Научно-практический журнал
Scientific and Practical Journal

ISSN 2307-9266
e-ISSN 2413-2241

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

PHARMACY & PHARMACOLOGY

2020 Том / Volume VIII

№ 3

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

Краткие сообщения
Brief Reports

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

www.pmedpharm.ru www.pharmpharm.ru

ПМФИ
Филиал ВолгГМУ

Scientific and Practical Journal

PHARMACY & PHARMACOLOGY

Scientific and practical journal
Volume VIII, Issue 3, 2020
The mass media registration certificate:
ПИ №ФС77–67428 от 13.10.2016
ISSN 2307-9266 e-ISSN 2413-2241

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

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

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

Научно-практический журнал

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

Периодичность 6 номеров в год

Том 8, Выпуск 3, 2020

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

ISSN 2307-9266 e-ISSN 2413-2241

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

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

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

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

CONTENTS / СОДЕРЖАНИЕ

Research Article / Оригинальные статьи

Pharmacology and Clinical Pharmacology / Фармакология и клиническая фармакология

Organization and Economy of Pharmacy / Организация и экономика фармацевтического дела

Reviews, Lectures / Обзоры, лекции

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

EFFICACY AND SAFETY OF SOME ETIOTROPIC THERAPEUTIC SCHEMES FOR TREATING PATIENTS WITH NOVEL CORONAVIRUS INFECTION (COVID-19)

L.A. Balykova, V.F. Pavelkina, N.V. Shmyreva, N.A. Pyataev, N.M. Selezneva, O.I. Shepeleva, R.Z. Almyasheva

National Research Ogarev Mordovia State University
26 (Bld.A), Ulyanov St., Saransk, Republic of Mordovia, Russian Federation

E-mail: larisabalykova@yandex.ru

Received 20 September 2020 Review (1) 15 October 2020 Review (2) 20 October 2020 Accepted 22 October 2020

The aim of the study is to assess the efficacy and safety of the Favipiravir (Areplivir) drug, compared to the standard etiotropic therapy in the patients hospitalized with COVID-19.

Material and methods. The research was conducted as a part of an open, randomized, multicenter comparative study of the efficacy and safety of Areplivir, 200 mg film-coated tablets ("PROMOMED RUS" LLC, Russia), in the patients hospitalized with COVID-19. The dosing regimen of Favipiravir was 1600 mg twice a day on the 1st day and 600 mg twice a day on days 2–14. Thirty nine patients were enrolled into the study with a laboratory-established diagnosis of a new type of Coronavirus infection caused by SARS-CoV-2 (confirmed) of moderate severity, with pneumonia. The group of comparison (22 patients) received standard etiotropic therapy, prescribed in accordance with the current version of the temporary guidelines for the diagnosis and treatment of COVID-19, represented mainly by Hydroxychloroquine with the dosage regimen of 800 mg on the 1st day, then 400 mg on days 2–7, and Azithromycin 500 mg once a day for 5 days. The main group (17 patients) received Favipiravir (Areplivir) as etiotropic therapy.

Results. In the main group, the time period until fever disappeared was found to be 1.36 days shorter than in the group of comparison ($p<0.05$); there was a higher rate of the reduction of inflammatory changes in the lungs according to the computer tomography data (38.4% vs 14.9%, $p<0.05$). By the end of the treatment, there was also a lower lactate level in the blood (27.1%, $p<0.05$) than in the patients of the group of comparison. The evaluation of the drug efficacy according to the Categorical Ordinal Scale of Clinical Improvement and measurements of oxygen saturation in the blood, manifested similar positive dynamics in the patients treated according to various etiotropic therapy regimens. By the end of the treatment, the RNA SARS-CoV-2 tests were also negative in all the patients. As for the overall frequency of adverse events (AEs), no relevant distinctions were found between the groups. A greater part of AEs was related to hepatotoxicity, with a predominantly clinically relevant increase in alanine aminotransferase (ALT). A clinically relevant prolongation of the corrected QT interval on the standard ECG was found to occur in the standard-therapy group on day 5, while no serious AEs were registered in the main group. No serious adverse reactions were registered in patients of the main group.

Conclusion. The efficacy of the Favipiravir (Areplivir) therapy for the novel coronavirus infection has proved to be superior to the efficacy of the standard etiotropic therapy in a number of aspects. Basing on the obtained findings, Favipiravir (Areplivir) drug can be recommended for treating patients with the novel coronavirus infection of moderate severity.

Keywords: novel coronavirus infection, COVID-19, etiotropic therapy, Areplivir, computer tomography, corrected QT-interval

Abbreviations: activated partial thromboplastin time (APTT); alanine aminotransferase (ALT); aspartate aminotransferase (AST); blood pressure (BP); upper limits of the norm (UHN); Temporary guidelines (TMR); a categorical ordinal scale of clinical improvement (CPSA); computed tomography (CT); corrected QT interval (QTc); creatine phosphokinase (CPK); adverse event (AE); polymerase chain reaction (PCR); prothrombin time (PTT); blood oxygen saturation (SpO_2); serious adverse events (SAEs); C-reactive protein (CRP); respiratory rate (RR); heart rate (HR); electrocardiogram (ECG).

For citation: L.A. Balykova, V.F. Pavelkina, N.V. Shmyreva, N.A. Pyataev, N.M. Selezneva, O.I. Shepeleva, R.Z. Almyasheva. Efficacy and safety of some etiotropic therapeutic schemes for treating patients with novel coronavirus infection (COVID-19). *Pharmacy & Pharmacology*. 2020;8(3):150-159. **DOI:** 10.19163/2307-9266-2020-8-3-150-159

Для цитирования: Л.А. Балыкова, В.Ф. Павелкина, Н.В. Шмырева, Н.А. Пятаев, Н.М. Селезнева, О.И. Шепелева, Р.З. Альмяшева. Сравнительная эффективность и безопасность различных схем этиотропной терапии у пациентов с новой коронавирусной инфекцией (COVID-19). *Фармация и фармакология.* 2020;8(3):150-159. **DOI:** 10.19163/2307-9266-2020-8-3-150-159

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-150-159

УДК 615.038

СРАВНИТЕЛЬНАЯ ЭФФЕКТИВНОСТЬ И БЕЗОПАСНОСТЬ РАЗЛИЧНЫХ СХЕМ ЭТИОТРОПНОЙ ТЕРАПИИ У ПАЦИЕНТОВ С НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИЕЙ (COVID-19)

Л.А. Балыкова, В.Ф. Павелкина, Н.В. Шмырева, Н.А. Пятаев, Н.М. Селезнева, О.И. Шепелева, Р. З. Альмяшева

Национальный исследовательский Мордовский государственный университет им. Н.П. Огарёва
Российская Федерация, республика Мордовия, г. Саранск, ул. Ульянова, д. 26а

E-mail: larisabalykova@yandex.ru

Получено 20.09.2020 Рецензия (1) 15.10.2020 Рецензия (2) 20.10.2020 Принята к печати 22.10.2020

Цель. Оценка эффективности и безопасности препарата Фавипиравир («Арепливир») в сравнении со стандартной этиотропной терапией у пациентов, госпитализированных с COVID-19.

Материалы и методы. Исследование проводилось в рамках открытого рандомизированного многоцентрового сравнительного исследования эффективности и безопасности препарата «Арепливир», таблетки 200 мг, покрытые пленочной оболочкой, (ООО «ПРОМОМЕД РУС», Россия), у пациентов, госпитализированных с COVID-19. Режим дозирования фавипиравира: 1600 мг 2 р/сут в 1-й день и 600 мг 2 р/сут в 2–14 дни. В исследование включено 39 пациентов, госпитализированных в стационар по поводу лабораторно подтвержденной новой коронавирусной инфекции, среднетяжелого течения с развитием пневмонии. Группа сравнения (22 больных) получала стандартную этиотропную терапию, назначенную в соответствии с действующей версией временных методических рекомендаций по диагностике и лечению COVID-19, представленную преимущественно Гидроксихлорохином (режим дозирования по 800 мг в 1-й день, далее по 400 мг в 2–7 дни) и Азитромицином по 500 мг 1 раз в день в течение 5 дней. Основная группа (17 пациентов) получала в качестве этиотропной терапии препарат Фавипиравир («Арепливир»).

