu L, Lupșe M. Toxoplasmosis Screening during Pregnancy in a Romanian Infectious Diseases Tertiary Center: Results of a 15 Years Follow-Up Program. Microorganisms. 2023;11(9):2189. DOI: 10.3390/microorganisms11092189

134. Schneider MO, Faschingbauer F, Kagan KO, Groß U, Enders M, Kehl S; AGG Section Maternal Diseases. Toxoplasma gondii Infection in Pregnancy – Recommendations of the Working Group on Obstetrics and Prenatal Medicine (AGG – Section on Maternal Disorders). Geburtshilfe Frauenheilkd. 2023;83(12):1431–1445. DOI: 10.1055/a-2111-7394

135. Avci ME, Arslan F, Çiftçi Ş, Ekiz A, Tüten A, Yildirim G, Madazli R. Role of spiramycin in prevention of fetal toxoplasmosis. J Matern Fetal Neonatal Med. 2016;29(13):2073–2076. DOI: 10.3109/14767058.2015.1074998

136. Felín MS, Wang K, Moreira A, Grose A, Leahy K, Zhou Y, Clouser FA, Siddiqui M, Leong N, Goodall P, Michalowski M, Ismail M, Christmas M, Schrantz S, Caballero Z, Norero X, Estripeaut D, Ellis D, Raggi C, Castro C, Moossazadeh D, Ramirez M, Pandey A, Ashi K, Dovgin S, Dixon A, Li X, Begeman I, Heichman S, Lykins J, Villalobos-Cerrud D, Fabrega L, Montalvo JLS, Mendivil C, Quijada MR, Fernández-Pirla S, de La Guardia V, Wong D, de Guevara ML, Flores C, Borace J, García A, Caballero N, Rengifo-Herrera C, de Saez MTM, Politis M, Wroblewski K, Karrison T, Ross S, Dogra M, Dhamsania V, Graves N, Kirchberg M, Mathur K, Aue A, Restrepo CM, Llanes A, Guzman G, Rebellon A, Boyer K, Heydemann P, Noble AG, Swisher C, Rabiah P, Withers S, Hull T, Su C, Blair M, Latkany P, Mui E, Vasconcelos-Santos DV, Villareal A, Perez A, Galvis CAN,

Montes MV, Perez NIC, Ramirez M, Chittenden C, Wang E, Garcia-López LL, Muñoz-Ortiz J, Rivera-Valdivia N, Bohorquez-Granados MC, de-la-Torre GC, Padrieu G, Hernandez JDV, Celis-Giraldo D, Dávila JAA, Torres E, Oquendo MM, Arteaga-Rivera JY, Nicolae DL, Rzhetsky A, Roizen N, Stillwaggon E, Sawers L, Peyron F, Wallon M, Chapey E, Levigne P, Charter C, De Frias M, Montoya J, Press C, Ramirez R, Contopoulos-Ioannidis D, Maldonado Y, Liesenfeld O, Gomez C, Wheeler K, Holfels E, Frim D, McLone D, Penn R, Cohen W, Zehar S, McAuley J, Limonne D, Houze S, Abraham S, Piarroux R, Tesic V, Beavis K, Abeleda A, Sautter M, El Mansouri B, El Bachir A, Amarir F, El Bissati K, de-la-Torre A, Britton G, Motta J, Ortega-Barria E, Romero IL, Meier P, Grigg M, Gómez-Marín J, Kosagisharaf JR, Llorens XS, Reyes O, McLeod R. Building Programs to Eradicate Toxoplasmosis Part I: Introduction and Overview. Curr Pediatr Rep. 2022;10(3):57–92. DOI: 10.1007/s40124-022-00269-w

137. Felín MS, Wang K, Moreira A, Grose A, Leahy K, Zhou Y, Clouser FA, Siddiqui M, Leong N, Goodall P, Michalowski M, Ismail M, Christmas M, Schrantz S, Caballero Z, Norero X, Estripeaut D, Ellis D, Raggi C, Castro C, Moossazadeh D, Ramirez M, Pandey A, Ashi K, Dovgin S, Dixon A, Li X, Begeman I, Heichman S, Lykins J, Villalobos-Cerrud D, Fabrega L, Montalvo JLS, Mendivil C, Quijada MR, Fernández-Pirla S, de La Guardia V, Wong D, de Guevara ML, Flores C, Borace J, García A, Caballero N, Rengifo-Herrera C, de Saez MTM, Politis M, Ross S, Dogra M, Dhamsania V, Graves N, Kirchberg M, Mathur K, Aue A, Restrepo CM, Llanes A, Guzman G, Rebellon A, Boyer K, Heydemann P, Noble AG, Swisher C, Rabiah P, Withers S, Hull T, Frim D, McLone D, Su C, Blair M, Latkany P, Mui E, Vasconcelos-Santos DV, Villareal A, Perez A, Galvis CAN, Montes MV, Perez NIC, Ramirez M, Chittenden C, Wang E, Garcia-López LL, Padrieu G, Muñoz-Ortiz J, Rivera-Valdivia N, Bohorquez-Granados MC, de-la-Torre GC, Hernandez JDV, Celis-Giraldo D, Dávila JAA, Torres E, Oquendo MM, Arteaga-Rivera JY, Nicolae DL, Rzhetsky A, Roizen N, Stillwaggon E, Sawers L, Peyron F, Wallon M, Chapey E, Levigne P, Charter C, De Frias M, Montoya J, Press C, Ramirez R, Contopoulos-Ioannidis D, Maldonado Y, Liesenfeld O, Gomez C, Wheeler K, Zehar S, McAuley J, Limonne D, Houze S, Abraham S, Piarroux R, Tesic V, Beavis K, Abeleda A, Sautter M, El Mansouri B, El Bachir A, Amarir F, El Bissati K, Holfels E, Frim D, McLone D, Penn R, Cohen W, de-la-Torre A, Britton G, Motta J, Ortega-Barria E, Romero IL, Meier P, Grigg M, Gómez-Marín J, Kosagisharaf JR, Llorens XS, Reyes O, McLeod R. Building Programs to Eradicate Toxoplasmosis Part IV: Understanding and Development of Public Health Strategies and Advances “Take a Village”. Curr Pediatr Rep. 2022;10(3):125–154. DOI: 10.1007/s40124-022-00268-x

138. Wei HX, Wei SS, Lindsay DS, Peng HJ. A Systematic Review and Meta-Analysis of the Efficacy of Anti-Toxoplasma gondii Medicines in Humans. PLoS One. 2015;10(9):e0138204. DOI: 10.1371/journal.pone.0138204

139. Montoya JG, Laessig K, Fazeli MS, Siliman G, Yoon SS, Drake-Shanahan E, Zhu C, Akbary A, McLeod R. A fresh look at the role of spiramycin in preventing a neglected disease: meta-analyses of observational studies. Eur J Med Res. 2021;26(1):143. DOI: 10.1186/s40001-021-00606-7

140. Valentini P, Buonsenso D, Barone G, Serranti D, Calzedda R, Ceccarelli M, Speziale D, Ricci R, Masini L. Spiramycin/cotrimoxazole versus pyrimethamine/sulfonamide and spiramycin alone for the treatment of toxoplasmosis in pregnancy. J Perinatol. 2015;35(2):90–94. DOI: 10.1038/jp.2014.161

141. Hotop A, Hlobil H, Gross U. Efficacy of rapid treatment initiation following primary Toxoplasma gondii infection during pregnancy. Clin Infect Dis. 2012;54(11):1545–1552. DOI: 10.1093/cid/cis234

142. Hansen MP, Scott AM, McCullough A, Thorning S, Aronson JK, Beller EM, Glasziou PP, Hoffmann TC, Clark J, Del Mar CB. Adverse events in people taking macrolide antibiotics versus placebo for any indication. Cochrane Database Syst Rev. 2019;1(1):CD011825. DOI: 10.1002/14651858.CD011825.pub2

143. You C, Zhang Y, Xu Y, Xu P, Li Z, Li H, Huang S, Chen Z, Li J, Xu HE, Jiang Y. Structural basis for motilin and erythromycin recognition by motilin receptor. Sci Adv. 2023;9(11):eade9020. DOI: 10.1126/sciadv.ade9020

144. Itoh Z, Suzuki T, Nakaya M, Inoue M, Mitsuhashi S. Gastrointestinal motor-stimulating activity of macrolide antibiotics and analysis of their side effects on the canine gut. Antimicrob Agents Chemother. 1984;26(6):863–869. DOI: 10.1128/AAC.26.6.863

145. Shim SR, Lee Y, In SM, Lee KI, Kim I, Jeong H, Shin J, Kim JY. Increased risk of hearing loss associated with macrolide use: a systematic review and meta-analysis. Sci Rep. 2024;14(1):183. DOI: 10.1038/s41598-023-50774-1

146. Vanoverschelde A, Oosterloo BC, Ly NF, Ikram MA, Goedegebure A, Stricker BH, Lahousse L. Macrolide-associated ototoxicity: a cross-sectional and longitudinal study to assess the association of macrolide use with tinnitus and hearing loss. J Antimicrob Chemother. 2021;76(10):2708–2716. DOI: 10.1093/jac/dkab232

147. Wu Y, Bi WT, Qu LP, Fan J, Kong XJ, Ji CC, Chen XM, Yao FJ, Liu LJ, Cheng YJ, Wu SH. Administration of macrolide antibiotics increases cardiovascular risk. Front Cardiovasc Med. 2023;10:1117254. DOI: 10.3389/fcvm.2023

148. Ostroumova OD, Goloborodova IV. Drug-induced pirouette-type tachycardia. Farmateka. 2019;26(9):11–20. DOI: 10.18565/pharmateca.2019.9.11-20

149. Volberg WA, Koci BJ, Su W, Lin J, Zhou J. Blockade of human cardiac potassium channel human ether-a-go-go-related gene (HERG) by macrolide antibiotics. J Pharmacol Exp Ther. 2002;302(1):320–327. DOI: 10.1124/jpet.302.1.320.

150. Wang X, Pan Z, Wang J, Wang H, Fan H, Gong T, Sun Q, Feng Y, Liang P. Characterization of the molecular mechanisms underlying azithromycin-induced cardiotoxicity using human-induced pluripotent stem cell-derived cardiomyocytes. Clin Transl Med. 2021;11(9):e549. DOI: 10.1002/ctm2.549148

DOI: 10.19163/2307-9266-2024-12-2-150-171

151. Prasil P, Sleha R, Kacerovsky M, Bostik P. Comparison of adverse reactions of spiramycin versus pyrimethamine/sulfadiazine treatment of toxoplasmosis in pregnancy: is spiramycin really the drug of choice for unproven infection of the fetus? J Matern Fetal Neonatal Med. 2023;36(1):2215377. DOI: 10.1080/14767058.2023.2215377

152. Descotes J, Vial T, Delattre D, Evreux JC. Spiramycin: safety in man. J Antimicrob Chemother. 1988;22 Suppl B:207–210. DOI: 10.1093/jac/22.supplement_b.207

AUTHORS

Olga I. Butranova – Candidate of Sciences (Medicine), Associated Professor of the Department of General and Clinical Pharmacology of the Medical Institute of Peoples' Friendship University of Russia named after Patrice Lumumba. ORCID ID: 0000-0001-7729-2169. E-mail: butranova-oi@rudn.ru;

Sergey K. Zyryanov – Doctor of Sciences (Medicine), professor, the Head of the Department of General and Clinical Pharmacology of the Medical Institute of Peoples' Friendship University of Russia named after Patrice Lumumba; deputy Chief Medical Officer of Municipal Clinical Hospital No. 24 of the Moscow City Health Department. ORCID ID: 0000-0002-6348-6867. E-mail: zyryanov_sk@rudn.university.

Anna A. Abramova – postgraduate student of the Department of General and Clinical Pharmacology of the Medical Institute of Peoples' Friendship University of Russia named after Patrice Lumumba. ORCID ID: 0009-0003-5739-4610. E-mail: abramova-aa@rudn.ru

REVIEWS

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Review of BRICS regulatory practices in the field of drugs compounding

D.S. Yurochkin[1], D.D. Mamedov[1], S.E. Erdni-Garyaev[1], A.V. Yarutkin[2], V.L. Bagirova[2], P.S. Guryanov[3], O. Ludiy[3], V. Li[4]

[1] St. Petersburg State Chemical and Pharmaceutical University, 14A Prof. Popov Str., St. Petersburg, Russia, 197022
[2] Scientific Centre for Expert Evaluation of Medicinal Products, Bld. 2, 8 Petrovsky Blvd, Moscow, Russia, 127051
[3] Perm State Pharmaceutical Academy, 101 Ekaterininskaya Str., Perm, Russia, 614990
[4] Independent Researcher, Beijing, China

E-mail: dmitry.yurochkin@pharminnotech.com

Received 03 March 2024 After peer review 19 Sep 2024 Accepted 08 Oct 2024

In the formation of a common market within the framework of interstate associations (Unions), the goal of improving the health of the member states population can be achieved, among other things, by providing an unimpeded access to safe, effective and quality medicines (drugs). One of the elements is activities in the field of these drugs compounding. This review has been prepared by authors due to the lack of information in the Russian-language literature on the regulation of this area in the current legal systems of the BRICS countries.

The aim of the work was to analyze the regulatory mechanisms and current approaches to the organization of activities in the field of drugs compounding, presented in the legislation of the BRICS interstate association (Union) member countries, including their structuring (systematization) in order to develop proposals for the convergence of these practices.

Materials and methods. PubMed, Google Scholar, elibrary.ru, and specialized databases of regulatory legal documents of the BRICS countries were used as search resources. The following keywords were used as search keywords: “drug”, “drug product”, “drugs compounding”, “pharmacy organization”, “medical organization”, “compounding”, “drug preparation”, “drug dilution (reconstitution)” in English, Portuguese, Spanish, Chinese, Arabic, Persian and Hindi. The paper uses empirical, theoretical, quantitative tools, including the analysis of a wide list of relevant sources - regulatory legal documents governing the activities of compounding pharmacies in the BRICS countries.