Результаты. В основной группе было отмечено сокращение времени исчезновения лихорадки (на 1,36 дней $p<0,05$), более высокая скорость редукции воспалительных изменений в легких по данным компьютерной томографии (38,4% против 14,9%, $p<0,05$) и более низкий уровень лактата (на 27,1%, $p<0,05$) крови к концу курса лечения по отношению к группе сравнения. Оценка эффективности терапии по категориальной порядковой шкале клинического улучшения и уровня сатурации кислорода крови выявили сходную положительную динамику у пациентов, получавших различные схемы этиотропной терапии. Также у всех пациентов тесты на наличие SARS-CoV-2 по завершению курса лечения показали отрицательные результаты. Значимые различия между группами терапии по общей частоте нежелательных явлений отсутствовали. Большая часть нежелательных явлений касалась гепатотоксичности, при этом преимущественно отмечалось клинически значимое повышение аланинаминотрансферазы. Кардиотоксическое действие в виде клинически значимого удлинения корригированного интервала QT (QTc) на стандартной электрокардиограмме имело место на 5-ый день лечения в группе стандартной терапии, тогда как в основной группе подобных нежелательный реакций отмечено не было. Серьезных нежелательных реакций у пациентов основной группы не зарегистрировано.

Заключение. Эффективность препарата Фавипиравир («Арепливир») в терапии новой коронавирусной инфекции по ряду изучаемых показателей превосходит эффективность стандартной этиотропной терапии. Учитывая полученные результаты, препарат Фавипиравир («Арепливир») может быть рекомендован для лечения больных новой коронавирусной инфекцией средней степени тяжести.

Ключевые слова: новая коронавирусная инфекция, COVID-19, этиотропная терапия, Фавипиравир («Арепливир»), компьютерная томография, корригированный интервал QT

Список сокращений: активированное частичное тромбопластиновое время (АЧТВ); аланинаминотрансфераза (АлТ); аспартатаминотрансфераза (АсТ); артериальное давление (АД); верхние границы нормы (ВГН); Временные методические рекомендации (ВМР); категориальная порядковая шкала клинического улучшения (КПШКУ); компьютерная томография (КТ); корригированный интервал QT(QTc); креатинфосфокиназа (КФК); нежелательные явления (НЯ); полимеразная цепная реакция (ПЦР); протромбиновое время (ПТВ); сатурации кислорода крови (SpO_2); серьезные нежелательные явления (СНЯ); С-реактивный белок (СРБ); частота дыхания (ЧД); частота сердечных сокращений (ЧСС); электрокардиограмма (ЭКГ).

INTRODUCTION

Three epidemics marked the beginning of the 21st century. They were appearing one after the other: a severe acute respiratory distress syndrome caused by SARS-CoV (atypical pneumonia), a Middle-East respiratory syndrome caused by MERS-CoV and, finally, a severe acute respiratory syndrome caused by SARS-CoV-2, the so-called novel coronavirus infection, or COVID-19 [1].

COVID-19 is greatly ahead of the former epidemics of coronavirus infection by the number of infected individuals. Currently, while vaccination against COVID-19 has not become available en masse and its long-term effects have not been evaluated, the effectiveness of

various etiotropic therapy regimens, as well as drugs for pathogenetic treatment, the action of which is aimed at suppressing the secondary effects of the cytokine storm and/or modulating the body's immune system or blocking some specific links in the pathogenesis of a new coronavirus infection (in particular, hypercoagulation), is widely studied [2].

It is known, that in most patients, COVID-19 has mild or moderately forms; however, about 5 to 10 percent of patients encounter serious, potentially life-threatening manifestations and complications. This creates an urgent need to develop and put into practice efficient etiotropic drugs [5, 6]. Despite several hundred already performed and on-going clinical trials assessing the efficacy and safety of various antiviral and immune-modulating medications, the World Health Organization states that at present, there are no drugs with unambiguously proven efficacy against the novel coronavirus infection.

In most conducted trials, only one group was enrolled for medical interference, with a control group absent, some medical drugs are being used up till now on the basis of either in vitro studies or on the basis of extrapolated data, or observational studies [7–9].

The following drugs have been better studied: the efficacy and safety of Hydroxychloroquine, Chloroquine, and Mefloquine both in monotherapy and in combination with Azithromycin, Umifenovir, Remdesivir, Lopinavir/Ritonavir with Interferon-1b, Favipiravir. Some of them have been studied in randomized clinical trials [10–16].

However, only Remdesivir and Favipiravir have not only a high efficiency, but also a selectivity of action, blocking the RNA-dependent RNA polymerase of the SARS-CoV-2 virus. However, Favipiravir has a dual mechanism of action, inducing lethal mutations of viral RNA, helping to reduce the viral load [17–19].

THE AIM of the study was to assess the therapeutic efficacy and safety of Favipiravir (Areplivir, film-coated tablets, OOO "PROMOMED RUS", Russia) compared to those of the standard etiotropic therapy administered in compliance with Temporary Methodological Recommendations of the Ministry of Health of RF aimed at the prevention, diagnostics and treatment of the novel coronavirus infection COVID-19 (version 6 of 28.04.2020 and version 7 dated 03/06/2020). Favipiravir is known to block RNA-dependent RNA-polymerase of SARS-CoV-2 virus [17–19].

MATERIAL AND METHODS

The study was conducted during the pandemic rise of COVID-19 in the Republic of Mordovia (in the period from 01.06.2020 to 01.08.2020) in the research center of "National Research Ogarev Mordovia State University" as part of an open randomized multicenter comparative study of the efficacy and safety of Areplivir, 200 mg film-coated tablets (PROMOMED RUS LLC, Russia), in patients hospitalized with COVID-19.

This article presents the data on the patients admitted only at the above-mentioned center. Thirty-nine patients, aged 21 to 73 years, were enrolled in the study, with a laboratory-confirmed diagnosis "Coronavirus infection, caused by SARS-CoV-2 (confirmed), of moderate severity, with the presence of bilateral pneumonia". The study was approved by the Local Ethics Committee at Ogarev Mordovia State University (Protocol No. 85 dated 27.05.2020), and was also reviewed in the international register of clinical trials (clinicaltrials.gov (NCT04542694)). The diagnosis was confirmed by PCR tests; RNA SARS CoV-2 was identified in the biomaterial of all the patients from the swabs taken in the nasopharynx and/or oropharynx. The diagnosis was established in compliance with the TMR. The patients were admitted at hospitals in Saransk and Ruzaevka.

The criteria of enrollment into the study were: signing and dating an Informed Consent Form of the Patient Information Sheet; male or female gender; the age from 18 to 80; a patient's hospitalization not exceeding 48 hrs prior to administering etiotropic therapy; a positive PCR test result for the presence of RNA SARS-CoV-2; a patient's consent to use reliable preventive measures during the study and for 3 weeks after their completion.

The exclusion criteria were: unavailability of a computed tomography(CT) procedure for some reason (for example, a plaster cast or metal constructions at the site under study); a need for a patient to be treated at the resuscitation and intensive therapy departments; an impaired liver function (AST and/or ALT≥2 upper limits of the norm (ULN) and/or the total bilirubin ≥ 1.5 of ULN) an impaired kidney function (creatinine clearance < 45ml/min); a positive test for HIV, syphilis, hepatitis B and /or C; a chronic heart failure of functional classes III-IV; the syndrome of malabsorption or some other clinically relevant disease of the gastrointestinal tract, which may affect the absorption of the studied drug; the patient's history of malignant neoplasms; alcohol, pharmacologic and/or drug (narcotic) addiction; mental pathology in the history or suspected pathology; severe decompensated or unstable somatic diseases that were life-threatening or deteriorated the patient's prognosis; pregnancy or its planning, breast-feeding.

All the patients were randomized into 2 groups. Group 1 (a group of comparison, n=22) received standard etiotropic therapy, administered in compliance with the treatment regimens stated in the TMR. Twelve patients (54.5%) received a combination of Hydroxychloroquine and Azithromycin as an antiviral therapy, 8 patients (36.4%) – Hydroxychloroquine (monotherapy), 2 patients (9.1%) – Lopinavir/Ritohavir (Calidavir). The dosage regimen was the following: for Hydroxychloroquine it was 800 mg on the first day (400 mg twice a day), then 400 mg/day (200 mg twice a day) for 2–7 days; for Azithromycin: 500 mg once a day for 5 days; for Lopinavir/Ritonavir: 400 mg+100 mg orally every 12 hours for 14 days. The patients were aged from 21 to 73

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-150-159

(the average age was 47.5±1.99 yrs). Group 2 (the main group), 17 patients, aged from 34 to 63 yrs (the average age was 47.12±2.26 yrs), received Favipiravir (Areplivir) as an etiotropic therapy: on day 1 – 1600 mg (8 tablets) twice a day; on days 2–14 of the therapy – 600 mg (3 tablets) twice a day. The main-group patients who received the drug under study, were not allowed to take other medications of the standard etiotropic therapy for COVID-19, in compliance with the TMR or any other antiviral therapeutic medicines.

Parameters under study

According to the study protocol, the following parameters were assessed: clinical status according to the Categorical Ordinal Scale of Clinical Improvement proposed by the World Health Organization (Table 1); test results for the presence of SARS-CoV-2 RNA; body temperature; assessment of changes in the lungs according to "Empirical" Visual Scale(Table 2); (the CT data); a need for the patients to be treated at the Resuscitation and Intensive therapy department; a need for non-invasive ventilation of the lungs; a need for artificial lung ventilation; an incidence of fatal cases; occurrence of undesirable phenomena (UP)/of serious undesirable phenomena (SUP); vital indices (BP, heart rate, respiration rate), the findings of the physical examination; a clinical blood test, a biochemical blood test (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, creatinine, urea, total bilirubin, glucose, C-reactive protein, creatine phosphokinase (CPK), ferritin, lactate); a coagulogram (activated partial thromboplastin time, thromboplastin time (PT), prothrombin time , fibrinogen, D-dimer): data on pulse oximetry with SpO2 measurement; general urinalysis (pH, specific weight, protein, erythrocytes, leukocytes); a test for pregnancy for women capable of childbearing, the ECG (with estimated corrected QT interval (QTc) by Bazett formula; by Framingham formula estimated HR below 60 or over 100 beats per minute); blood test dana for HIV, syphilis, hepatitis B and C. The following equipment was used to perform the studies: clinical blood analysis – hematological analyzer Micros ES 60, Horiba ABX (France), biochemical analysis – biochemical analyzer HUMASTAR 600, Human GmbH (Germany), coagulogram – analyzer-coagulometer KS 1 Delta, Tcoag (Ireland), urine analysis – uroanalyzer Combilyzer 13, Human GmbH (Germany). The analyses were performed using certified reagent kits according to the manufacturer's protocols. Coagulogram and lung CT data were analyzed at the patient's admission at the hospital and on the 15th day of therapy, the duration of the QT interval and laboratory parameters were evaluated on the day of admission at the hospital, on the 5th and 15th day of therapy, virus elimination was judged by the absence of SARS-CoV-2 RNA on the 10th and 11th day from the start of treatment.

Statistical processing of results

Statistical processing of the findings was conducted on a personal computer with the help of Statistica 6.0 and Microsoft Excel programs. Processing of the descriptive statistical data was conducted by estimating the mean arithmetic value (M) and an error to the mean arithmetic value (±m). The average values were compared using the t-Student criterion (for normal distribution of the trait) or the nonparametric Wilcoxon criteria (for paired samples) and Mann-Whitney criteria (for unpaired samples). The selection of the criterion (parametric or nonparametric) was carried out after checking the type of the data distribution for compliance with the normal distribution law using the Shapiro-Wilk criterion. The relevance and significance of the differences for qualitative signs between the compared groups, were determined by the analysis of contingency tables, with computation of exact $\chi 2$ criterion according to Pearson. The results at $p<0.05$ were considered significant.

RESULTS OF THE STUDY AND THEIR DISCUSSION

A comparative evaluation of time periods (in days) had been made until fever disappeared. A criterion for this indicator was the body temperature lower than 37.2 for 3 days in succession, without an intake of antipyretic drugs. In the group of comparison, a period until fever disappeared was 6.36±0.56 days; in the main group it was 5.00 ± 0.34 days. The distinction between the opposed groups was statistically significant ($p<0.05$). This is an evidence of a higher therapeutic effect of Favipiravir (Areplivir) on fever in the patients who were receiving this drug.

The efficacy of the conducted therapy was also assessed by Categorical Ordinal Scale for Clinical Improvement (COSCI), its parameters are presented in Table 1. The assessment by this scale was conducted daily.

The assessment of the treatment efficacy by COSCI showed that the condition of the comparison-group patients on admission to the in – patient department corresponded to 3.36±0.10 category; on day of the surveillance to 1.95±0.15 category ($p<0.001$). Similar positive dynamics in patient condition, evaluated by COSMI was recorded in the main group. At the beginning of the surveillance the condition of the patients was evaluated as 3.24±0.11 category, on day 15 as 1.59±0.17 ($p<0.001$). The conducted therapy was similarly effective in the studied groups ($p>0.05$).

On the 15th day of the observation in the main group, the condition of the patients was assessed at 1.59±0.17 points (categories) according to the Categorical Ordinal Scale of Clinical Improvement (COSCI), versus 3.24±0.11 categories at the admission to hospital ($p<0.001$). In the comparison group, a similar positive dynamic was observed. On the 15th day of therapy, the condition of the patients according to COSCI, was assessed in 1.95±0.15 categories versus 3.36±0.10 at the beginning of the observation ($p<0.001$). There were no significant differences between the groups ($p>0.05$).

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Table 1 – Categorical Ordinal Scale for Clinical Improvement [20]

Patient condition	Description	Category
Uninfected	Clinical and virological signs of infection are absent	0
Out-patient	Restrictions on human activity are absent	1
	Restriction on human activity are present	2
In-patient	Hospitalized. oxygen therapy	3
Moderate course of disease	Absent	
	Oxygenation with a mask or nasal cannula	4
Severe course of disease	Non-invasive ventilation or high – flow oxygenation	5
	Intubation or mechanical ventilation	6
	Ventilation + an additional support of organs: vasopressors. replacement therapy for kidneys. extracorporal membrane oxygenation (ECMO)	7
Deceased	Death	8

Table 2 – "Empirical" Visual Scale for assessing pronounced changes in the lungs by the CT data [21]

Description	Value
Absence of characteristic manifestations	CT-0
Minimal volume/prevalence. <25% of lung volume	CT-1
Medium volume/prevalence. 25–50% of lung volume	CT-2
Considerable volume/prevalence. 50–75% of lung volume	CT-3
Crucial volume/prevalence. >75% of lung volume	CT-4

Table 3 – Assessment of changes in the lungs by CT data

CT data	Group of comparison M±m (n=22)		Main group M±m (n=17)		P
	On admission	On day 15 of therapy	On admission	On day 15 of therapy	
Area of lung damage. %	31.41±2.27	26.73±3.11	26.62±2.59	16.4±1.98*	<0.05
Area of lung damage by EVS	1.73±0.097	1.59±0.14	1.65±0.12	1.24±0.11	<0.05

Notes: p – statistical significance of differences in the indices of the main and groups on day 15 of therapy; * – significance of the differences in the indices associated with the dynamics of the disease during the treatment.