Results. The study presents key regulatory legal acts and documents, analyzes them and describes the main provisions of the legislative framework for the organization of activities in the field of drugs compounding. The identified peculiarities determine the need to rethink the current state of the Russian regulation of the drugs compounding sector. The study emphasizes the need to improve regulatory approaches in Russia. The BRICS countries can strive to develop the best practices and “gold” standards for the organization of this socially important activity in the field of drugs compounding. Such an approach can led to the creation of a unified good practice in compounding and dispensing of drugs.

Conclusion. The authors of the study consider it advisable to carry out a further and more detailed elaboration of the convergence issues of regulatory practices of both health care systems and pharmaceutical industries in the BRICS member states. It has been proposed to develop and form a “Roadmap” (an action plan) for the development of the cooperation between the BRICS member states in the field of health care and pharmaceutical industry in order to intensify integration processes and build a modern model of the public health and drug market, including joint research and development by world-class scientific centers for technological development of the interstate association (Union) countries.

Keywords: legislation; BRICS; manufacturing of pharmaceuticals; Diluting (Reconstitution) of pharmaceuticals; compounding pharmacies; drugs compounding; quality of pharmaceuticals; regulatory practice; personalized medicine; Brazil; India; China; Republic of South Africa; United Arab Emirates; Iran; Egypt; Ethiopia

Abbreviations: PO – pharmacy organization; SP – stock preparation; WTO – World Trade Organization; EEU – Eurasian Economic Union; DF – dosage form; FDF – finished dosage form; RPPs – radiopharmaceutical preparations; SOP – Standard Operation Procedure; MO – medical organization; SCO – Shanghai Cooperation Organization; DS – drug substance; CDP – compounded drug product; GMP – Good Manufacturing Practice; GPhP – Good Pharmacy Practice.

Для цитирования: Д.С. Юрочкин, Д.Д. Мамедов, С.Э. Эрдни-Гаряев, А.В. Яруткин, В.Л. Багирова, П.С. Гурьянов, О. Лудий, В. Ли. Обзор практик нормативного правового регулирования стран БРИКС в сфере изготовления лекарственных препаратов. *Фармация и фармакология.* 2024;12(2):172-194. **DOI:** 10.19163/2307-9266-2024-12-2-172-194

For citation: D.S. Yurochkin, D.D. Mamedov, S.E. Erdni-Garyaev, A.V. Yarutkin, V.L. Bagirova, P.S. Guryanov, O. Ludiy, V. Li. Review of BRICS regulatory practices in the field of drugs compounding. *Pharmacy & Pharmacology.* 2024;12(2):172-194. **DOI:** 10.19163/2307-9266-2024-12-2-172-194

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОБЗОРЫ

DOI: 10.19163/2307-9266-2024-12-2-172-194

Обзор практик нормативного правового регулирования стран БРИКС в сфере изготовления лекарственных препаратов

Д.С. Юрочкин[1], Д.Д. Мамедов[1], С.Э. Эрдни-Гаряев[1], А.В. Яруткин[2], В.Л. Багирова[2], П.С. Гурьянов[3], О. Лудий[3], В. Ли[4]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Санкт-Петербургский химико-фармацевтический университет» Министерства здравоохранения Российской Федерации, 197022, Россия, г. Санкт-Петербург, ул. Профессора Попова, д. 14, лит. А
[2] Федеральное государственное бюджетное учреждение «Научный центр экспертизы средств медицинского применения» Министерства здравоохранения Российской Федерации, 127051, Россия, г. Москва, Петровский б-р, д. 8, стр. 2
[3] Федеральное государственное бюджетное образовательное учреждение высшего образования «Пермская государственная фармацевтическая академия» Министерства здравоохранения Российской Федерации, 614990, Россия, г. Пермь, ул. Екатерининская, д. 101
[4] Независимый исследователь, г. Пекин, Китай

E-mail: dmitry.yurochkin@pharminnotech.com

Получена 03.03.2024 После рецензирования 19.09.2024 Принята к печати 08.10.2024

При формировании общего рынка в рамках межгосударственных объединений (союзов) цель укрепления здоровья населения государств-членов может достигаться, в том числе, путём обеспечения беспрепятственного доступа к безопасным, эффективным и качественным лекарственным препаратам (ЛП). Одним из элементов выступает деятельность в сфере изготовления ЛП. Ввиду недостаточного объёма сведений в русскоязычной литературе о регулировании этой сферы в действующих правовых системах стран БРИКС, авторами подготовлен настоящий обзор.
Цель. Провести анализ действующих подходов и механизмов регулирования к организации деятельности в сфере изготовления ЛП, представленных в законодательстве стран-участниц межгосударственного объединения (союза) БРИКС, включая их структурирование (систематизацию) для выработки предложений по сближению данных практик.
Материалы и методы. В качестве поисковых ресурсов были использованы базы данных PubMed, Google Scholar, elibrary.ru, а также специализированные базы данных нормативных правовых документов стран БРИКС. В качестве ключевых слов для поиска использовали: «лекарственное средство», «лекарственный препарат», «изготовление лекарственных препаратов», «аптечная организация», «медицинская организация», «компаундинг», «приготовление лекарств», «разведение (восстановление) лекарственных препаратов» на английском, португальском, испанском, китайском, арабском, персидском языках и хинди. В работе использованы эмпирические, теоретические, количественные инструменты, включая анализ широкого перечня релевантных источников – нормативных правовых документов, регулирующих деятельность производственных аптек в странах БРИКС.
Результаты. В исследовании представлены ключевые нормативные правовые акты и документы, проведён их анализ, а также описаны основные положения законодательной базы по организации деятельности в сфере изготовления ЛП. Выявленные особенности определяют потребность в переосмыслении текущего состояния российского регулирования сферы изготовления ЛП. Исследование подчеркивает необходимость совершенствования регуляторных подходов, действующих в России. В сфере изготовления ЛП страны БРИКС могут стремиться к выработке лучших практик и «золотых» стандартов по организации данного социально значимого вида деятельности. Такой подход может привести к созданию единой надлежащей практики изготовления и отпуска ЛП.
Заключение. Авторы исследования считают целесообразным выполнение дальнейшей и более детальной проработки вопросов сближения регуляторных практик как систем здравоохранения, так и фармацевтических отраслей стран-участниц БРИКС. Предложено разработать и сформировать «Дорожную карту» (план мероприятий) по развитию взаимодействия государств-участников БРИКС в области здравоохранения и фармацевтической отрасли. В целях интенсификации интеграционных процессов и выстраивания современной модели общественного здоровья и рынка обращения ЛС, включающего проведение совместных исследований и разработок научными центрами мирового уровня для технологического развития стран межгосударственного объединения (союза).

Ключевые слова: законодательство; БРИКС; изготовление лекарственных препаратов; разведение (восстановление) лекарственных препаратов; производственные аптеки; экстемпоральные лекарственные препараты; качество лекарственных средств; регуляторная практика; персонализированная медицина; Бразилия; Индия; Китай; Южно-Африканская Республика; Объединенные Арабские Эмираты; Иран; Египет; Эфиопия

Список сокращений: АО – аптечная организация; ВАЗ – внутриаптечная заготовка; ВТО – Всемирная торговая организация; ГЛФ – готовая лекарственная форма; ЕАЭС – Евразийский экономический союз; ЛП – лекарственный препарат; ЛС – лекарственное средство; ЛФ – лекарственная форма; РФЛП – радиофармацевтические лекарственные препараты; СОП – стандартная операционная процедура; МО – медицинская организация; ШОС – Шанхайская организация сотрудничества; ФС – фармацевтическая субстанция; ЭЛП – экстемпоральный лекарственный препарат; GMP – Надлежащая производственная практика; НАП – надлежащая аптечная практика.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

INTRODUCTION

An interstate association (Union), the BRICS (BRICS+) transregional partnership (community) was founded on the initiative of the Russian Federation (RF) in June, 2006. In 2013, the Concept of the Russian Federation's participation in the BRICS[1] association was adopted, which is one of the strategically significant directions of long-range foreign policy activities, and the National Committee on the BRICS Research was established. Among the priority areas of the Russian Federation's state policy and BRICS initiatives, the cooperation in the sphere of health care, public health protection, development, introduction of new medical technologies and medicines (drugs), is of particular importance. According to this concept, the main aims in the development of the interaction and cooperation with other BRICS member states have been established: in the pharmaceutical industry (the development and production of modern types of drugs); in the field of health care (strengthening health care systems and expanding an access of the population to high-quality, effective and safe medicines, vaccines and other drugs).

As of October 2024[2], the BRICS comprises 9 states: the Federative Republic of Brazil (Brazil), Russian Federation, Republic of India (India), People's Republic of China (China), Republic of South Africa (South Africa), United Arab Emirates (UAE), Islamic Republic of Iran (Iran), Arab Republic of Egypt (Egypt), and Federal Democratic Republic of Ethiopia (Ethiopia). At the time of this study completion, the XVIth BRICS summit was held from October 22 to 24, 2024, in Kazan, Russia. At the same time, the countries invited to join the association included the Kingdom of Saudi Arabia, which had not confirmed its membership in BRICS, and the Republic of Argentina, which had declined to join. At the BRICS summit in Russia in 2024, a new category of BRICS-related states was proposed – 13 states (Algeria, Belarus, Bolivia, Cuba, Indonesia, Kazakhstan, Malaysia, Nigeria, Thailand, Turkey, Uganda, Uzbekistan, Vietnam) were granted the status of "BRICS partner countries". Following the XVIth BRICS Summit, on 23 October 2024, the "Kazan Declaration on Strengthening Multilateralism for Equitable Global Development and Security"[3] was signed, according to which the decision of the Consultative Group on the New Industrial Revolution to establish seven working groups, comprising the ones on medical devices and pharmaceuticals, was supported. The declaration welcomes the establishment of closer ties between the BRICS health institutions responsible for sanitary and epidemiological health and well-being, the prevention of infectious diseases, and calls for a further exploration of opportunities to share knowledge and expertise, and to implement joint projects in the health sector. The BRICS countries have made a great contribution to establishing a close cooperation in the tuberculosis and antibiotic resistance control, capacity building in the infectious disease prevention and other areas such as non-communicable diseases, research and development, exchange of experience, including the problems on traditional medicine, digital health, nuclear medicine, radiopharmaceuticals (with a special emphasis on strengthening radiopharmaceutical supply chains and expanding an isotope production, as well as contributing to the development of advanced digital solutions).

The above-mentioned areas, together with stimulating the development of health care systems, are a harmonious continuation of the human capital development aims set out in the structure of priority areas of the BRICS Economic Partnership Strategy up to 2025[4]. The BRICS countries, including the Russian Federation, have created all the necessary conditions for the development of scientific and technological competencies in the production of medicines, raw materials, materials, equipment, components, as well as drugs compounding, including radiopharmaceutical preparations (RPPs)[5]. The growing and significant role of the unique interstate association (Union) BRICS has been regularly emphasized in the works devoted to the development of diplomatic relations of Russia, speeches at forums, summits of various levels and Addresses of the President of the Russian Federation to the Federal Assembly since 2011[6,7] [1].

[1] CONCEPT of participation of the Russian Federation in BRICS. Available from: http://kremlin.ru/events/president/news/17715

[2] BRICS Intergovernmental Organization. Available from: https://brics-russia2024.ru/about/

[3] Kazan Declaration "Strengthening multilateralism for just global development and security". Available from: http://static.kremlin.ru/media/events/files/ru/MUCfWDg0QRs3xfMUiCAmF3LEh02OL3Hk.pdf

[4] The BRICS Economic Partnership Strategy until 2025. Available from: https://www.economy.gov.ru/material/file/636aa3edbc0dcc2356ebb6f8d594ccb0/1148133.pdf

[5] Decree of the Government of the Russian Federation dated 7 June 2023 No. 1495-r «On the Strategy for the development of the pharmaceutical industry of the Russian Federation for the period up to 2030». Available from: https://docs.cntd.ru/document/1301897806

[6] Message of the President of the Russian Federation to the Federal Assembly of the Russian Federation at 22 Dec 2011. Available from: http://kremlin.ru/events/president/news/14088

[7] Message of the President of the Russian Federation to the Federal Assembly of the Russian Federation at 29 Feb 2024. Available from: http://www.kremlin.ru/acts/bank/50431

DOI: 10.19163/2307-9266-2024-12-2-172-194

As the practice of international organizations of regional and economic integration has shown, when forming a common market, the goal is to improve the population health in the member states of the interstate association (Union). This goal can be achieved, among other things, by providing an access to safe, effective and high-quality medicines, recognizing the expediency of a coordinated policy in the sphere of the drug circulation, taking into account mutual interest, due to the fact that medicines are socially important products. One of the elements in the sphere of the drug circulation is the activity in the sphere of drugs compounding, which has a high potential in the transition from the accounting of "packages" to the accounting of the volume of course prescriptions in the use of drug therapy, which simultaneously contributes to the optimization of resources and the development of personalized medicine, high-tech health care and health-saving technologies [2]. The greatest need among pediatric patients is in the segment of liquid oral dosage forms that provide an age-appropriate dosing and the administration convenience of [3]. Herewith, drugs compounding can be carried out by both pharmacy organizations (POs) and medical organizations (MOs). In the world practice, it is defined by the concept of "hospital exemptions" [4, 5]. Due to the personalization of dosages, combinations of active substances, dosage forms (DFs) and packaging volumes, compounded drug products (CDPs) belong to unregistered types of drugs.

A number of works by Russian researchers have been devoted to the study of various aspects of drug circulation in the BRICS countries. In terms of the peculiarities of an expanded access for unregistered drugs within the framework of a compassionate use and provision of therapy in the context of early access programs, including the BRICS countries, the study by Omelyanovsky V.V. et al. is of interest. [6]. The paper shows approaches to the accelerated registration and registration of medicines with insufficient clinical data, describes the practices of the countries under consideration for early access programs. The issues of quality assurance and safety, the organization and implementation of a quality control in the BRICS countries are of particular importance. An up-to-date review of the current joint activities of the Federal Service for Supervision of Health Care (Roszdravnadzor), the Ministry of Health of Russia and the Ministry of Industry and Trade of Russia on the results of the participation in specialized activities of the BRICS countries, in particular, in consultations to discuss the draft Memorandum of Understanding and Cooperation in the field of the medical products regulation between the regulatory authorities of the BRICS countries, is presented in the work by Samoylova A.V. and Kudryavtseva E.M. [7]. From the point of view of the pharmaceutical industry, a special role is played by the requirements for the quality system in the structure of Good Manufacturing Practice (GMP) and GMP inspectorate procedures, which is presented in the work by V.N. Shestakov and Y.V. Podpruzhnikov. [8]. This study summarizes international recommendations in this area; an additional professional retraining program for specialists "Rules of organizing production and quality control of drugs – theory and practice of a GMP-inspection / audit" has been developed, approved of and tested. An equally important issue is also the order and principles of price regulation systems for registered MPs in the BRICS countries, the study and identification features of which the work by Gorin S.F. et al. [9] is devoted to. Despite the fact that the main group of domestically produced MPs is represented by reproduced MPs, the researchers come to the conclusion that confirms the need for a further cooperation between Russia and major economic alliances – EEU and BRICS [10] on the issues of organizing the production of raw materials of a pharmaceutical quality for the MPs manufacturing.

To study the normative legal regulation of health care systems in the BRICS countries, the research on the example of Brazil t has been conducted. For example, the work by S.A. Belousov and E.A. Tarasova [11] notes the country's experience in developing a modern public health care system and creating conditions of an equal accessibility to medical care for all segments of the population, highlighting the experience of individual social systems. In the light of the provisions of a number of normative and judicial acts of general and special character in the field of the public health care, the provision of drugs to the population and the protection of industrial property rights, the issues of a patent law validity are one of the decisive ones when exporting technological solutions or products. In this regard, in relation to pharmaceutical products, the key legal problems and ways to solve them before and after the accession of one of the BRICS countries (Brazil) to the World Trade Organization (WTO) are detailed in the work by Belikova K.M. [12]. Speaking about the diversification of partnerships and the intensification

of the already existing international contacts, including the expansion of institutional partnerships within the Russian Federation, the focus on a deeper interaction with friendly partners (EEU countries, BRICS, Shanghai Cooperation Organization – SCO) in the context of this study, the work by Belov F.D. and Zvolinskaya O.V. presents the results of monitoring the activities of world-class research centers in the priority area – "Personalized medicine, high-tech healthcare and health-saving technologies" for 2020–2022 [13].

The recent works devoted to the prospects of improving the legislation on manufacturing medicines in the Russian Federation, should be also noted. A profound and strategically significant assessment of the work in this direction is presented by the head of the working group on the revival of compounding pharmacies at the Health Protection Committee of the State Duma of the Federal Assembly of the Russian Federation, the deputy of the State Duma of the VIIIth convocation, Doctor of Sciences (Medicine), Associate Professor, Honored Physician of the Republic of Tatarstan, A.Z. Farrakhov. [14]. Modern and prospective pharmacopoeial requirements for the quality of compounded drug products (CDPs) are presented in the work by Shishova L.I. et al. [15]. Nevertheless, due to the insufficient amount of information in the Russian-language literature on the regulation of drug manufacturing in the current legal systems of the BRICS countries, it was concluded that the actual aim of this study was to improve the understanding and knowledge of Russian regulators, specialists in the field of health care organizations, public health, pharmacy, as well as medical and pharmaceutical professionals regarding the legal requirements for POs and MOs engaged in manufacturing activities on the territory of the BRICS countries, including requirements for the creation of a modern, high-tech healthcare infrastructure capable of manufacturing necessary and demanded types of MPs from highly toxic (hazardous) substances.

This article provides systematized information on the key features of regulation in manufacturing of drugs, as well as allows to develop an effective list of priority measures to achieve prospective and joint goals of the BRICS commonwealth in the development of the interaction between the MPs research and the development sectors, building cooperation ties covering the entire life cycle of pharmaceutical products between the countries of the interstate association (Union) in the field of personalized, predictive and preventive medicine, high-tech healthcare and health-saving technologies, including the rational use of MPs (primarily antibacterial drugs). The present study meets one of the key aims of the Russian cooperation in the BRICS association – to strengthen scientific and technological independence through the development of a hyperlocal healthcare infrastructure in the field of drug manufacturing, which also stimulates a domestic production of MPs, medical devices, machinery and equipment, and thus contributes to the achievement of the targets for increasing a trade turnover between the states of the interstate association (Union). In case decisions are made on the formation of common principles and rules for the drug circulation in the BRICS countries to form a common market, the results of the work can serve as a basis for the harmonization of regulatory approaches for MOs and POs in the organization of activities for manufacturing of drugs. The study promotes the progress towards the convergence of regulatory requirements for the drug circulation in the BRICS member countries, allows to deepen understanding and familiarize with the experience of organizing activities in the field of individual and small-scale drug manufacturing of different classes (radiopharmaceuticals, biologics, biotechnology, high-tech and other types of drug therapy). The joint efforts of the authors are aimed at developing balanced proposals to stimulate research and development of technologies for individual drug manufacturing, personalized reconstitution (dilution) and in-pharmacy packaging of registered drugs (finished dosage forms, FDFs), as well as in general to improve the competitiveness of domestic technological solutions and developments, to accelerate the development of new competencies in the domestic pharmaceutical industry.

Turning to the study of the of the legal regulation experience of the BRICS countries in drug manufacturing, it is important to note that a further development of pharmaceutical activities with the right of manufacturing drugs can be considered in terms of different ways of classification and organization of the licensing system. To date, there are two main approaches to the regulation of compounding pharmacies:

1. In the structure of the North American regulation, where POs are divided into two lists — 503A and 503B, taking into account that the latter must comply with GMP requirements, where a number of GMP provisions and requirements are inapplicable to the activities of compounding

DOI: 10.19163/2307-9266-2024-12-2-172-194

pharmacies due to the peculiarities of pharmacy technologies, quality control methods used and an individualization of pharmacotherapy to the needs of a particular patient.

2. In the European regulatory structure, which uses the concept of risk levels based on which different requirements are set for processes, premises, equipment, analytical methods, a quality assurance system and, accordingly, the requirements for different classes of active substances are separated according to their toxicity level within the framework of the Good Pharmacy Practice (GPHP) system.

The above models are discussed in detail in the previous works by the author's teams [16–19], including the Russian experience of organizing drug manufacturing activities [16, 20].

In the vast majority of the BRICS countries, similar to the Russian practice, there is a two-tier system of education of pharmaceutical specialists. At the same time, a few certain differences which are not the main subject of this study and not included in the final scope of work, have been identified. Therefore, the authors decided to simplify the reader's perception of these features by using in the text the definitions "pharmacist" to denote specialists with higher pharmaceutical education, and "pharmacist assistant", as specialists with secondary pharmaceutical education, as well as their common aggregate in the form of a "pharmaceutical specialists". In addition, the BRICS countries have created conditions for recording information about staffing and employment of these specialists. This information is organized in the relevant state registers: employees are required to undergo the accreditation procedure to obtain the admission to pharmaceutical activities and to master periodic professional development programs, which conceptually also corresponds to the procedures adopted in Russia [21]. This paper presents some key features of pharmaceutical education and conditions of specialists admission to pharmaceutical activities. The article by Mandrik M.A. et al. details the state of educational programs, the international experience and current trends in drug manufacturing as the factors that initiate the transformation of pharmaceutical education [22].

For the purpose of the unification, it was also decided to use the definitions "a hospital pharmacy" to denote a pharmacy, which is a structural subdivision of a medical organization of any ownership form, and "a public pharmacy", as a pharmacy that carries out a retail trade (dispensing), primarily in outpatient settings, which also meets the international conceptual apparatus.

THE AIM of the work was to analyze the regulatory mechanisms and current approaches to the organization of activities in the field of drugs compounding, presented in the legislation of the BRICS interstate association (Union) member countries, including their structuring (systematization) in order to develop proposals for the convergence of these practices.

MATERIALS AND METHODS

Empirical, theoretical, quantitative methodological tools were used in the work. In particular, a wide list of relevant information sources was analyzed; information from regulatory legal documents governing the activities of compounding pharmacies in the BRICS countries, implemented by a bibliometric method, was also obtained.

The authors analyzed regulatory legal documents and databases of Brazil[8,9], India[10], China[11,12], Republic of South Africa[13,14], UAE[15], Iran[16], Egypt[17], Ethiopia[18,19] available in the open sources.

The search was performed using the following keywords: "drug", "drug manufacturing", "pharmacy organization", "medical organization", "compounding", "drug preparation", "drug dilution (reconstitution)" in English, Portuguese, Spanish, Chinese and Arabic.

To analyze the results of studies by other authors, relevant sources of information and data from the following search engines — biomedical research PubMed, a scientific electronic library elibrary.ru, Google Academy, were used. Similar key queries were

[8] The president of the Republic. Available from: https://www.gov.br/planalto/pt-br
[9] Brazilian Health Regulatory Agency. Available from: https://antigo.anvisa.gov.br/
[10] Ministry of Health and Family Welfare, Government of India. Available from: https://mohfw.gov.in/
[11] State Administration of Market Regulation. Available from: https://www.samr.gov.cn/
[12] National Health Commission of the PRC. Available from: http://en.nhc.gov.cn/
[13] South African Government. Available from: https://www.gov.za/
[14] The Southern African Legal Information Institute. Available from: https://www.saflii.org/
[15] UAE Legislation. Available from: https://uaelegislation.gov.ae/
[16] The Ministry of Health and Medical Education. Available from: https://behdasht.gov.ir/
[17] Egyptian Drug Authority. Available from: https://edaegypt.gov.eg/
[18] Ethiopian Food and Drug Authority. Available from: http://www.efda.gov.et/
[19] Ethiopian Legal Information Portal. Available from: https://www.lawethiopia.com/

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

also used for the search.

The literature and normative legal documents were searched for the period of 1900–2024; the choice of the period was due to the specifics of the publication of legislative acts in the BRICS countries. The key, but not exhaustive, criteria for recognizing normative legal documents, legislative acts as relevant and for their further consideration, were the presence of provisions on (about) in them: a regulation of MPs circulation issues, organizing medical and POs, specifics of the procedure and requirements for licensing (premises, equipment, processes, personnel, etc.), rules / practices for manufacturing, a quality control and dispensing of medicines.

According to the specified directions and keywords, 1875 sources of information were found; after excluding invalid data, the final review included 50 most relevant works in relation to the above criteria.

RESULTS AND DISCUSSION

Brazil

According to Brazilian Law No. 5991 dated 17 December 1973[20], there are two kinds of POs: the ones with the right of compounding drugs and the POs dealing with FDFs.

The performance of POs pharmaceutical activities in Brazil is regulated by two main legal regulations:

– Decision of the Collegial Council of the National Agency for Health Surveillance of Brazil No. 44 dated August 17, 2009[21] – describes the basic requirements for FDFs retail sale and is identical in its content to the Rules of Good Pharmacy Practice of Drugs (hereinafter – Order No. 647)[22] in Russia;
– Decision of the Collegial Council of the National Health Surveillance Agency of Brazil No. 44 dated October 8, 2007[23] (hereinafter – GMP of Brazil) – represents the GMP Rules for manufacturing MPs.

The Brazilian GPhPs contains an extensive conceptual framework dedicated to drug manufacturing activities, including the following definitions: “pharmaceutical care”, “pharmaceutical services”, “drug dispensing”, “regulatory documentation”, “standard operating procedures” (SOPs) [23], “officinal” and “magistral formulation” [16], “classified premises”, “validation”, “verification”, etc. [16].

Annex No. 1 of Brazilian GPHP structurally and substantively repeats the main chapters of GMP, and also discloses the specifics related to POs activities in the field of drug compounding. For example, basic provisions and requirements are established for (including, but not limited to): premises of the compounding pharmacy, equipment and SOPs, labeling of raw materials, etc.

The key features of the above Annex No. 1, as applied to the present study, are:

1. Possibility to use scientific literature in the absence of necessary pharmacopoeial monograph (general and (or) private), as well as the realized right of POs to carry out an independent development of necessary specifications for raw materials, quality control methods for CDPs.
2. Purified water is subjected to a full pharmacopoeial analysis at least once a month.
3. Mandatory kinds of a quality control for non-sterile DFs compounded for a particular patient by prescription, are carried out according to the following indicators: a description, organoleptic properties, an average mass (volume), pH (if applicable), mass (volume) of CDPs, mass (volume) of semi-finished products before packaging. At the same time, a full pharmacopoeial quality control of such drugs is carried out at least once every 3 months.
4. Each series of MPs compounded as a stock preparation (SP) shall be evaluated for the following: organoleptic properties; pH (if applicable); an average weight and/or volume; viscosity (if applicable); ethanol content (if applicable); density (if applicable); a quantitative analysis of a drug substance (DS); microbiological purity (if applicable).

In this case, a PO must necessarily have a technical possibility, necessary equipment and materials to ensure the quality of compounded drugs in accordance with the above subparagraphs. An assessment of a quantitative composition and microbiological purity can be carried out in an outsourcing laboratory (center) of a MP quality control. A documented in-pharmacy control shall be

[20] Law No. 5991 of 17 December 1973. Available from: https://www.planalto.gov.br/ccivil_03/leis/l5991.htm?hidemenu=true
[21] Resolution of the Collegial Council of the RDC No. 44 dated August 17, 2009. Available from: https://antigo.anvisa.gov.br/legislacao#/visualizar/28425
[22] Order of the Ministry of Health of the Russian Federation No. 647n dated August 31, 2016 “On Approval of the Rules of Good Pharmacy Practice of Medicines for medical use”. Available from: https://docs.cntd.ru/document/420377391
[23] Resolution of the RDC Board No. 67 dated November 08, 2007. Available from: https://antigo.anvisa.gov.br/legislacao#/visualizar/28030

DOI: 10.19163/2307-9266-2024-12-2-172-194

carried out during compounding stock SPs. There is no minimum sample size for quality control purposes and it should be statistically representative for the size of the series to be compounded.