Table 4 – Undesirable phenomena recorded in the study

Undesirable phenomena (UPs)	Group of comparison. m (n. %)	Main group. m (n. %)	P
Total number of UPs	31 (16, 72.7%)	23 (11, 64.7%)	>0.05
UPs probably associated with etiotropic drug intake	5 (5, 22.7%)	0 (0, 0%)	<0.05
UPs possibly associated with etiotropic drug intake	27 (15, 68.2%)	23 (11, 64.7%)	>0.05
Clinically relevant ALT elevation	11 (11, 50%)	10 (10, 58.8%)	>0.05
Clinically relevant AST elevation	5 (5, 22.7%)	5 (5, 22.7%)	>0.05
Skin rash	5 (5,22.7%)	5 (5, 22.7%)	>0.05
Clinically relevant prolongation of QTc	5 (5, 22.7%)	0 (0, 0%)	<0.05
Clinically relevant hyperglycemia	4 (4, 18.2%)	2 (2, 11.8%)	>0.05
Clinically relevant elevation of creative phosphokinase	1 (1, 4.5%)	0 (0, 0%)	>0.05
Clinically relevant leukocyturia	1 (1, 4.5%)	0 (0, 0%)	>0.05
Clinically relevant erythrocyturia	1 (1, 4.5%)	1 (1, 5.9%)	>0.05

Notes: m – the number of UPs; n – the number of patients with UPs in the group (percentage is estimated to the total number of patients in the group); P – statistical significance of differences in comparison – group indices and main – group indices

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-150-159

Table 5 – Assessment of the QTc duration against the background of different therapeutic options

Study timing	QTc duration. ms		P
	Group of comparison. M±m (n=22)	Main group. M±m (n=17)	
On admission	394.65±3.99	400.71±6.41	>0.05
On day 5 of treatment	411.08±6.71*	392.33±5.19	<0.05
On day 15 of treatment	396.44±4.37	398.26±5.49	>0.05

Notes: p – statistical significance of differences in indices in the group of comparison and the main group; * – significance of differences compared to the values at admission

Table 6 – Coagulogram indices against the background of various treatment methods

Indices (reference intervals)	Group of comparison. M±m (n=22)		Main group. M±m (n=17)		p
	At admission	On day 15 of therapy	At admission	On day 15 of therapy	
APTT (24–34 sec)	28.78±1.71	27.45±1.73	30.31±1.66	26.029±1.27	>0.05
PTT (9–16 sec)	13.90±0.32	13.49±0.37	13.67±0.32	13.02±0.30	>0.05
Fibrinogen (200–400 mg/dl)	342.52±24.87	337.69±16.02	330.51±23.95	372.29±25.49	>0.05
D-dimer (0–386 ng/ml)	484.59±135.30	422.95±118.38	471.35±156.30	409.47±131.92	>0.05

Note: p – significance of differences between the indicators of the main and comparison groups on day 15th of therapy

Table 7 – Biochemical data of blood analysis against the background of different therapeutic options

Indices (reference intervals)	Group of comparison. M±m (n=22)			Main group. M±m (n=17)			p
	At admission	On day 5 of therapy	On day 15 of therapy	At admission	On day 5 of therapy	On day 15 of therapy	
Bilirubin (2.7–21 mcmol/l)	9.90±0.98	10.93±0.93	12.10±1.19*	10.52±0.97	10.73±1.24	11.50±1.39	>0.05
ALT (5–41 units/l)	32.85±3.57	49.77±6.93*	91.57±26.81*	33.58±4.01	70.88±11.89*	102.2±20.0*	>0.05
AST (3–35 units/l)	31.82±2.68	33.61±2.50	36.76±4.17	33.94±2.83	38.88±5.99	50.06±9.27	>0.05
Urea (3.5–8.3 mmol/l)	5.68±0.40	6.07±0.38	5.92±0.45	5.15±0.31	5.46±0.46	5.62±0.54	>0.05
Creatinine (51–115 mcmol/l)	85.50±4.12	86.41±4.84	90.36±3.97	98.65±14.75	90.81±5.11	91.06±5.54	>0.05
Lactate (0.5–2.2 mmol/l)	3.57±0.46	5.12±0.54*	5.42±0.60*	3.20±0.24	3.99±0.47	3.95±0.37	<0.05
CRP (0–6 mg/l)	22.90±4.75	15.93±3.89*	11.19±3.56*	25.43±6.28	10.38±2.16*	12.51±2.51*	>0.05
Ferritin (20–250 mcg/l)	150.0±18.66	155.1±24.58	192.9±28.06	220.3±30.93	265.1±37.76	242.6±32.84	>0.05
Uric acid (200–420 mmol/l)	243.0±17.64	240.9±13.46	280.1±14.71*	250.3±13.99	243.1±12.62	322.4±28.8*	>0.05
CPK (24–171 unit/l)	95.0±38.68	87.64±31.82	54.68±8.66	80.76±26.87	47.88±8.95	38.24±6.07	>0.05
Glucose (3.5–6.4 mmol/l)	6.16±0.65	7.12±0.85	7.47±1.06	5.80±0.37	6.52±0.59	5.79±0.66	>0.05
Total protein (64–87 g/l)	69.68±1.07	68.64±1.11	67.18±1.27	70.41±0.86	68.18±1.37	66.76±1.92	>0.05

Note: p – significance of difference between the indices of the main and comparison groups on day 15 of the treatment; * – significance of differences in disease dynamics during the conducted therapy. It is important to note that not a single patient has been transferred to the departments of resuscitation and intensive therapy. There have been no cases of non-invasive or artificial ventilation of the lungs. and no deaths either

The study of the indicator "blood oxygen saturation" is very important. To assess an intensity of hypoxemia and reveal respiratory failure distress, pulse oximetry was conducted to all the patients with measurements of blood oxygen saturation (SpO_2). Positive dynamics of blood oxygen saturation was notified in both groups.

In the main group, this index was 94.47±0.47% at the initiation of the therapy for infection, significantly rising during the ongoing therapy up to 97.88±0.26% ($p<0.001$). At the admission to the hospital, SpO_2 value in patients of the comparison group was 94.68±0.31%; by day 15 of the surveillance a significant increase in SpO_2 had risen up to 97.86±0.20% ($p<0.001$).

A computed tomography, as a highly sensitive device for detecting COVID-19 characteristic changes in the lungs, was used in the study to assess such changes. The use of the CT is reasonable for an initial evaluation of the state of the chest organs in patients with severely progressing forms of the disease and, for both differential diagnostics of detected changes and assessment of dynamics of the process, too. The CT makes it possible to reveal characteristic lung changes in COVID-19 patients prior to the availability of positive laboratory tests for infection done with nucleic acid amplification technique. To unify the rapid visual assessment of the volume of the lung tissue compaction according to the CT data, WHO has proposed an "empirical" visual scale [21], which makes it possible to determine the degree of lung damage (Table 2).

The study of the CT data by "Empirical" Visual Scale showed the following. The scale indices for the comparison group were 1.73±0.097 and 1.59±0.14 against the background of treatment. In the main group they were significantly lower: 1.61±0.12 and 1.24±0.11, respectively ($p<0.05$). This is another proof of a high efficacy of the therapy if it includes Favipiravir (Areplivir) as an antiviral drug.

At admission to hospital, there were no significant differences in lung lesions between the main group and the comparison group. By the 15th day of treatment with Favipiravir (Areplivir), a decrease in the area of lesion of the pulmonary parenchyma by 38.4% ($p<0.05$) had been notified, and in the group of traditional therapy – by 14.9% from the initial level ($p>0.05$). In the main group of patients at the end of the course of treatment, a smaller area of lung tissue damage was found in relation to the comparison group (16.4±1.98 and 26.73±3.11, respectively, $p<0.05$), indicating the superiority of treatment with Favipiravir (Areplivir) compared to the recommended etiotropic therapy.

In all the patients, the percentage of virus elimination during the treatment was assessed. The elimination of the virus was determined by two negative lab tests for the presence of RNA SARS-CoV-2 done with an interval of 24 hrs on days 10 and 11 of hospital therapy. On the completion of the therapy, the test results were negative in the both studied groups, which points to the elimination of the virus and the efficacy of the conducted etiotropic therapy.

An important indicator of an emerging pathological process associated with oxygen deficiency (for instance, in pneumonia), is accumulation of lactate due to hypoxia. With a reduced oxygen delivery to the cells, lactate production rises, thus making blood lactate level elevated [22]. It is known that blood lactate level helps to monitor an extent of tissue hypoxia. An elevated lactate level is an early sensitive indicator of imbalance between oxygen demand and its delivery to the tissues. Elevation of blood lactate level may pose a risk of complications [23].