Annex No. 2 of the Brazilian GPHP establishes requirements for compounding drugs with "a low therapeutic index" (port. Substâncias de Baixo Índice Terapêutico), i.e. the drugs characterized by a high biological activity with a minimal change in dosage (valproic acid, aminophylline, carbamazepine, cyclosporine, clindamycin, etc.). Thus, for example, in the specified document, there is a requirement to use the smallest size of capsules containing drugs of this type.

Annex 3 of the Brazilian GPHP, which establishes the minimum requirements necessary for handling the CDPs made from hormonal MPs, antibiotics and cytostatic substances, is of particular importance. The Annex prescribes a mandatory availability of different compounding premises with an airlock for all classes of the listed substances and independent air supply systems (heating, ventilation and air conditioning (HVAC)). Such premises must be negatively pressurized in relation to the adjacent compounding premises and designed to prevent bulk substances from entering other areas of the compounding pharmacy.

Annex No. 4 of the Brazilian GPHP is devoted to the compounding of sterile DFs, which is structurally and content-wise fully similar to Chapter 797 of the US Pharmacopoeia. The requirements for compounding premises in terms of a microbiological purity and the number of particles in the air correspond to the GMP requirements. The same document specifies the main parameters of compounding highly toxic MPs corresponding to Chapter 800 of the US Pharmacopoeia. A detailed review of the US Pharmacopeia in terms of drug compounding is presented in the monograph [16] and the article on pharmacy drug compounding in the USA [17].

Annex No. 5 of the Brazilian GPHP describes the basic requirements established for homeopathic MPs. Annex 6 of the Brazilian GPHP establishes requirements for MP reconstitution (dilution) processes.

According to the Decision of the Collegial Council of the National Agency for Health Surveillance No. 63 dated December 18, 2009[24], RPPs compounding is carried out in hospital (at a MO) (hereinafter - hospital pharmacy), or retail / outpatient (outside a MO) (hereinafter – compounding pharmacies) in compliance with the requirements of a radiation safety and the rules of good practice in RPPs compounding. The admission to this type of activity is carried out by undergoing additional professional education.

India

When considering the legislation of India, the peculiarities of circulation, organization of production and manufacturing "traditional" MPs, which occupy a significant part in the structure of the Indian health care system – Ayurvedic, Siddha and Unani MPs [24–26], were not included in the present study.

According to clause "f)" of Article 3 of the Indian Medicines and Cosmetics Act dated 10 April 1940[25], the concepts of "manufacturing" and "production" are separated in the meaning, similar to the Russian legislation on the circulation of MPs.

According to Sections 6, 12, 33 and 33-N of the Act referred to, the Central Government of India is empowered to frame rules for the circulation of MPs. One of the main documents is the "MPs and Cosmetics Rules" (hereinafter referred to as the Indian Rules)[26].

To obtain a license for opening a pharmacy, the licensee must meet the requirements for premises, equipment, staff qualifications and other features set out in Annex No. N of the Indian Rules. When issuing a license for opening a pharmacy, the licensing authority shall take into account the average of licenses issued or reinstated during the last three years, i.e. the rule of limiting the number of pharmacies by a geographical area may be applied.

According to Article 3 of the Indian Pharmacy Act dated 4 March 1948[27] (hereinafter referred to as the Indian Pharmacy Act), the Central Government of India forms the Pharmacy Council of India, which has the power to make regulations in accordance with the Act. The core business of manufacturing MPs is governed by Good Pharmacy Practice Rules of India (hereinafter referred to as GPHP of India)[28] approved by the Pharmacy Council of India.

GPHP of India interprets "compounding" as a

[24] Resolution of the CRD No. 63 dated December 18, 2009. Available from: https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2009/rdc0063_18_12_2009.html

[25] The Drugs And Cosmetics Act, 1940. Available from: https://indiankanoon.org/doc/1891720/

[26] The Drugs and Cosmetics Rules, 1945. Available from: https://indiankanoon.org/doc/16293633/

[27] The Pharmacy Act, 1948. Available from: https://indiankanoon.org/doc/549550/

[28] REGD. No. D. L.-33004/99. Available from: https://www.pci.nic.in/pdf/Pharmacy%20Practice%20Regulations.pdf

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

process of preparation, mixing, packaging or labeling an MP or an article used: according to a prescription for an MP written by a health care provider, or at the request of an individual [18, 19] for an over-the-counter MP; or for the purpose of carrying out research, development, including training processes, conducting clinical trials, chemical analysis of an MP, but not intended for sale or dispensing.

It seems appropriate to pay special attention to the accepted qualifications of pharmacists in India. According to the Indian Pharmacy Act, the following types of accredited professionals are recognized:

1. "Public pharmacist" – provides pharmaceutical counseling and dispensing of prescription drugs in a public pharmacy.
2. "Hospital pharmacist" - functions within a PO as a structural subdivision of the MO, provides the latter with the necessary goods of the pharmacy assortment, takes part in the pharmaco-economic substantiation of the drug therapy choice.
3. "Consultant pharmacist" – provides pharmaceutical counseling and dispensing of over-the-counter drugs in a public and/or hospital pharmacy.
4. "Clinical pharmacist" - provides pharmaceutical counseling, providing patients with information on indications, contraindications for use, precautions, features, possible adverse reactions in the use of drugs, and promotes health, well-being and prevention of diseases in order to optimize treatment.

The provisions of Article 65 of the Indian Regulations stipulate that MPs compounding must be carried out under the supervision of a pharmacist, and only a pharmacist may prepare MPs containing substances from Annexes H (a list of prescription MPs) and X (e.g. amphetamine) of the Indian Regulations in accordance with Article 8.1 of the GPHP of India.

According to the survey conducted by the Indian Institute of Health Management and Research, Bangalore [27], it is shown that only 66 hospitals out of 107 follow the recommendations of FIP/W.H.O. [28, 29] or the GPHP of India, which is only 62% of the total sample.

Having comprehensively studied various aspects of the organization of drug compounding activities, the authors came to the conclusion that the regulation of this activity in India is in the initial stage of development, which began in 2015 – the GPHP of India was adopted, which established requirements for education programs, qualification levels of pharmaceutical specialists, the order of interaction of pharmaceutical boards. In addition, in India, there are various types of methodological guidelines from professional associations, compliance with which has a recommendatory nature.

China

In the People's Republic of China, Articles 69–76 of the Law dated 20 September 1984, on the Control of Medicines (hereinafter referred to as the Chinese Law)[29] are devoted to drug compounding, according to which this type of activity can be carried out exclusively by MOs. At the same time, the latter must obtain a license for drug compounding, which is a part of the medical activity. Only pharmacists are allowed to be involved in drug compounding processes. MOs holding a license for drug compounding must develop and implement a quality assurance system. However, a substitution of any raw material in a prescription may only be made after a consultation with the treating physician. In addition, a transfer of CDPs between different unrelated MOs is allowed. According to Clause 27 of Order No. 26[30] of the State Food and Drug Administration of China (hereinafter referred to as CFDA) [30] dated 31 January 2007, dispensing of compounded MPs is allowed to patients in the MOs where they are compounded, and the transfer of CDPs to third-party MOs must be approved by the regional health authority. At the same time, the sale of CDPs to wholesale organizations and POs is prohibited.

Based on the provisions of Articles 20–27 of China's Law Enforcement Regulations[31], China has a two-tiered process for obtaining a license for drug compounding, where the immediate licensing procedure is preceded by an inspection of MPs by regional health authorities.

CFDA Order No. 20 dated 22 June 2005 (hereinafter referred to as Order No. 20)[32] established a ban on the drug compounding of registered MPs; the MPs containing

[29] Drug Administration Law of the People's Republic of China at 20 Sep 1984. Available from: https://www.gov.cn/xinwen/2019-08/26/content_5424780.htm

[30] Order of the State Food and Drug Administration No. 26 at 31 Jan 2007 "Measures for the Supervision and Administration of Drug Circulation". Available from: https://www.gov.cn/ziliao/flfg/2007-02/15/content_527789.htm

[31] Implementing Regulations of the Drug Administration Law of the People's Republic of China at 2 March 2019. Available from: https://www.gov.cn/gongbao/content/2019/content_5468873.htm

[32] Order of the State Food and Drug Administration No. 20 at 22 June 2005 "Administrative Measures for the Registration of Preparations in Medical Institutions (trial implementation)". Available from: https://www.gov.cn/gongbao/content/2006/content_292146.

DOI: 10.19163/2307-9266-2024-12-2-172-194

narcotic drugs, psychotropic and toxic substances; and RPPs.

In view of clauses 7, 19–25 of Order No. 20, the specific composition of the CDPs compounded in MOs must be registered by the regional health authorities, for which a special form must be filled out and sent to these state authorities. The CDPs registered for the first time must undergo clinical trials on the basis of MOs on at least 60 patients, upon the completion of which a summary report on the results of clinical trials must be submitted to the regional health authorities.

In case of natural calamities, epidemics, emergencies or clinical cases, when MPs (deficiency) required for treatment of the population are not available within one province [31], by order of the regional public health authorities, the MO is allowed to manufacture and dispense all types of MPs without any restrictions, which is established in item 26 of Order No. 20. Drug manufacturing must be carried out according to the approved SOPs, which are submitted together with a special form when CDPs are registered by the regional public authorities.

The quality of MPs manufactured in medical organizations shall comply with the Chinese Pharmacopoeia[33] and the requirements of the Good Manufacturing Practice for MPs in MOs approved by CFDA Order No. 27 dated 13 March 2001 (hereinafter referred to as China's GPhP)[34].

China's GPHP is an adaptation of GMP rules applicable to MPs compounding activities, which establishes: a personal responsibility of the chief physician for safety and quality in the use of prescribed CDPs; the need to allocate separate premises for MPs compounding and a ban on combining the head of compounding and a quality control in one person; the formation of different production zones (premises) for MPs compounding using active substances of different classes and DFs; measures to prevent cross-contamination; and the introduction of a cleanroom classification compliant with GMP requirements, etc.

In general, China's GPHP is similar to the regulation of POs type 503A in the USA [16, 17]. As noted above, compounding of RPPs without a manufacturing license is prohibited in China.

United Arab Emirates

The UAE is a constitutional federation consisting of seven emirates [32]. Medical and pharmaceutical licensing processes are regulated by the UAE Ministry of Health and Prevention [33], the Abu Dhabi Department of Health and the Dubai Health Authority. Until 2014, each authority had separate and independent requirements for licensing procedures[35] and in general approaches to the organization of the health care system [34], which have now been unified into one set of standards and requirements for all health care professionals, including pharmaceutical specialists.

In the scientific search of the regulatory legal documents governing the health sector throughout the UAE, it was found that there are 29 legislative acts, executive decrees and regulatory requirements of subordinate organizations, 23 of which have been adopted since 2019 (an active phase of the centralization of regulation) until now[36,37,38,39,40].

Article 1 of the UAE Federal Law No. 8 dated 19 December 2019. "On Medicines, Pharmaceutical Activities and Pharmaceutical Organizations"[41] (hereinafter – UAE Law) establishes the main definitions and concepts used in the field of the drug circulation. The definition of "Pharmaceutical profession" fixed by the legislation is a health profession (specialties) aimed at improving the level of health of citizens through the implementation of pharmaceutical advice on the correct or optimal use of MPs based on scientific specialized knowledge. As follows from the definition, a pharmaceutical profession in the UAE has a systemic role, functions and tasks that correspond to a deep integration into the activities of the health care sector, which, from the point of view of the Russian legislation, could be enshrined in the legislation on the fundamentals of a public health protection as pharmaceutical care. Types of pharmaceutical profession are defined by the

[33] National Pharmacopoeia of China. Available from: https://ydz.chp.org.cn/

[34] Order No. 27 "Quality management specifications for the preparation of preparations in medical institutions (trial implementation)" at 13 March 2001. Available from: https://www.gov.cn/zhengce/2021-06/30/content_5723541.htm

[35] Ministry of Health, Health Authority of Abu Dhabi, & Dubai Health Authority (2014) Healthcare professional qualification requirements 2014, United Arab Emirates: Author; P. 1–123. Available from: https://ru.scribd.com/doc/276006215/Healthcare-Professionals-Qualification-Requirements-PQR-2014-1

[36] Federal Law on the Public Health. Available from: https://uaelegislation.gov.ae/en/legislations/1456

[37] Federal Law Concerning the Use of the Information and Communications Technology in Health Fields. Available from: https://uaelegislation.gov.ae/en/legislations/1209

[38] Federal Law Concerning Private Health Facilities. Available from: https://uaelegislation.gov.ae/en/legislations/1204

[39] Federal Law Regulating the Practice of the Medical Profession. Available from: https://uaelegislation.gov.ae/en/legislations/1201

[40] Federal Law on Veterinary Products. Available from: https://uaelegislation.gov.ae/en/legislations/1207

[41] Federal Law on Medical Products, Pharmacy Profession and Pharmaceutical Establishments. Available from: https://uaelegislation.gov.ae/en/legislations/1426

Order of Implementation of the UAE Law (hereinafter – Resolution No. 90)[42]. Its Article 22 specifies the provision of a wide range of pharmaceutical and clinical consulting services both in technical and scientific terms. With regard to the qualification of pharmaceutical specialists, Article 6 of Decree No. 90 specifies the requirements for the necessity to undergo the admission procedure to perform pharmaceutical activities, and there is a reference to the relevant procedure. Subspecies of a pharmaceutical profession (activities) or pharmaceutical specialties are also defined by Unified Healthcare Professional Qualification Requirements[43] and Clause 5.2.2.9.1. of the DoH normative document[44,45]. Attention should be paid to the presence of provisions on "health care practitioners"[46] in the UAE Federal Law No. 6 dated May 31, 2023 "On the Employment of Certain Health Professions by Non-Medical and Pharmaceutical Professionals"[47]. The concept of "an authorized ("responsible") pharmaceutical specialist", a pharmacist responsible for the quality of pharmaceutical services provided by the PO, should be highlight separately, the information about it is included in the composition of the license for a pharmaceutical activity. The authors separately distinguish the concept of "an authorized ("responsible") pharmaceutical specialist", a pharmacist responsible for the quality of pharmaceutical services provided by the PO, information about which is included in the license for a pharmaceutical activity.

Drug compounding is a type of the performed work, the rendered services, constituting a pharmaceutical activity, which is established by subparagraph "b" of paragraph 1 – Article 22, paragraph 1 of Decree No. 90. According to the available data, drug compounding is performed in 32% of pharmacies in the UAE [34]. The provisions of the UAE Law divide POs into public and hospital ones, which refer to the collective concept of "a pharmaceutical institution".

To obtain a pharmaceutical license for both retail and drug compounding activities, a PO must comply with the technical and health conditions specified in UAE Ministerial Resolution No. (228) dated 13 October 2023, "Technical and health conditions for compounding pharmacies"[48] (hereinafter referred to as Resolution No. (228). Herewith, the director (head) of the compounding pharmacy must necessarily appoint a full-time pharmacist who has an appropriate authorization to carry out activities and, among other things, acts as a responsible pharmacist. The authors found that the pharmacies exclusively engaged in retail sales of registered MPs must meet certain sanitary and hygienic conditions set forth in the UAE Minister of Health and Prevention Decree No. 932, dated 2012, "On sanitary conditions to be observed in public pharmacies".

Regulation No. 228 establishes specialized definitions and concepts for the following pharmacies: "MPs traceability system" (requirements for the labeling of compounded MPs, which define the mandatory presence of a serial number or non-repeating symbols for each compounded MP, the date of compounding [month and year], an indication of the category of a compounded MP [individual – compounded by prescription or SP, a series number [batch], a composition, number of doses in the package, pharmacy details and a quality assurance analysis number [QAA] of the compounded MP); "A quality assurance system of a manufacturing pharmacy" (an internal system of requirements and rules developed and approved by each compounding pharmacy, compliant with current regulatory legal documents, which allow to ensure a proper quality of an implementation of all organization processes of the drug compounding activities, including activities on traceability after dispensing); "Record on starting and raw materials" (detailed information about the starting and raw materials in the CDPs, including sources of acquisition, and references to documented quality methodologies – a shelf life of any starting and raw material ingredient cannot be less than the "beyond-use date" – BUD of the CDPs); "MP Compounding Record" (documenting the fulfillment of technological processes); "Use-by date" (the date that the

[42] Cabinet Resolution Concerning the Executive Regulations of Federal Law Concerning Medical Products, Pharmacy Profession and Pharmaceutical Establishments. Available from: https://uaelegislation.gov.ae/en/legislations/1523

[43] Unified Healthcare Professional Qualification Requirements (PQR). Available from: https://www.doh.gov.ae/en/pqr

[44] Pharmacist and Pharmacy Technician Scope of Practice. Available from: https://www.doh.gov.ae/-/media/E160783B819C479D90E4DF8BAA108737.ashx

[45] Unified Healthcare Professional Qualification. 3rd Version, – 146, – 2022. Available from: https://www.dha.gov.ae/uploads/072022/Unified%20Healthcare%20Professional%20Qualification202273235.pdf

[46] Federal Law on the Practice of Some Medical Professions by Persons Other Than Physicians and Pharmacists. Available from: https://www.dha.gov.ae/uploads/092023/Federal%20Law%20no2023944635.pdf

[47] Renewal of a License to Practice as a Pharmacist. Available from: https://mohap.gov.ae/ar/services/renewal-of-a-license-to-practice-as-a-pharmacist

[48] Ministerial Resolution No. (228) of 2023 AD Technical and health conditions for the compound pharmacy, 28 Rabi ' al-awwal 1445h-13 October 2023. Available from: https://www.dha.gov.ae/uploads/102023/Ministerial%20Decision%20no2023107514.pdf

DOI: 10.19163/2307-9266-2024-12-2-172-194

compounding pharmacy sets for the compounded MP after which its use is prohibited – BUD), "Analysis (SOA)" (a certificate containing the results of a laboratory analysis of the compounded MP), "Pharmacy SOPs" (internal standards approved by the compounding pharmacy, which must not contradict an applicable law and which must be complied with by all employees of the compounding pharmacy; "Compounded drug product", "Annual Report", etc. For the last item, it should be specified that the report should contain information on each compounded MP: a composition of active substances and their concentration per unit; information on the starting and raw materials used; DF, dosage and route of administration; description of packaging; a number of units compounded or produced; a tracking identification code for each CDP; information on CDPs release (in case of release by prescription – patient data, a patient identification number, a copy of the prescription; in case of release at the request of a MO – a MO name, a copy of the contract for CDPs compounding services, a copy of the MO's request; information on the doctor and his license number; a report on a patient's medication treatment plan (upon a request) and patient information, including an identification number); information on the expiry date and period of the CDPs use; the date of release to the PO, a delivery to the PO, the time of the CDP receipt, including the time of the receipt affected by the time of compounding (which is significant for CDPs). The annual report must be submitted by the PO to the appropriate UAE Minister of Health and Prevention department not later than January 31 of the following year following the year during which the activity was performed and also upon a request by the licensing control authority.

The authors emphasize that a compounded drug product is an MP obtained (compounded or manufactured) by collecting or mixing raw materials, materials, or changing the qualitative or quantitative composition of active ingredients (in compounding) from FDFs, and dispensed by a compounding pharmacy for retail and wholesale sale for a domestic circulation to meet the needs of patients as prescribed by a physician or the needs of the MO in which it will be used, including dispensed raw materials in unprocessed (prepackaged mono-component doses) or partially processed. Resolution No. 228 specifies that hospital compounding pharmacies or POs belonging (by an ownership form) to public and private MOs are allowed to compound registered MPs ("to perform technological operations with registered MPs"). In this case, the composition of the compounded MP must *qualitatively* correspond to that stated in the registration certificate and subject to the prescription of such an MP in accordance with the indications for use established in the instructions for the medical use approved by the UAE Ministry of Health. In such cases, the hospital compounding pharmacy is required to obtain a UAE GMP certificate similar to that for MP manufacturers as stipulated in Article 23 of the UAE Law, including the requirements of Article 88 regarding quality management standards. The UAE GMP inspection and certification procedure is carried out by the MP Department of the UAE Ministry of Health. These provisions should be linked to the provisions of par. 1.4.2. part 1 of Resolution No. 228, which establishes a mechanism that allows the MO, in special cases and upon an approval of the authorized health authority, to enter into a service contract with a compounding PO for the production of pilot series of registered MPs intended for clinical trials of a "special nature", provided that the quality composition is maintained and the indications for the use of such MPs are consistent with the instructions for a medical use approved by the UAE Ministry of Health. In such cases, an executed contract between the MO and the compounding PO is required, as well as an application to the UAE competent health authority for the authorization (approval request). The application (request for approval) requires a justification of the reasons for the contract and a description of the cohort of patients for whom the therapeutic or pharmacoeconomic benefits are expected to be realized based on their treatment plan. In this case, the agreement made between the MO and the compounding PO should specify the mechanism and requirements for the transportation, shipping and storage of CDPs in accordance with the regulations governing these requirements. The authors believe that they are referring to the need to comply with the standards of good practice for storage, distribution, transportation and shipping (distribution) of MPs in the UAE, the Gulf Cooperation Countries, the procedure for issuing a Certificate of Compliance which is carried out by the UAE Ministry of Health and Prevention[49], in the manner prescribed by the UAE Minister of Health and

[49] Issue a Certificate of Compliance with the good Practice Standards of a Pharmaceutical Establishment. Available from: https://mohap.gov.ae/ar/services/issue-a-certificate-of-compliance-with-the-good-practice-standards-of-a-pharmaceutical-establishment

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Prevention Decree No. (22)[50] dated 15 February 2022. As required by Regulation No. 228, a compounding PO is responsible for the quality of CDPs and a MO is responsible for verifying the stability, safety and efficacy of CDPs. Thus, medical professionals who write a prescription or a request from the MO for CDPS share the responsibility with pharmaceutical professionals to ensure the safety of a dose selection and when taking the compounded MP, as well as in case of adverse reactions, effects or any symptomatic abnormalities in the patient, are obliged to inform the UAE Ministry of Health. It is considered, that this mechanism is particularly significant and promising for providing MPs to patients with rare (orphan) diseases.

Currently, compounding registered MPs is completely prohibited for registered pharmacies in the Russian Federation, and the "contractual" mechanism or model for determining MPs risk levels described in the previous paragraph, has not been implemented in the legislation. Although in compounding and dispensing under drug prescriptions of monocomponent CDPS containing monocomponent DSs without any excipients, which have undergone the procedure of grinding and in-pharmacy filling (e.g. streptocide powder, glucose powder, etc.), the standard prescription formulations of CDPSs do not contain any excipients (e.g. streptocide powder, glucose powder, etc.). in standard formulations "da tales doses numero"), as well as in case of dispensing according to the requirements of the MO of FDFs, which have undergone the procedure of intra-pharmacy filling (a procedure of breaking the primary packaging), the qualitative and quantitative composition of the registered MP does not change either.

It is important to pay attention to the technical conditions of p. 1.1.3. part 1. Decree No. 228, according to which compounding pharmacies may compound and dispense OTC MPs in the amount calculated according to the average monthly quantity of MPs manufactured according to doctors prescriptions and requirements of the Ministry of Health for the 3 previous months. The condition for compounding and dispensing of OTC CDPs (on a requirement of an individual) [18, 19] is also a due compliance with all technical and hygienic conditions of Decree No. 228 with fulfillment of requirements for dispensing of safe and quality CDPs within the established limit of MPs use (BUD). The same paragraph establishes the regulatory elements important from the point of view of a scientific exchange and increasing the availability of CDPs:

- compounding POs are prohibited from compounding formulations for which there is no scientific data or pharmacopoeial articles in the UAE approved pharmacopoeias according to Article 1(1) of the UAE Law;
- in addition, if the pharmacist is aware of the practice of another compounding PO with regard to the prescribed CDPs, it is acceptable to use the reference formulations of some other compounding PO, as well as those CDPs and DF formulations approved for use by the MO or public authority, provided that there is a signed non-disclosure agreement or contract between the compounding PO to disclose full information on the formulation, available regulations, CDPs and the necessary documentation for their compounding, quality control procedures, and the safety of use information, including the condition that these compounding POs have not been issued warning letters or restrictive measures by the competent health authority in the reference state of the UAE. With respect to this mechanism, according to Resolution No. 228, the UAE Ministry of Health reserves the right to determine the level of its responsibility for physical, territorial and price accessibility to provide safe and quality CDPs.

General requirements for compounding pharmacies are set forth in part 2 of Resolution No. 228; in addition to the above requirements, the key ones are:

- clause 2.4. indicates that compounding POs are prohibited to compound MPs in the form of transdermal therapeutic systems, MPs of the plant origin, dosed aerosols, as well as powder and dry mixtures for inhalation, except in cases where a deficiency or defect with regard to registered MPs is established;
- clause 2.8. specifies that all formulations (prescriptions) shall be manufactured in accordance with the requirements of the current pharmacopoeia and shall comply with PO quality assurance documents;
- clause 2.9. stipulates that the raw materials used in the drug compounding, shall be approved

[50] Ministerial Resolution No. (22) of 2022 AD regarding the regulation of the transportation, storage and distribution of medical products or raw materials used in their manufacture. Available from: https://www.dha.gov.ae/uploads/082022/Ministerial%20Decision%20no2022856380.pdf

DOI: 10.19163/2307-9266-2024-12-2-172-194

by the UAE authorized health authority, have a remaining shelf life of at least two thirds of the total shelf life, be subject to control at least once a year in an accredited laboratory;
– clause 2.18. defines storage conditions for raw materials and finished products, which must meet the requirements of the current pharmacopoeia or the manufacturer's instructions.

The chapter "General requirements" of Decree No. 228 to compounding POs defines the provisions that in order to perform pharmaceutical expertise of drug prescriptions and MOs requirements, verification of medical prescriptions, the manufacturing pharmacy is obliged to use current scientific literature, including information on active ingredients in the structure of documentation for registered MPs, reflecting the use of the data in the quality assurance system documents as a mechanism (regulation) of compounding POs. Also, the quality assurance system documents of compounding POs must also reflect the mechanism approved by the authorized ("responsible") pharmacist, providing for the recall of compounded MPs or CDPs series from the circulation in accordance with the pharmacovigilance guidelines approved by the UAE Ministry of Health.

Decree No. 228 consists of 9 parts with annexes, however, in its content part, it is not an independent GPHP of MPs compounding and dispensing, it determines general requirements and technical conditions for the implementation of MPs compounding activities. The authors came to the conclusion that, from the point of view of norms in the organization of pharmaceutical activities with the right to compound MPs, the above-mentioned system is partly comparable to the approaches in the current Russian legislation, where Order No. 647 applies exclusively to the retail sale of MPs and ignores the peculiarities of MPs compounding activities in POs. Herewith, the main provisions and design of UAE Order No. 228 are highly comparable to the current Russian Rules for Manufacturing and Dispensing MPs[51], except for certain provisions allowing POs to compound registered MPs while complying with GMP requirements. Thus, this model of regulation of compounding POs in the UAE is highly comparable to the model implemented in the USA.

[51] Order No. 249n of the Ministry of Health of the Russian Federation dated 22 May 2023 "On Approval of the Rules for the manufacture and release of medicines for medical use by pharmacy organizations licensed for pharmaceutical activities". Available from: https://docs.cntd.ru/document/1301699481

South Africa

Pharmacy compounding of MPs in Republic of South Africa is governed by the Medicines and Related Substances Act of South Africa No. 101 dated 1965[52], where the activities of POs must comply with the Pharmacy Organizations Act of South Africa No. 53 dated 1974[53] [35]. Based its provisions, all pharmacists and POs must comply with the South African Good Pharmacy Practice Regulations (hereinafter referred to as the South African GPHP)[54].

The South African GPHP in its preamble harmonizes with the European Union's concept of pharmaceutical care as a set of pharmaceutical services, and also exposes the role of pharmaceutical specialists in the South Africa public health.