At the onset of the surveillance, the lactate level was notified to elevate in both-group patients: up to 3.57±0.46 mmol/L in the comparison group and up to 3.20±0.24 mmol/L in the main group (Table7). However, during the treatment, lactate accumulation was found to occur in patients of standard – therapy group (5.42±0.61 mmol/L). Meanwhile, in Favipiravir (Areplivir) group its level did not change (3.95±0.37 mmol/L; $p<0.05$) but it was lower than in the group of comparison.

Thus, no correlation was found between the lactate concentration and the degree of lung tissue damage in patients with moderate COVID-19. This may be due to the extrapulmonary mechanisms of hypoxia development and progression in patients with COVID-19 and the peculiarities of the mechanism of action of Favipiravir and Hydroxychloroquine, which prevent the interaction of the virus with hemoglobin hematoporphyrin and the development of hemic hypoxia [24]. In addition, hyperlactatemia in this case may be associated with the production of lactate in the lung tissue itself [25].

In the course of the study, all the data were collected on the undesirable phenomena (UPs) associated with an intake of the standard-therapy drugs and Favipiravir (Areplivir) (Table 4). On the whole, 54 undesirable phenomena were recorded in both groups: 31UPs in 16 patients (72.7%) in the comparison group and 23 UPs in 11 patients (64.7%) of the main group. All the recorded UPs were mild in form. The association with the intake of etiotropic drugs was suggested in 5 cases of UPs as probable (in the comparison group), in 48 cases as possible (26 UPs in the comparison group and 23 in the main group).

It is important to note, that there has not been a single case of a serious undesirable phenomenon (SUP) recorded, and not a single case of an early termination of participation in the study due to UPs or to SUPs associated with an intake of the studied drugs or drugs of comparison. None of the recorded cases of UPs have led to the withdrawal of any etiotropic drug or to a change in the dosage of the administered drugs, either.

Associated with an intake of etiotropic drugs, undesirable phenomena of this type were recorded in five

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-150-159

comparison-group patients (22.7%). These phenomena were manifested as a significant prolongation of the corrected QT interval (QTc) on the ECG. Similar UPs were not notified in the main group (p<0.05).

Most of the undesirable phenomena were associated with hepatotoxicity, manifested mainly as elevated ALT and ACT, to a lesser degree: in 11 patients of the comparison group (50%) and in 5 (22.7%); in lo (58.8%) and in 8 (47.1%) main-group patients, respectively. The following undesirable phenomena were observed less frequently: skin rash, a clinically relevant increase in creatine phosphokinase (CPK), hyperglycemia, leukocyturia, erythrocyturia. However, no statistically significant intergroup differences were noted in accidence of above-mentioned undesirable phenomena (p>0.05).

It is well known that Hydroxychloroquine and Azithromycin, when used in monotherapy or in a combination, prolong the QT interval. Their use may cause drug-induced ventricular «pirouette» – a type of Tachycardia (torsades de pointes, Td P). Although Td P occurs only in a small proportion of patients with a prolonged QTc interval (longer than 500 ms), the drug QT prolongation may increase a risk of death from arrhythmic or non- arrhythmic causes. For this reason, this indicator is very important for sachet of drugs [26, 27]. The analysis of the Multinational Register conducted in late May 2020 with enrollment of patients with severe COVID-19 showed that Hydroxychloroquine use is associated with an increased tick of intrahospital mortality. The association of the use of Hydroxychloroquine (including its combination with Macrolide) with occurrence of ventricular arrhythmias during hospitalization, was also confirmed [19].

Taking the above into account, the lack of Favipiravir (Areplivir) effect on the duration of the corrected QTc interval observed in our study, is of vital importance. The QTc value in a standard-therapy group averaged 394.65±3.99 ms on the admission. 54.4% patients received a combination of Hydroxychloroquine and Azithromycin, 36.4% – only Hydroxychloroquine. On day 5 of the treatment, the QTc duration went up, amounting to 411.08±6.71 ms (p<0.05). While in the Favipiravir (Areplivir) group the value of the corrected QTc interval did not change (400.71±6.41 ms on admission and 392.33±5.19 by day 5 of the therapy (p>0,05). Hence, an observed difference between the groups turned out to be statically significant (p>0.05), which is an evidence of a safer use of Favipiravir (Areplivir) with regard to the heart.

By day 15 of the treatment, the QTc duration in the comparison group had returned to the initial one, making 396.44±4,37 ms. No changes in the QTc value were observed in the main group (398.26±5.49 ms). Eventually, statistically significant intergroup difference recorded on day 5 of the Surveillance was leveled out by day 15 (p>0.05). This may be apparently explained by quite a long period of withdrawal of Hydroxychloroquine and Azithromycin by that time (the duration of Hydroxychloroquine intake was 7 days, of Azithromycin – 5 days).

Evaluation of the indicators of the general blood test, coagulogram and simple urine test in the moderate course of COVID-19, did not reveal statistically significant dynamics during the study and intergroup differences. The coagulation parameters were presented in Table 6. No difference in the number of clinically significant deviations of these indicators from the norm, has been notified either.

The data on biochemical blood analyses of both group patients, are presented in Table 7. As previously mentioned, our observation showed an extensive involvement of the liver into the pathological process. The syndrome of hepatocyte cytolysis was recorded on day 5 of the surveillance: the enzymatic ALT activity in a group of comparison equaled to 49.77±6.93 units/L, in the main group to 70.88±19.89 units/L. By day 15 of the treatment, a significant rise of ALT activity had been observant in both groups, up to 91.57±26.81 and 102.2±20.0 units/L, respectively. However, no difference in hepatotoxicity was found in the drugs user in the studied groups (p<0.05).

CONCLUSION

The results obtained in our study, have shown that the efficacy of Favipiravir (Areplivir) drug for the treatment of the novel coronavirus infection is superior to the efficacy of standard etiotropic therapy in a number of aspects (indices). The time period until fever disappeared, had been shorter in the group with administered Favipiravir (Areplivir) (the body temperature <37,2 within 3 days in succession, without any antipyretic drugs). The lactate level in the blood of this group of patients, was lower than in the patients who received standard antiviral therapy prescribed in accordance with temporary guidelines.The greater effectiveness of therapy in the main group is also indicated by CT data, which showed a more significant reduction in the area of pulmonary parenchyma lesion on the 15th day of therapy, in relation to the comparison group.

The conducted study also bears evidence that, the safety of Favipiravir (Areplivir) use for treating patients with novel coronavirus infection of moderate severity, is comparable to the safety of standard therapy. Of vital importance is the fact, that by its effect on the corrected QTc interval, Favipiravir (Areplivir) is safer than standard therapy represented mostly by Hydroxychloroquine and Azithromycin.

On the basis of the results obtained in the study, Favipiravir (Areplivir) can be recommended for the treatment of patients with the novel coronavirus infection of moderate severity.

FINANCE

The clinical study was carried out with the support of "Promomed RUS" LLC. The sponsor had no influence on the choice of the material for publication, the analysis and interpretation of the data.

AUTHOR'S CONTRIBUTIONS

All authors have contributed to the research equally.

CONFLICT OF INTERESTS

The authors declare no conflicts of interests.