In general, the South African GPHP is a comprehensive document dealing with all aspects of POs pharmacy operations, including quality assurance system requirements, and in relation to compounding pharmacies, the document contains the following sections with their own peculiarities:

1. Compounding all types of sterile DFs:
   – in compliance with GMP requirements for compounding sterile MPs;
   – with the possibility of a retrospective microbiological CDPs control.
2. Reconstitution (dilution) of non-toxic parenteral DFs:
   – when a laminar flow cabinet is available.
3. Compounding and reconstitution (dilution) of parenteral cytostatics:
   – with at least a Class 2 biosafety box and uniformity of regulations for POs and MOs;
   – with compliance with protective clothing and proper cleaning requirements;
   – with additional toxic substance training for the staff.
4. Compounding of non-sterile drugs:
   – at a minimum, under the supervision of a pharmacist;
5. Intra-pharmacy packaging of registered MPs:
   – in compliance with GMP regulations, in terms of primary MPs packaging, and Good Distribution Practice regulations, according to storage and transportation requirements.

[52] Medicines and Related Substances Act 1965. Available from: https://www.saflii.org/za/legis/consol_act/marsa1965280/
[53] Pharmacy Act 1974. Available from: https://www.saflii.org/za/legis/consol_act/pa197498/
[54] Rules relating to good pharmacy practice. Available from: https://www.saflii.org/za/legis/consol_reg/rrtgpp362/

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

In accordance with the South African GPHP, compounding of RFLPs in POs is carried out in accordance with the GMP requirements for the production of radiopharmaceutical MPs and sterile MPs.

The described system of normative legal regulation of MPs compounding activities is comparable to that in the USA [16, 17].

Ethiopia

In reviewing the regulatory framework for MPs compounding in Ethiopia, it should be noted that its health governance structure is based on the North American experience of the public administration, both in terms of the existence of a single mega-regulator with a similar name (Ethiopian Food and Drug Authority) and the publication of explanatory documents in the form of guidelines [16, 36].

In Ethiopia, the circulation of MPs is regulated by Proclamation No. 1112 dated 28 February 2019[55], and according to Article 34, of which compounding of MPs is carried out from drugs. Despite the fact that according to the text of the proclamation there is no prohibition on compounding parenteral drugs, the authors of this study were not able to identify any legal acts regulating this type of activity, which can also be traced from the data of scientific publications on pharmacy MPs compounding in Ethiopia [37–40].

However, in 2022, the now defunct Ethiopian MPs Control Authority issued Good Practice Guidelines on compounding[56]. In its introductory part, it declared that compounding MPs in the form of DFs for a local use was a common practice of POs and MOs. For the latter, the document fragmentarily increased the requirements for the necessary quality assurance system, premises, labeling, personnel, equipment, documentation, raw materials.

In 2020, the Ministry of Health of Ethiopia issued three-chapter National Guidelines for the Administration of Dermatological MPs[57], which also applies to POs and MOs. The first chapter describes the background of the document, the second chapter is devoted to the direct regulation of MPs compounding activities and is a direct translation of the provisions of the US Pharmacopeia for non-sterile DFs (503A type pharmacies) [16, 17], the third chapter contains a list of dermatologic CDPs formulations with a composition and suggested methodology for their compounding.

The authors of this study were unable to ascertain from available sources of information the availability of the infrastructure and regulations for radiopharmaceutical MPs compounding activities in Ethiopia.

Iran

In Iran, the main regulator of the pharmaceutical market and MPs circulation is the Ministry of Health and Medical Education (hereinafter – the Ministry of Health of Iran), its executive bodies are: Food and Drug Administration of Iran (hereinafter referred to as IFDA) and the Supreme Council of Health Insurance (SCoHI) [41]. According to the latest IFDA statistics, there are about 11 036 functioning pharmacies in Iran, 10,028 of which are public pharmacies and the rest are hospital pharmacies[58].

Pharmaceutical legal framework in Iran is divided into 5 separate levels: constitutional; long- and medium-range planning, including relevant legislation; pharmaceutical legislation; subordinate regulations; international rules and agreements [42]. The general elements of the MPs regulation system of MPs circulation of Iran, relevant for the purpose of this study, are:

1. According to Article 3 of the Iranian Law on Medical, Pharmaceutical, Food and Drinking Products (hereinafter referred to as the Iranian Law) and subsequent amendments, import, export, sale and purchase of MPs without obtaining a license from the Iranian Ministry of Health are prohibited, Ch. 4 defines the requirements for the production and import.
2. IFDA exercises the authority to register MPs and maintains the relevant state register[59].
3. In a certain part it can be said that the analog of the list of Vital and Essential Drugs of the Russian Federation is the List of Iranian Medicines (hereinafter – IML)[60], which is formed and revised by the Iranian Drug Selection Committee (IDSC), which is a part of IFDA and has pharmaceutical specialists [43].
4. Iranian law restricts the production, import,

[55] Proclamation No. 1112/2019. Available from: http://www.fmhaca.gov.et/wp-content/uploads/2020/06/Food-and-Medicine-Administration-Proclamation-1112.pdf

[56] Good Pharmaceutical Compounding Laboratories. Available from: https://www.ethiopianreview.com/pdf/001/Labcomp.pdf

[57] Ministry of Health-Ethiopia. National guideline for compounding of dermatological preparations; 2020.

[58] Ministry of Health. The Food and Drug Administration. The business of pharmacies; 2018. Available from: https://www.fda.gov.ir/

[59] IFDA. Available from: https://www.fda.gov.ir/ورود-هرادا-ی‌اه-کنی‌ل/روش‌ک-ی‌ی‌ورود-ی‌م‌س‌ر-ت‌س‌رهف

[60] Ibid.

DOI: 10.19163/2307-9266-2024-12-2-172-194

distribution and prescribing MPs not included in the IML, while there is a mechanism under Iran's Emergency Pharmaceutical Care Centers (EPC) Law to provide MPs not included in the IML in cases of shortage (deficiency) of approved MPs or for the treatment of patients with life-threatening conditions. In such cases, Art. 2 of the Law stipulates that the physician and the patient must sign a consent that they understand the risk of possible lack of efficacy in the use of such MPs and (or) the occurrence of a certain amount of side effects.

5. In addition to the retail markup for MPs, POs of Iran are authorized to impose a surcharge for MPs vacation[61];
6. Based on the Iranian Law on Supply of Medicines, in case of lack of interest from the private sector to invest and open POs in remote and disadvantaged areas, the Iranian Ministry of Health is obliged to provide relevant MPs through subordinate MOs to ensure the availability of MPs.
7. Iran has not acceded to the WTO and has not signed the Agreement on Trade-Related Aspects of Intellectual Property Rights [44], but has accepted the World Intellectual Property Organization agreement and adopted the Law on Patents, Industrial Designs and Trademark Registration[62].

According to the Iranian Law, a pharmacy is a health care institution that dispenses MPs and provides various pharmaceutical services to the public. According to Article 2 of the above law, the fulfillment of all regulatory requirements imposed on POs should be supervised only by the responsible official (pharmacist).

According to Article 4 of the Iranian Pharmacy Law (hereinafter referred to as the Iranian Pharmacy Law) [42], the types of services of community pharmacies are specified, and each qualified pharmaceutical specialist is allowed to obtain only one pharmacy license. Unqualified pharmaceutical personnel ("pharmacy technicians") are included in the pharmaceutical specialists, in respect of whom the legislation does not establish any requirements on the need to complete pharmaceutical education programs and (or) obtain admission to pharmaceutical activities, while unqualified pharmaceutical personnel are trained directly on the job. The duration of higher pharmaceutical education programs is 5.5 years[63].

The Iranian Pharmacy Law defines restrictions on the maximum possible number of pharmacies based on the number of urban or rural population.

The duties of a pharmacist are set out in Article 25 of the Iranian Pharmacy Law, which includes: performing technological operations for in-pharmacy packaging of MPs; compounding and dispensing MPs, including galenic ones.

In addition, Article 33 of the Iranian Pharmacy Law defines the minimum areas for POs, including 24-hour POs, as well as storage conditions.

The authorse have established the studies in which various Iranian authors have repeatedly noted the problems of the need to improve the level of safety and health protection of medical and pharmaceutical specialists involved in the compounding of MPs. It is also necessary to note the problem of the negative impact of cytotoxic MPs on the medical personnel in the implementation of activities for the recovery (dilution) of MPs and the lack of specialized POs that would carry out the compounding of MPs from highly toxic and hazardous substances [45, 46]. The authors note significant technical availability limitations of the open sources and regulatory legal documentation of Iran on the issue under study, difficulties in translation from Persian (Farsi) [47], an insufficient level of the representation in the public domain of regulatory legal documents of the Ministry of Health of Iran and IFDA, including blocking an access to a number of resources on the Internet, also through virtual private networks (VPNs). At the same time, a relevant study [48] was analyzed, which shows the experience of Iranian pharmacists in establishing the first compounding pharmacy under the Ministry of Health of Iran for the drug compounding that meets the GMP principles, as well as Chapters 797 and 800 of the US Pharmacopeia. The paper describes the main stages of creating a modern, high-tech pharmacy infrastructure, including a construction of clean premises, adopted approaches to the human resource management, building process principles of a quality assurance and a quality control system, the development and SOPs implementation,

[61] Principles notified by the Food and Drug Organization under No. 57412/655 dated 17/06/2019 «Notification of instructions on how to obtain the tariff for pharmaceutical services in the year 1400». Available from: https://arakmu.ac.ir/vcfd/fa/news/18004/هفرعت-زاسوراد-لاعف-روضح-رب-یدج-تراظن-م وزل-هناخوراد-رد-یئوراد-تامدخ هناخوراد-رد-ینف-لویئسم

[62] Patents, Industrial Designs and Trademarks Registration Act. Available from: http://www.wipo.int/wipolex/en/details.jsp?id=7706

[63] Pharmacy Education and Regulations in Iran. Available from: https://irimc.org/en/Regulations/Pharmacy-Education

the documentation and automation processes. The study refers to two standards (not available in the public domain) that govern clean premises specifications for cytotoxic MPs compounding, established requirements to locate the facilities either within a hospital pharmacy structure or in a close proximity to an injectable MPs administration unit for chemotherapy (a centralized MPs dilution (reconstitution) room as a part of a medical license [16]). In addition, the article postulates the existence of guidelines for the management of chemotherapy services adopted by the Iranian Ministry of Health, which stipulate the need for such an infrastructure in every MO that has anti-tumor drug therapy (chemotherapy) departments with 12 or more beds.

In the Russian-language literature, the presence of a study of the pharmaceutical sector in Iran, which reflects the standing and prospects of the market, but is also limited to references to primary sources of the regulatory framework, was found [49]. The work devoted to the peculiarities of introducing Russian pharmaceutical products to the Iranian market and based on the practical experience, which also concluded that it is necessary to conduct a comprehensive and sufficiently deep study and the analysis of political, socio-cultural, economic, regulatory and legal aspects of business activities in Iran, is of particular interest[64].

Egypt

The Egyptian Drug Authority (EDA)[65] is the national regulatory authority for MPs. The EDA reports directly to the Prime Minister[66] and its activities are supervised by the Ministry of Health and Population of Egypt (Ministry of Health and Population). The functional structure of the EDA includes three independent organizations:

1. The Central Administration for Pharmaceutical Affairs (CAPA), which covers the registration, pricing, regulation of promotion and/or advertising of MPs, as well as control and supervision functions of participants in the sphere of the drug circulation.
2. The National Organization for Drug Control and Research (NODCR), responsible for the quality control of circulating pharmaceutical products, cosmetics, medicinal plants, insecticides, raw materials and products of the natural origin.
3. The National Organization for Research and Control of Biologicals (NORCB), which carries out the procedures for authorizing the sale (trade, dispensing) and licensing of various professional activities.

It can be said that for the Egypt's pharmaceutical regulatory system, the national regulatory authority for MPs is the specialized body of the African Union – the African Medicines Agency (AMA)[67,68] whose objectives include the development and support of a harmonized regulatory framework, the development of the pharmaceutical industry, including MPs, traditional medicines and medical devices, including the ones within the framework of the African Continental Free Trade Area ("Africa Continental Free Trade Area" AfCFTA).

Egypt's main legislative documents in the sphere of circulation of medicines include the following:

1. Law No. 127 dated 10 March 1955 on Pharmaceutical Activities (hereinafter referred to as Egyptian Law No. 127)[69,70].
2. Law No. 15 dated 5 March 2017 "On simplifying the procedure for issuing licenses to industrial facilities"[71].
3. Resolution of the Minister of Industry and Trade No. 1082 dated 16 August 2017. "On the implementing regulations to Law No. 15 dated 5 March 2017. "On Simplifying the Procedure for Issuing Licenses to Industrial Facilities"[72].

[64] Tsatsyn N. Features of the introduction of pharmaceutical products to the Iranian market (from practical experience). In: GxP News. Available from: https://gxpnews.net/2019/10/osobennosti-vyvoda-farmacevticheskoj-produkcii-na-rynok-irana-iz-prakticheskogo-opyta/?ysclid=m1m9k8z262569120004

[65] Law No. 101 of 1971 on Medicine, Medical Technology Management and the Egyptian Drug Authority (with changes at 25.08.2019). Available from: https://edaegypt.gov.eg/media/52vavp5k/2019-151.pdf

[66] Prime Ministerial Resolution No. 777 of 2020 Issuing the executive regulations of the law establishing the Egyptian Authority for Unified Procurement, Supply, Medical Agents, Medical Technology Management and the Egyptian Drug Authority. Issued by Law No. 101 of 1971. Available from: https://edaegypt.gov.eg/media/es5a1awn/قرار-رئيس-الوزراء-رقم-777-لسنة-2020-باصدار-اللائحة-التنفيذية.pdf

[67] Treaty for the Establishment of the African Medicines Agency. Available from: https://au.int/sites/default/files/treaties/36892-treaty-0069_-_ama_treaty_e.pdf

[68] The Treaty on the Establishment of the African Medicines Agency was adopted at the 38th ordinary session of the Assembly of the African Union on 11 February 2019, which entered into force on 5 November 2021 (currently 38 of the 55 member States of the African Union have signed and/or ratified the said treaty). Russian

[69] Egyptian Pharmacy Practice Law Number 127. Available from: https://edaegypt.gov.eg/media/v0wfy2bk/1955-127.pdf

[70] Amendments to Law No. 127 of 1955. Available from: https://edaegypt.gov.eg/ar/القوانين-والقرارات-والقواعد-المنظمة-و-الإشعارات/القوانين-واللوائح-التنفيذية/تعديلات-قانون-رقم-127-لسنة-1955/

[71] Law No. 10 of 2017 Issuing the Law to Facilitate Procedures for Granting Industrial Establishments Licenses. Available from: https://edaegypt.gov.eg/media/4t1j33qb/2017-15.pdf

[72] Ministry of Commerce and Industry Decision No. 1082 of 2017 issuing the executive regulations of the law regulating the procedures for the inspection of hazardous materials issued by Law No. 15 of 2017. Available from: https://edaegypt.gov.eg/media/zs4ja0ex/2017-1082.pdf

DOI: 10.19163/2307-9266-2024-12-2-172-194

4. Other laws regulating the clinical trials, operating procedures, collection of blood and plasma for the production and export.

The key subordinate regulatory legal documents in the field of organization of pharmaceutical activities with the right to compound MPs include:

– Ministerial Decree No. 265 dated 16 April 1981 "On sanitary and technical requirements to be observed in pharmaceutical enterprises"[73];
– Ministerial Decision No. 25 dated 18 January 2009 "On requirements for the licensing of pharmacies"[74];
– Ministerial Decision No. 114 dated 3 February 2017 "Regarding union certificates required in the licensing of public pharmacies"[75];
– Decision of the Chairman of the Authority No. 271 dated 25 May 2021 "Regarding the conditions to be observed in pharmacy organizations"[76];
– Decision No. 265 dated 21 May 2024 of the President of the Authority "On issuing a methodological guide on the mechanisms, procedures for verifying and monitoring the implementation of the correction plan submitted by stores, warehouses and pharmacies"[77].

According to the Egyptian Pharmacists Syndicate (EPS), the total number of community pharmacies in Egypt had reached the figure of 95 000 by the end of 2023. Herewith, the number of pharmacists reached 313 000, up from 175 000 in 2013 and 71 000 in 2003. Egyptian Law No. 127 stipulates that public pharmacies in Egypt (dispensing only FDFs) can operate not only with pharmacists, but also with other non-regulated pharmacy personnel, which includes their assistants who have neither medical nor pharmaceutical education. Although the law restricts the formation of pharmacy networks, the provisions on "pharmacy management" allow a pharmacy technician to manage a group of pharmacies, thus forming pharmacy networks [50].

There is a significant number of regulatory documents[78] for Egyptian POs that reflect modern approaches in the organization of MPs compounding activities. In particular, compounding pharmacies are subject to the regulations of the "Egyptian Guidelines for Oncology Pharmacy Practice" (EGOPP) in the format of good manufacturing practice for MPs used for the treatment of cancer. The guidelines consist of 2 volumes[79].

"MPs compounding" refers to the process by which unit operation steps are carried out to produce the MPs suitable for use by the patient.

The first volume contains "Guidelines for the Manufacture of Sterile MPs, Non-sterile MPs and Safe Handling of Highly Active (Hazardous) Substances". Its content and provisions directly refer to the regulatory requirements of the US Pharmacopeia, Chapters 795, 797 and 800 [16, 17].

Sterile MPs made from non-hazardous substances include: parenteral nutrition, hydration protocols, chemotherapy premedication, and antibiotics.

For sterile MPs made from hazardous substances, the Guidelines include: antineoplastic drugs; any drugs identified in the "List of Antineoplastic and Other Hazardous Drugs in Health Care Settings," compiled by the National Institute for Occupational Safety and Health (NIOSH)[80,81].

The minimum requirements for the facilities and quality assurance system of a compounding pharmacy are presented in Chapter 3 of the Guidelines.

For non-sterile MPs, the Guidelines include topical MPs for the extravasation and MPs used for oral mucositis caused by chemotherapy and/or radiotherapy.

In a separate volume, there are "Guidelines for compounding biological MPs and biosimilars"[82].

[73] Ministerial Decree No. 265 of 16/04/1981 on the health and technical requirements to be met in pharmaceutical factories. Available from: https://edaegypt.gov.eg/media/0flb3fyk/1981-265.pdf

[74] Ministerial Decision No. 25 of 18/01/2009 On the requirements required for licensing pharmaceutical stores. Available from: https://edaegypt.gov.eg/media/cw5ozyh4/2009-25.pdf

[75] Regarding the Union certificates required during the licensing of public pharmaciesministerial Decision No. 114 of 02/03/2017. Available from: https://edaegypt.gov.eg/media/xrek4hlt/2017-114.pdf

[76] Decisions of the Ministry of Health and population decision no. 271 of 25/05/2021. Available from: https://edaegypt.gov.eg/media/قرارئيس-الهيئة-قرم-271-2021.pdf

[77] Guidelines for File Assessment for Pharmaceutical Products for Human Use. Available from: https://edaegypt.gov.eg/media/zpidyytc/guidelines-for-file-assessment-for-human-pharmaceutical-product.pdf

[78] Regulatory evidence. Central Pharmaceutical Care Administration Guidelines. Available from: https://www.edaegypt.gov.eg/ar/القوانين-والتشريعات-والقواعد-المنظمة-لإشراف-الأدلة-الإرشادية-للممارسة-الصيدلية-في-مجال-علاج-السرطان

[79] Egyptian Guide for Oncology Pharmacy Practice. Available from: https://edaegypt.gov.eg/media/hrqcj435/edrexgl-cap-care-011-egyptian-guide-for-oncology-pharmacy-practice-volume-1-2022-2.pdf

[80] NIOSH List of Antineoplastic and Other Hazardous Drugs in Healthcare Settings, 2016. Available from: https://www.cdc.gov/niosh/docs/2016-161/pdfs/2016-161.pdf

[81] Hazardous Drugs: Draft NIOSH List of Hazardous Drugs in Healthcare Settings, 2020; Procedures; and Risk Management Information. Available from: https://www.federalregister.gov/documents/2020/05/01/2020-09332/hazardous-drugs-draft-niosh-list-of-hazardous-drugs-in-healthcare-settings-2020-procedures-and-risk

[82] Egyptian Guide for Oncology Pharmacy Practice. Available from: https://edaegypt.gov.eg/media/vkvbbcmd/eda-guide-for-oncologu-pharmacy-practice-vol-2-biosimilars-chapter_1.pdf

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

CONCLUSION

For the first time, this study presents generalized materials on the main regulatory approaches and requirements for the implementation of activities in the field of MPs compounding applied in the regulatory systems of the BRICS interstate association (Union) member countries. This study has no analogues in domestic and foreign literature. The study presents key regulatory legal acts and documents, analyzes them and describes the main provisions of the legal framework for organizing the activities of compounding pharmacies. The results of the study determine the need to rethink the current state of the Russian regulation of MPs compounding. The work emphasizes the need for the development and implementation of a single, harmonized good practice of MPs compounding and dispensing, applicable to any of the types of compounded MPs, the FD type, the intra-pharmacy filling and reconstitution (dilution) of registered MPs both within pharmaceutical and medical activities, including in the format of " hospital exemptions". The implementation of the only currently missing element of the good practices system is particularly important in achieving the national health care development goals established in the Russian Federation in accordance with the project "New Health Saving Technologies", a transition to modern, advanced practices of mastering critical technologies, personalized, predictive and preventive medicine, high-tech health care and health saving technologies, including the ones through the rational use of MPs, the use of genetic and genetic technologies, the use of genetic data and technologies, mastering the compounding of biomedical cellular products and tissue engineering products, which are of high interest for medical and pharmaceutical science.

In the sphere of MPs compounding, the BRICS countries can strive to develop the best practices and "gold" standards for the organization of relevant socially important activities, regulation and accessibility of CDPs. The principle of the BRICS alliance does not initially impose on its members one or another way of the state governance and normative legal regulation, but seeks to improve the quality and living standard of the member countries citizens.

In this sense, there is no need for total the harmonization or unification of activities regulation in the field of MPs compounding, but it makes sense to study the best practices in this area in the BRICS countries, to highlight and implement them, and for the BRICS governance bodies to strive to achieve high indicators of the member countries of the association: organization, regulation, accessibility, innovativeness of CDPs. This approach could lead to the creation of a single GPHP, but does not require it at the moment due to the need to focus on the main parameters of MPs circulation. The study of the best practices and development of "gold" standards can become the task of a world-class scientific center. Such practices and standards may include the following:

– Brazilian experience of using scientific literature in the absence of the necessary pharmacopoeial monograph, as well as the POs right to independently develop necessary specifications for raw materials, quality control methodologies on CDPs;

– experience of India, when the Central Government of India forms the Pharmaceutical Council;

– UAE's experience, which allows the compounding of registered MPs only in cases where no change in their qualitative composition is carried out;

– quantitative indicators of an availability of CDPs to the residents of the country, etc.

Given the fact that by now, there is information about the interest of additional 34 countries in the activities of the association (the creation of BRICS partner states is being discussed), the Russian Federation, which is chairing BRICS this year, faces the task of facilitating the fastest possible integration of the new member countries into all mechanisms of the association. From the point of view of improving the interaction efficiency between representatives of the BRICS countries in the healthcare and pharmaceutical industries, the authors note the presence of national medical research centers and world-class scientific centers established and operating in Russia, created in accordance with the Decree of the President of the Russian Federation No. 939 dated 22 June 1993 and p. 2, art. 5 of Federal Law No. 127-FZ dated 23 August 1996. Their activities, in accordance with the priorities of scientific and technological development of the Russian Federation and the directions of development of the most important science-intensive technologies provided for by the Decree of the President of the Russian Federation No. 529 dated 18 June 2024, are aimed at research and development in the field of preventive and personalized medicine, ensuring healthy longevity, development of high-tech healthcare, health-saving technologies, and MPs. It is also possible to initiate the process of creating a World-Class Scientific and Educational Center in the field of drugs manufacturing, organized on the basis of integration of higher education institutions, scientific organizations and their cooperation with organizations who's operating in the real sector of the economy. In

DOI: 10.19163/2307-9266-2024-12-2-172-194

general, as for the BRICS member countries, the results of this study necessitate a further in-depth elaboration of the convergence issues of regulatory practices of both health care systems and pharmaceutical industries including clinical trials, MPs inspection and registration, the MPs production and compounding, including radiochemistry and radiation safety.

Another promising direction of the interaction is the pharmacopoeias requirements harmonization of the member countries association – it is an exchange of experience in the CDPs compounding and the nomenclature analysis of the most demanded for CDPs compounding between the member countries, which will also contribute to ensuring the uniformity of approaches and requirements to the quality of the MPs compounded in pharmacies. In line with the goals and objectives of the Concept of Russia's Participation in BRICS, the results of this study raise the question of the need to develop and form a "Roadmap (an action plan) for the development of the interaction between the BRICS member states in the field of health care and the pharmaceutical industry", which could become the basis for the establishment (expansion of existing centers) in the form of a national pharmaceutical research center or a world-class scientific center in order to perform the functions of promoting the most rapid integration and building a modern model of the common MPs market, joint research and development for technological development of the interstate association (Union) countries. The implementation of the above proposals is aimed at achieving the national goals established in the Decree of the President of the Russian Federation dated 07 May 2024 № 309, and their consideration is recommended to the Government of the Russian Federation in the formation of the "Unified plan to achieve the national development goals of the Russian Federation for the period up to 2030 and in the perspective up to 2036".

FUNDING

This study had no financial support from outside organizations.

AUTHORS' CONTRIBUTION

All the authors have made equivalent and equal contributions to the publication. All the authors confirm that their authorship meets the ICMJE international criteria (all the authors have made substantial contributions to the conceptualization, research and preparation of the article, and have read and approved of the final version before the publication).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Putin VV. Russia and the changing world. Public Service. Bulletin of the Coordinating Council on Personnel Issues, State Awards and Public Service under the Plenipotentiary Representative of the President of the Russian Federation in the North-Western Federal District. 2012;(1):99–117.
2. Narkevich IA, Medvedeva DM, Nemyatykh OD, Kuznetsova PV, Trushnikova IO. Pharmaceutical compounding in Russia: analysis of open competitive procurement and segment prospects. Medical Technologies. Assessment and Choice. 2023;(4):64–74. DOI: 10.17116/medtech20234504164
3. Kuznecova PV, Narkevich IA, Medvedeva DM. The use compounding pharmacy of medicines in pediatrics (literature review). Topical issues of the development of Russian pharmacy – Ilyinsky readings: Materials of the XIII Annual Interuniversity Interregional Scientific Conference; St. Petersburg: Military Medical Academy named after S.M. Kirov; 2024. P. 82–92. Russian
4. Mamedov DD. Hospital exceptions in the manufacture of medicines. Pavlovsky Readings 2023: Proceedings of the IX International Scientific and Practical Conference dedicated to the 130th anniversary of the birth of Professor N.K. Vereshchagin, Kursk, 13 October 2023; Kursk: Kursk State Medical University; 2023. P. 64–9. Russian
5. Melnikova EV, Merkulov VA, Merkulova OV. Regulation for the Translation of Gene and Cell Therapy into Medical Practice in East Asian Countries. Bulletin of the Scientific Centre for Expert Evaluation of Medicinal Products. Regulatory Research and Medicine Evaluation. 2024;14(1):29–41. DOI: 10.30895/1991-2919-2024-14-1-29-41
6. Omelyanovskiy VV, Rukavitsyna NP, Mukhortova PA, Kingshott AA, Zinadinov SI, Kharitonova AG, Minakova EI, Krekhtunova LO, Barysheva VO. Medicinal product early access programs: experience of the BRICS group, European Union, and United States of America. Medical Ethics. 2023;(4):7–15.
7. Samoylova AV, Kudryavtseva EM. Organizing and conducting of quality control of medicines in the new circumstances. Vestnik Roszdravnadzora. 2023;4:6–16.
8. Shestakov VN, Podpruzhnikov YuV. Topical issues of

training of GMP INSPECTORS. Drug development & registration. 2016;(2):196–200.