REFERENCES

1. e Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. Nat Rev Microbiol. 2016; 14(8):523–34. DOI: 10.1038/nrmicro.2016.81.
2. Yang L, Tian D, Liu W. [Strategies for vaccine development of COVID-19]. Sheng Wu Gong Cheng Xue Bao. 2020; 36(4):593–604. Chinese. DOI: 10.13345/j.cjb.200094.
3. McCreary EK, Pogue JM. Coronavirus Disease 2019 Treatment: A Review of Early and Emerging Options. Open Forum Infect Dis. 2020 Mar 23;7(4): ofaa105. DOI: 10.1093/ofid/ofaa105.
4. Giovane RA, Rezai S, Cleland E, Henderson CE. Current pharmacological modalities for management of novel coronavirus disease 2019 (COVID-19) and the rationale for their utilization: A review. Rev Med Virol. 2020; e2136. DOI:10.1002/rmv.2136
5. Mitjà O, Clotet B. Use of antiviral drugs to reduce COVID-19 transmission. Lancet Glob Health. 2020;8(5):e639–40. DOI: 10.1016/S2214-109X(20)30114-5.
6. Wu R, Wang L, Kuo HD, Shannar A, Peter R, Chou PJ, Li S, Hudlikar R, Liu X, Liu Z, Poiani GJ, Amorosa L, Brunetti L, Kong AN. An Update on Current Therapeutic Drugs Treating COVID-19. Curr Pharmacol Rep. 2020; 11:1–15. DOI: 10.1007/s40495-020-00216-7.
7. Esposito S, Noviello S, Pagliano P. Update on treatment of COVID-19: ongoing studies between promising and disappointing results. Infez Med. 2020;28(2):198–211.
8. Bhimraj A, Morgan RL, Shumaker AH, Lavergne V, Baden L, Cheng VC, Edwards KM, Gandhi R, Muller WJ, O'Horo JC, Shoham S, Murad MH, Mustafa RA, Sultan S, Falck-Ytter Y. Infectious Diseases Society of America Guidelines on the Treatment and Management of Patients with COVID-19. Clin Infect Dis. 2020: ciaa478. DOI: 10.1093/cid/ciaa478.
9. Kalil AC. Treating COVID-19-Off-Label Drug Use, Compassionate Use, and Randomized Clinical Trials During Pandemics. JAMA. 2020;323(19):1897–1898. DOI: 10.1001/jama.2020.4742.
10. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 2020; 395:1569-1578. DOI:10.1016/S0140-6736(20)31022-9
11. Cao B, Wang Y, Wen D, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med. 2020;382(19):1787–1799. DOI: 10.1056/NEJMoa200128.
12. Zhu Z, Lu Z, Xu T, Chen C, Yang G, Zha T, Lu J, Xue Y. Arbidol monotherapy is superior to lopinavir/ritonavir in treating COVID-19. J Infect. 2020;81(1):e21–e23. DOI: 10.1016/j.jinf.2020.03.060.
13. Hung IF, Lung KC, Tso EY, et al. Triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. Lancet. 2020;395(10238):1695-1704. DOI: 10.1016/S0140-6736(20)31042-4
14. Zhou Q, Chen V, Shannon C.P, et al. Interferon-α2b Treatment for COVID-19. Front Immunol. 2020; 11:1061. DOI:10.3389/fimmu.2020.01061
15. Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J Crit Care. 2020; 57:279–283. DOI: 10.1016/j.jcrc.2020.03.005.
16. Chen C., ZhangY., HuangJ. et al. Favipiravir versus Arbidol for COVID-19: A Randomized Clinical Trial. MedRxiv. 2020:2020.03.17.20037432.
17. Cai Q., Yang M., Liu D., Chen J., Shu D., Xia J., Liao X., Gu Y., Cai Q., Yang Y., Shen C., Li X., Peng L., Huang D., Zhang J., Zhang S., Wang F., Liu J., Chen L., Chen S., Wang Z., Zhang Z., Cao R., Zhong W., Liu Y., Liu L. Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study. Engineering (Beijing). 2020;6(10):1192-1198. DOI: 10.1016/j.eng.2020.03.007
18. [Vremennye metodicheskie rekomendacii MZ RF «Profilaktika, diagnostika i lechenie novoj korona-virusnoj infekcii COVID-19»]. 2020 Apr 28;6: 165
19. [Vremennye metodicheskie rekomendacii MZ RF «Profilaktika, diagnostika i lechenie novoj korona-virusnoj infekcii COVID-19»]. 2020 Oct 3; 7: 166.
20. WHO R&D Blueprint – COVID-19 Therapeutic Trial Synopsis Draft, February 18, 2020. Available from: https://www.who.int/blueprint/priority-diseases/key-action/COVID-19_Treatment_Trial_Design_Master_Protocol_synopsis_Final_18022020.pdf
21. Inui S, Fujikawa A, Jitsu M, et al. Chest CT Findings in Cases from the Cruise Ship "Diamond Princess" with Coronavirus Disease 2019 (COVID-19). Radiol Cardiothorac Imaging. 2020;2(2):e200110. DOI: 10.1148/ryct.2020200110
22. [Klinicheskie rekomendacii MZ RF «Vnebol'nichnaya pnevmoniya u vzroslyh»]. 2019:97.
23. Anaev EK. Blood lactate and the lungs: from theory to practice. Pulmonologiya. 2014;(6):108–114. DOI: 10.18093/0869-0189-2014-0-6-108-114
24. Wenzhong L; Hualan L. COVID-19: Attacks the 1-Beta Chain of Hemoglobin and Captures the Porphyrin to Inhibit Human Heme Metabolism. ChemRxiv. Preprint. https: // DOI.org/10.26434/chemrxiv.11938173.v7.
25. Voennov O.V., Zagrekov V.I., Boyarinov G.A., Geras'kin V.A., Boyarinova L.V. Mechanisms of development of pulmonary injury in patients with new coronavirus infection. Medicinskij al'manah. 2020; 3 (64); 15–27.
26. Chugh SS, Reinier K, Singh T, Uy-Evanado A, Socoteanu C, Peters D, Mariani R, Gunson K, Jui J. Determinants of prolonged QT interval and their contribution to sudden death risk in coronary artery disease: the Oregon Sudden Un-

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-150-159

expected Death Study. Circulation. 2009;119(5):663–70. DOI: 10.1161/CIRCULATIONAHA.108.797035.

27. Simpson TF, Salazar JW, Vittinghoff E, Probert J, Iwahashi A, Olgin JE, Ursell P, Hart A, Moffatt E, Tseng ZH. Association of QT-Prolonging Medications with Risk of Autopsy-Defined Causes of Sudden Death. JAMA Intern Med. 2020;180(5):698–706. DOI: 10.1001/jamainternmed.2020.0148.

AUTHORS

Larisa A. Balykova – Doctor of Sciences (Medicine), Professor, the Head of the Department of pediatrics; corresponding member of the Russian Academy of Sciences. Director of the National Research Ogarev Mordovia State University. ORCID: 0000-0002-2290-0013. E-mail: larisabalykova@yandex.ru

Vera F. Pavelkina – Doctor of Sciences (Medicine), Professor, the Head of the Department of infectious diseases with courses in epidemiology, phthisiology, skin and venereal diseases. National Research Ogarev Mordovia State University. ORCID ID: 0000-0001-9582-9986. E-mail: pavelkina@rambler.ru

Natalya V. Shmyreva – Candidate of Sciences (Medicine), Associate Professor of the Department of Pharmacology and Clinical Pharmacology with a course in pharmaceutical technology. National Research Ogarev Mordovia State University. ORCID ID: 0000-0001-9331-7979. E-mail: shmyrevanv@yandex.ru

Nikolay A. Pyataev – Doctor of Sciences (Medicine), Associate Professor and the Head of the Department of Anesthesiology and Reanimatology. National Research Ogarev Mordovia State University. ORCID ID: 0000-0002-9688-7640. E-mail: pyataevna@mail.ru

Natalya M. Selezneva – Candidate of Sciences (Medicine), Associate Professor of the Department of Hospital Therapy. National Research Ogarev Mordovia State University. E-mail: nata_rm@mail.ru. ORCID ID:0000-0002-3004-2063

Olga I. Shepeleva – Candidate of Sciences (Medicine), Associate Professor of the Department of Faculty therapy with courses of physiotherapy, physiotherapy exercises. National Research Ogarev Mordovia State University. ORCID ID: 0000-0001-8307-2787. E-mail: shepeleva-oi@rambler.ru

Rimma Z. Almyasheva – Candidate of Sciences (Medicine), Associate Professor of the Department of infectious diseases with courses in epidemiology, phthisiology, skin and venereal diseases. National Research Ogarev Mordovia State University. ORCID ID: 0000-0001-6727-9083. E-mail: almiasheva.rimma@yandex.ru

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

CLINICAL-IMMUNOLOGICAL EFFECTIVENESS OF RIBOMUNYL IN CHILDREN WITH VIRUS-INDUCED BRONCHIAL ASTHMA

E.B. Belan, E.M. Nikiforova, T.E. Zayachnikova, I.N. Shishimorov, O.V. Magnitskaya

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

E-mail: belan.eleonora@yandex.ru

Received 18 March 2020 Review (1) 15 June 2020 Review (2) 20 July 2020 Accepted 30 September 2020

The aim of the study is to research the effects of immunostimulant Ribomunyl in virus-induced bronchial asthma (VBA) children.

Materials and methods. 14 virus-induced bronchial asthma (VBA) children were agministrated with immunostimulant Ribomunyl as a part of complex therapy in a 18-month trial (3 cycles of treatment). The comparison group consisted of 16 patients who received only standard therapy for bronchial asthma. At the end of the study, against the background of basic BA therapy, the following parameters were estimated: the frequency of acute respiratory viral infections (ARVI), the need for antibacterial therapy, the frequency of IgG to respiratory-syncytial virus (RSV) prevalence, the serum level dynamics of total IgE, IFN-γ, interleukin-4 (IL-4), interferon gamma (IFN-γ).

Results. The inclusion of Ribomunyl into the basic therapy complex in virus-induced bronchial asthma (VBA) children, made it possible to reduce the need for the VBA basic therapy complex by 50% and by 12,5% (p=0,0279). At the same time, as for the frequency of acute respiratory viral infections (ARVI), there was a comparable decrease in both groups, but in the main group the number of cases requiring antibiotic therapy decreased from 78.6% to 42.9% (p=0.0199). The inclusion of Ribomunyl into the basic therapy complex resulted in the decrease of the total IgE serum level; in the patients with the initial presence of IgG to the respiratory syncytial virus (RSV), the IL-4 level decreased and the IFN-γ level increased.

Conclusion. Ribomunyl improves the treatment of virus-induced bronchial asthma (VBA) children, herewith the dynamics of immunological indicators is more in RSV-seropositive patients.

Keywords: bronchial asthma, children, Ribomunyl, virus-induced bronchial asthma

Abbreviations: VBA – virus-induced bronchial asthma; ICS – inhaled corticosteroids; INF – interferon; IL – interleukin; ARVI – acute respiratory viral infection; RSV – respiratory syncytial virus; Ig – immunoglobulin; TLR – Toll-like receptors.

КЛИНИКО-ИММУНОЛОГИЧЕСКАЯ ЭФФЕКТИВНОСТЬ РИБОМУНИЛА У ДЕТЕЙ С ВИРУС-ИНДУЦИРОВАННОЙ БРОНХИАЛЬНОЙ АСТМОЙ

Э.Б. Белан, Е.М. Никифорова, Т.Е. Заячникова, И.Н. Шишиморов, О.В. Магницкая

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

E-mail: belan.eleonora@yandex.ru

Получено 18.03.2020 Рецензия (1) 15.06.2020 Рецензия (2) 20.08.2020 Принята к печати 30.09.2020

For citation: E.B. Belan, E.M. Nikiforova, T.E. Zayachnikova, I.N. Shishimorov, O.V. Magnitskaya. Clinical-immunological effectiveness of ribomunyl in children with virus-induced bronchial asthma. *Pharmacy & Pharmacology*. 2020;8(3):160-168. **DOI:** 10.19163/2307-9266-2020-8-3-160-168

Для цитирования: Э.Б. Белан, Е.М. Никифорова, Т.Е. Заячникова, И.Н. Шишиморов, О.В. Магницкая. Клинико-иммунологическая эффективность рибомунила у детей с вирус-индуцированной бронхиальной астмой. *Фармация и фармакология.* 2020;8(3):160-168. **DOI:** 10.19163/2307-9266-2020-8-3-160-168

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-160-168

Цель: изучить клинико-иммунологическую эффективность рибомунила у детей с вирус-индуцированной бронхиальной астмой (БА).
Материалы и методы. 14 детей с вирус-индуцированной БА получали в составе комплексной терапии препарат рибомунил (3 курса, 18 месяцев); группу сравнения составили 16 больных, получавших только стандартную терапию вирус-индуцированной БА. На момент окончания исследования у детей оценивали на фоне базисной терапии БА частоту острых респираторных вирусных инфекций (ОРВИ), потребность в антибактериальной терапии, частоту определения иммуноглобулина G (IgG) к респираторно-синцитиальному вирусу (РСВ), динамику сывороточного уровня общего IgE, интерлейкина-4 (IL-4), интерферона-гамма (ИФНγ).
Результаты. Включение рибомунила в терапевтический комплекс при вирус-индуцированной БА у детей позволило в 50% случаев и 12,5% (p=0,0279) снизить потребность в базисной терапии БА. В то же время частота ОРВИ сопоставимо уменьшилась в обеих группах, однако в основной группе снизилось с 78,6% до 42,9% (p=0,0199) количество случаев, требующих назначения антибактериальной терапии. Включение рибомунила в терапевтический комплекс привело к снижению сывороточного уровня общего IgE; у больных с исходным наличием IgG к РС-вирусу снизился уровнь IL-4 и повысился уровень ИФНγ.
Заключение. Рибомунил улучшает течение вирус-индуцированной БА у детей, при этом динамика иммунологических показателей более выражена у РСВ-серопозитивных пациентов.
Ключевые слова: бронхиальная астма, дети, рибомунил, вирус-индуцированная БА
Список сокращений: БА – бронхиальная астма; ИКС – ингаляционные кортикостероиды; ИНФ – интерферон; ИЛ – интерлейкин; ОРВИ – острая респираторная вирусная инфекция; РСВ – респираторно-синтициальный вирус; Ig – иммуноглобулин; TLR – Toll-подобные рецепторы.

INTRODUCTION

Despite the significant progress made in the treatment and prevention of bronchial asthma (BA), the search for new approaches to the treatment of patients who fail to achieve a controlled course of the disease, is currently ongoing [1, 2].

Numerous epidemiological studies indicate that one of the common reasons for the development of broncho-obstructive conditions and the absence of a controlled course of asthma in children, is a respiratory tract damage. In this case, the greatest role is assigned to respiratory syncytial and rhinovirus infections [3].

In general, it is the respiratory viruses in children in 90% of cases that cause an asthma exacerbation and an increase in the severity of symptoms of the disease [4]. In addition, viral lesions of the respiratory epithelium can increase bacterial colonization and infection of the respiratory tract, increasing the need for antibacterial therapy; the greatest role in this is assigned to *Streptococcus pneumoniae, Moraxellacatarrhalis* and *Haemophilusinfluenza* [4].

The difficulty of managing patients with virus-induced broncho-obstructive diseases is due to the lack of specific antiviral therapy and prophylaxis of most of them, including respiratory syncytial viruses (RSVs) and rhinoviruses. In this regard, it seems appropriate to study the possibility of using drugs with an immunostimulating effect, the potential effectiveness of which is 40% of cases in children with recurrent respiratory tract diseases [5].

One of the most promising groups among them is represented by preparations based on bacterial lysates, which combine vaccine properties in relation to the most frequent pathogens of inflammatory diseases of the respiratory tract with a non-specific immunostimulating activity [5–8].

The latter is based on the ability of bacterial lysates to interact with a number of innate immunity structures. Thus, the induction of Toll-like TLR2/3/4 receptors causes the activation of mechanisms of (**без артикля**) antibacterial immunity [9, 29]. At the same time, a high clinical efficacy associated with the activation of macrophages (stimulation of adhesion, phagocytic activity and cytotoxicity), polinuklear cells (activation of chemotaxis, migration and adhesion), natural killer cells (activation of the antiviral effect as well as an increase in the production of gamma interferon), B-lymphocytes (with an increase in IgG, IgA and IgM titers), dendritic cells (with the synthesis reinforcement of Th1 cytokines and activation of lymphocytes) [10]. It should be borne in mind that ribosomes, the extract of which is a part of ribomunil, are 1000 times more immunogenic than whole bacteria [9, 11].