9. Gorin SF, Parfeynikov SA, Gabrielyan NV, Koshel MS. Examine and identify features of the systems regulating the prices of medicines in the European Union countries, the BRICS and CIS. Modern problems of science and education. 2014;(3):731–731.
10. Zurnadjyants Y, Bliznyak O. Prospects for strategic partnership between the EAEU and BRICS countries in the pharmaceutical industry. Krasnoyarsk Science: Economic Journal. 2024;13(1):22–43. DOI: 10.12731/2070-7568-2024-13-1-224
11. Belousov SA, Tarasova EA. Legal regulation of the health care system of the federative republic of Brazil. Bulletin of Kutafin University. 2023;3(106):132–41. DOI: 10.17803/2311-5998.2023.106.6.132-141
12. Belikova KM. The force of patent law in Brazil applicable to pharmaceutical products: legal issues and ways for their solution prior and after Brazil's membership in WTO. Law and Politics. 2019;(7):1–12. DOI: 10.7256/2454-0706.2019.7.29922
13. Belov FD, Zvolinskaya OV. World-Class Research Centers in the Personalized Medicine, Advanced Healthcare, and Health Preservation Technologies Priority Field: Monitoring 2020–2022 Performance Results. Science Governance and Scientometrics. 2023;18(4):831–5. DOI: 10.33873/2686-6706.2023.18-4.811-835
14. Farrakhov AZ. Compounding Pharmacy Restoration as a Current Healthcare Priorit. Regulatory Research and Medicine Evaluation. 2024;14(4):380–5. DOI: 10.30895/1991-2919-2024-14-4-380-385
15. Shishova LI, Yarutkin AV, Bagirova VL. Current and Future Pharmacopoeial Requirements for the Quality of Extemporaneous Medicinal Products: A Review of Regulatory Standards. Regulatory Research and Medicine Evaluation. 2024;14(4):386–99. DOI: 10.30895/1991-2919-2024-14-4-386-399
16. Narkevich IA, Fisenko VS, Golant ZM, Yurochkin DS, Mamedov DD, Erdni-Garyaev SE, Leshkevich AA. Fundamentals of the formation of a unified harmonized system of normative legal regulation in the field of circulation of drugs manufactured by pharmacy organizations: Monograph; St. Petersburg: Mediapapir; 2023. 292 p. Russian
17. Mamedov DD, Yurochkin DS, Leshkevich AA, Erdni-Garyaev SE, Golant ZM, Narkevich IA. Compounding pharmacy regulations: experience of the North American pharmaceutical market. Farmakoekonomika. Modern Pharmacoeconomics and Pharmacoepidemiology. 2023;16(1):80–6. DOI: 10.17749/2070-4909/farmakoekonomika.2022.155
18. Erdni-Garyaev SE, Mamedov DD, Yurochkin DS, Zelikova DD, Golant ZM, Fisenko VS, Narkevich IA. Pharmacy Compounding Regulation in the German Pharmaceutical Market. Part 1. Basic Regulatory Provisions (Review). Bulletin of the Scientific Centre for Expert Evaluation of Medicinal Products. Regulatory Research and Medicine Evaluation. 2024;14(1):91–109. DOI: 10.30895/1991-2919-2024-14-1-91-109
19. Erdni-Garyaev SE, Mamedov DD, Yurochkin DS, Zelikova DD, Golant ZM, Fisenko VS, Narkevich IA. Pharmacy Compounding Regulation in the German Pharmaceutical Market. Part 2. Organisational Features (Review). Regulatory Research and Medicine Evaluation; 2024. DOI: 10.30895/1991-2919-2024-590
20. Mamedov DD, Yurochkin DS, Golant ZM, Fisenko VS, Alekhin AV, Narkevich IA. Past, current and future of legal regulation of drugs compounding in the Russian Federation. Pharmacy & Pharmacology. 2023;11(3):176–92. DOI: 10.19163/2307-9266-2023-11-3-176-192
21. Fisenko VS, Solomatina TV, Farrakhov AZ, Mamedov DD. Analysis of the conditions and development of ways to improve the system of training of pharmaceutical and medical workers aimed at developing the potential of compounding pharmacies in the Russian Federation. Bulletin of Roszdravnadzor. 2023;(4):29–42.
22. Mandrik MA, Sadkovskii IA, Korol LA, Egorova SN, Krasnyuk II, Bykov AV. Development of Extemporaneous Compounding as an Initiating Factor in the Transformation of Pharmaceutical Education: International Experience and Current Trends. Regulatory Research and Medicine Evaluation. 2024;14(4):419–36. DOI: 10.30895/1991-2919-2024-14-4-419-436
23. Savchenko IA, Korneeva IN, Luksha EA, Shmalts MA. Development of an algorithm for making a standard operating procedure (SOP) under conditions of production pharmacies. Journal of Pharmaceuticals Quality Assurance Issues. 2020;2(28):48–55. DOI: 10.34907/JPQAI.2020.17.53.007
24. Matveev SA Tradition Indian medicine and its preventive meaning in the modern world. Aspirantskiy Vestnik Povolzhiya. 2014;14(7–8):32–6. DOI: 10.17816/2072-2354.2014.0.7-8.32-36
25. Kotova NI. The "revival" of traditional Indian medicine in the 19th and 20th centuries: the phenomenon of "modern" Ayurveda. Siberian Historical Research. 2018;4:218–37.
26. Sharma AK, Pundarikakshudu K. Regulatory Aspects of Traditional Indian Medicines (TIM) in India and in International Purview. J AOAC Int. 2019;102(4):993–1002. DOI: 10.5740/jaoacint.18-0379
27. Roy M. Good Pharmacy Practice in India: Its Past, Present and Future with Need and Status in COVID 19. Bioethical Issues in Healthcare. 2022:149. DOI: 10.5772/intechopen.100635
28. Joint FIP, FIP WHO. J. WHO guidelines on good pharmacy practice: standards for quality of pharmacy services from the WHO technical report series, No. 961; 45th report of the WHO Expert Committee on specifications for pharmaceutical preparations. World Health Organization; 2011. Vol. 20.
29. Hallit S, Sacre H, Sarkis H, Dalloul N, Jaoude CA, Nahhas Z, Dagher J, Sili G, Salameh P. Good Pharmacy Practice Standardized for Community Pharmacists: The

Lebanese Order of Pharmacists Initiative. J Res Pharm Pract. 2019;8(1):29–32. DOI: 10.4103/jrpp.JRPP_18_96

30. Tang W, Huang Y, Zhou D, Huang Y, Chen Y, Ren S, Li Y, Wu S, Zhao X, Song X, Wang H, Jin Y, Yu H, Zhang L, Li Y, Boulton D, Shen K. Evolving drug regulatory landscape in China: A clinical pharmacology perspective. Clin Transl Sci. 2021;14(4):1222–30. DOI: 10.1111/cts.12987
31. Zuenko I. Development of heilongjiang's administrative and territorial system: 1982-2014. Russia and Pacific RIM. 2015;3(89):187–209.
32. Ivanova YuA, Dolgiy MO. United Arab Emirates (UAE): constitutional legislation. Bulletin of Economic Security. 2023;(1):83–9.
33. Alshamsi AI. A review of the United Arab Emirates healthcare systems on medical tourism and accreditation. Front Health Serv. 2024;4:1329252. DOI: 10.3389/frhs.2024.1329252
34. Hassan R, Alam Sher H, Khokhar R, Hussain R. Pharmaceutical Policy in the UAE. Pharmaceutical Policy in Countries with Developing Healthcare Systems. 2017:365–379. DOI: 10.1007/978-3-319-51673-8_18
35. Gray A, Riddin J, Jugathpal J. Health Care and Pharmacy Practice in South Africa. Can J Hosp Pharm. 2016;69(1):36–41. DOI: 10.4212/cjhp.v69i1.1521
36. Suleman S, Woliyi A, Woldemichael K, Tushune K, Duchateau L, Degroote A, Vancauwenberghe R, Bracke N, De Spiegeleer B. Pharmaceutical Regulatory Framework in Ethiopia: A Critical Evaluation of Its Legal Basis and Implementation. Ethiop J Health Sci. 2016;26(3):259–76. DOI: 10.4314/ejhs.v26i3.9
37. Assefa D, Tekassa T, Alemu S. Extent and barriers to providing effective pharmaceutical compounding services in hospital and community pharmacies of a low-income country: Case of Southwest Ethiopia. J Med Access. 2023;7:27550834231183753. DOI: 10.1177/27550834231183753
38. Selam MN, Ababu A, Bayisa R, Abdella M, Diriba E, Wale M, Alemu T, Marew T, Baye AM. Prescribing pattern of dermatological compounding in Ethiopia: The case of ALERT Hospital. Integr Pharm Res Pract. 2022;11:1–8. DOI: 10.2147/IPRP.S346395
39. Selam MN, Ababu A. Extemporaneous Compounding Practice for Dermatologic Preparations in Ethiopian Public Hospitals: Regulatory Requirements and Quality Concerns. Risk Manag Healthc Policy. 2021;14:1933–8. DOI: 10.2147/RMHP.S300906
40. Mohammed AS, Woldekidan NA, Mohammed FA. Knowledge, attitude, and practice of pharmacy professionals on generic medicines in Eastern Ethiopia: A cross-sectional study. PLoS One. 2020;15(7):e0235205. DOI: 10.1371/journal.pone.0235205
41. Ansaripour A, Uyl-de Groot CA, Steenhoek A, Redekop WK. The Drug Reimbursement Decision-Making System in Iran. Value Health Reg Issues. 2014;3:174–81. DOI: 10.1016/j.vhri.2014.04.010
42. Zaboli P, Hashemi-Meshkini A, Varmaghani M, Gholami H, Vazirian I, Zekri HS, Eslamitabar S, Kebriaeezadeh A. Pharmaceutical laws and regulations in Iran: An overview. J Res Pharm Pract. 2016;5(3):155–61. DOI: 10.4103/2279-042X.185709
43. Zargaran M, Nikfar S, Cheraghali AM. Evaluation of prescriptions of medicines not included in Iran medicine list: A cross-sectional study. J Res Pharm Pract. 2016;5(4):234–7. DOI: 10.4103/2279-042X.192456
44. Hashemi Meshkini A, Kebriaeezadeh A, Dinarvand R, Nikfar S, Habibzadeh M, Vazirian I. Assessment of the vaccine industry in Iran in context of accession to WTO: a survey study. Daru. 2012;20(1):19. DOI: 10.1186/2008-2231-20-19
45. Shahrasbi AA, Afshar M, Shokraneh F, Monji F, Noroozi M, Ebrahimi-Khojin M, Madani SF, Ahadi-Barzoki M, Rajabi M. Risks to health professionals from hazardous drugs in Iran: a pilot study of understanding of healthcare team to occupational exposure to cytotoxics. EXCLI J. 2014;13:491–501.
46. Abbasi K, Hazrati M, Mohammadbeigi A, Ansari J, Sajadi M, Hosseinnazzhad A, Moshiri E. Protection behaviors for cytotoxic drugs in oncology nurses of chemotherapy centers in Shiraz hospitals, South of Iran. Indian J Med Paediatr Oncol. 2016;37(4):227–31. DOI: 10.4103/0971-5851.195748
47. Ayati MH, Ardekani MRS, Baghbani M, Zare F. Pathology of Nomenclature of Compounded Drugs in Persian Medicine Based on Gharabadin-e-Salehi, Gharabadin-e-Kabeer, and Gharabadin-e-Azam. Iranian Journal of Medical Ethics and History of Medicine; 2023. DOI: 10.18502/ijme.v16i2.15810
48. Ayazkhoo L, Sistanizad M, Darvishzadeh M, Babaeian M. Strategic insights and practical tips for establishing the aseptic chemotherapy drug compounding unit in a Tehran Hospital: A Comprehensive Guide to Operational Success // Journal of Medical Device Technology. 2024;3(1):40–4. DOI:10.11113/jmeditec.v3.52
49. Mamedyarov ZA. Pharmaceutical Sector in Iran: Current Status and Prospecs. MEMO Journal. 2018;62(7):57–62. DOI: 10.20542/0131-2227-2018-62-7-57-62
50. Youssef A, Amin MEK. Egyptian community pharmacies and self-care: Context, challenges and opportunities. Explor Res Clin Soc Pharm. 2023;12:100384. DOI: 10.1016/j.rcsop.2023.100384

AUTHORS

Dmitry S. Yurochkin – Deputy Head of Laboratory of Regulatory Relations and Good Practices of St. Petersburg State Chemical and Pharmaceutical University. ORCID ID: 0000-0003-4609-0155. E-mail: dmitry.yurochkin@pharminnotech.com

Devi D. Mamedov – junior researcher of

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Laboratory of Regulatory Relations and Good Practices of St. Petersburg State Chemical and Pharmaceutical University. ORCID ID: 0000-0001-5061-0729. E-mail: devi.mamedov@mail.ru

Sergey E. Erdni-Garyaev – Senior Lecturer at the Department of Physical and Inorganic Chemistry, St. Petersburg State Chemical and Pharmaceutical University. ORCID ID: 0000-0001-6297-9304. E-mail: sergey.erdny-garyaev@pharminnotech.com

Aleksei V. Yarutkin – Deputy Director of the Institute of Pharmacopoeia and Medicinal Product Standardisation of Scientific Center for Evaluation of Medical Products. ORCID ID: 0000-0002-3156-5958. E-mail: yarutkin@expmed.ru

Valeria L. Bagirova – Doctor of Sciences (Pharmacy), Professor, Director of the Institute of Pharmacopoeia and Medicinal Product Standardization of Scientific Center for Evaluation of Medical Products. ORCID ID: 0000-0003-0379-6158. E-mail: bagirova@expmed.ru

Pavel S. Guryanov – Candidate of Sciences (Pharmacy), Deputy Dean of the Faculty of Training of Foreign Citizens of Perm State Pharmaceutical Academy. ORCID 0000-0003-0275-9937. E-mail: laboratory.iot@gmail.com

Omar Loudiyi – student of Perm State Pharmaceutical Academy. ORCID ID: 0009-0009-3768-4315. E-mail: omarloud1008@gmail.com

Li Wen – independent researcher, Beijing, China. ORCID ID: 0009-0000-2770-1868. E-mail: wen890425@outlook.com

ПМФИ

ВолгГМУ

MENTE ET ANIMO