In recent studies it has nevertheless been shown that ribomunyl can induce receptors responsible for the activation of antiviral immunity, including TLR7 / 8, responsible for the induction of interferon-gamma (IFNg), and can also “cancel” the virus-mediated inhibition of TLR 9-mediated synthesis of type I INF [12].

The broad immunological activity of bacterial lysates served as the basis for their study in patients with a predisposition to perverted immune responses, in particular, in allergic diseases. Thus, in the work by Matricardi et al., an analysis of 12 placebo-controlled studies of microbial drugs in patients with allergic diseases (bronchial asthma, atopic dermatitis, allergic rhinitis) was carried out [13]. A decrease in the frequency of exacerbation and severity of symptoms was notified in half of the studies.

THE AIM of the study was to research the clinical and immunological efficacy of ribomunil in children with virus-induced BA.

MATERIALS AND METHODS

The work was carried out in the design of a prospective randomized simple comparative study in parallel groups.

The monitored parameters were:

- the need for basic therapy of BA and its volume (the stage at which a controlled course of the disease is achieved);
- a number of acute respiratory viral infections (ARVIs) per year;
- the need for ARVI antibacterial therapy (in all cases; no more than 50% of cases; not required);
- serum interleukin-4 (IL-4) levels);
- serum IFNg level;
- the presence of IgG to RSV.

The study comprised 30 children (the mean age was 4.3=0.12 years) with virus-induced BA in the disease controlled outside of ARVI with the use of pharmacotherapy corresponding to the 2nd stage (low doses of inhaled corticosteroids). The patients were managed in accordance with the Federal Clinical Guidelines "Bronchial asthma in children" and GINA 2019[1,2]. The work was performed as an initiative study with no conflict of interest. It was approved by the Regional Ethics Committee (Protocol No. 256-2016 dated 25 March, 2016). The informed consent was obtained from the legal representatives of all patients for all studies.

The inclusion criteria were as follows: the presence of foci of a chronic infection; any immunotropic therapy conducted for 6 months before the inclusion in the study.

The exclusion criteria were as follows: a refusal to participate in the study for any reason; drug intolerance.

Monitoring of the children's condition was carried out every 3 months during the entire follow–up period (with a revision of the basic therapy volume -after 1 month and 3 months).

Group 1 (n = 14) consisted of the children who were administrated, in addition to the standard BA therapy [1,2], with ribomunyl (Ribomunyl® PierreFabre; France; ATC code L03AX Other immunostimulants; Registration number: P No. 011369/01; P No. 011369/02). It was prescribed for 18 months at the dose of 0.75 mg / day according to the scheme recommended by the manufacturer (in the first month of treatment and / or daily 4 days a week for 2–5 months; 3 courses). The drug includes bacterial ribosomes as active components, titrated up to 70% of ribonucleic acids (including ribosomes *Klebsiellapneumoniae* – 3.5 shares, *Streptococcus pneumoniae* – 3.0 shares, *Streptococcus pyogenes* – 3.0 shares and *Haemophilusinfluenzae* – 0.5 shares).

Group 2 (n = 16) consisted of the children in whom a controlled course of asthma was previously achieved with the use of low doses of inhaled glucocorticosteroids as a basic therapy.

The presence of polyclonal virus-specific IgG in blood serum was detected by enzyme-linked immunoassay in accordance with the attached instructions (LLC "CNDO", St. Petersburg).

The determination of IFNg and IL-4 serum levels was performed by enzyme-linked immunoassay in accordance with the attached instructions ("CYTIMMUNE", USA; "Multiscan", Labsystem, Finland).

The determination of the total IgE level in blood serum was carried out by the enzyme-linked immunoassay method in accordance with the attached instructions (JSC "Vector-Best" (Novosibirsk); reader "Multiscan", Labsystem, Finland). If necessary, the sera were stored at –20°C for more than 2 months.

Statistical processing

To characterize quantitative indicators in the normal distribution, the arithmetic mean with a standard deviation (M±s), or the median value with an interquantile span in the nonparametric distribution (Me[Q1;Q3]), was used.

The differences between the values were considered significant at $p < 0.05$, which were determined using the Student's test for a normal distribution, Wilcoxon's test for related groups with a nonparametric distribution, Fisher's test, or X2 (depending on the sample size) to compare the frequencies.

Statistical processing of the material was carried out using the STATISTICA6.0 software package.

RESULTS AND DISCUSSION

The expediency of studying immunotropic drugs (in our study it is ribomunyl) as a part of a therapeutic complex of patients with virus-induced BA, is determined by the presence of a secondary immunodeficiency state, manifested in the recurrent nature of the infectious process, leading, among other things, to an exacerbation of asthma and / or a high frequency of bacterial complications requiring a prescription of antibacterial drugs.

In general, the course of asthma was somewhat different in the patients administrated with ribomunil, and in the comparison group.

Thus, by the end of the study, every fourth patient ($p=0.0047$) who received a standard treatment, had had its volume revised; at the same time, it had been increased in 2/16 patients, and 2/16 patients had been given a possibility to refuse it. The number of ARVI episodes had slightly decreased in patients of the both groups, which could be probably due to age characteristics, but the need for antibiotic therapy in them had remained unchanged.

The results of this study are presented in Tables 1 and 2.

The results obtained indicate that during the follow-up period, in the group of children receiving ribomunyl, the need for basic BA therapy did not increase in any case, while in the comparison group, 2 patients were prescribed the 3rd step volume therapy on the 4th and 12th months of the follow-up (Table 1).

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-160-168

At the same time, the number of patients who needed basic therapy in the volume of stage 2, decreased in the main group twice (14/14, respectively, 7/14, p=0.028). However, in the comparison group, only 2 of 16 patients could refuse regular anti-asthma therapy, which was significantly less frequent than in the main group (p=0.0279) (Table 1).

In parallel with the assessment of the BA course, the number of ARVI episodes for the 1st year (Table 1) prior to the start of the research and throughout the entire follow-up period, was studied. It turned out that the incidence of diseases had significantly decreased in both groups (p=0.045), which can be explained by the age-related aspects of the pathology. However, the median value of the number of the episodes against the background of treatment was 4 in the main group and 5 in the comparison group. In addition, the number of patients who did not have ARVI complications by a bacterial infection, increased almost 3 times, and they did not need any antibiotics. These facts can be considered the evidence of the formation of more effective anti-infective resistance in the patients with virus-induced BA, if the basic therapy is supplemented with bacterial lysates, in particular, ribomunil. The data obtained are consistent with the results of a meta-analysis of 11 randomized controlled trials of ribomunil efficacy in sickly children, which has shown a 43.5% reduction in the incidence of upper respiratory tract infections [95% CI 33.7–53.2%] [29].

It has been established that at an early age, one of the most common causes of ARVI is an RSV infection. According to some authors [3, 4, 20], by the age of 5, up to 100% of children had been in contact with this type of pathogen.

At the same time, clinical manifestations of infection vary from mild diseases of the upper respiratory tract to severe lesions of the lower respiratory tract (bronchiolitis, pneumonia), accompanied by a syndrome of bronchial obstruction and virus-induced exacerbation of BA [20]. In general, in 2015, the number of registered cases of the RVC infection in the world amounted to 33.1 million people; 3.2 million patients required hospitalization; the total number of deaths in hospital was 59.600 and 149.400 outside it [21]. Up to 80% of ARVI-associated broncho-obstructive syndromes in preschoolers, is believed to be associated with this pathogen [3, 4, 17], and the annual pharmacoeconomic losses due to the RSV infection are estimated at 50–57 million pounds sterling and are associated with the incidence of preschool children [22].

In the present study, all the groups were comparable to each other in terms of the detection frequency of IgG to RSV (Table 2).

When evaluating the pharmacodynamic effects of ribomunyl, some dependence of the results on the initial presence of IgG to RSV was revealed.

In general, at the beginning of the study, antibodies were detected in 17/30 people, which corresponds to the modern data on the epidemiology of an RSV infection in children and its role in the development of BA. There were no new cases of seroconversion during the follow-up period.

During the follow-up process, in the intervention group in 4/14 cases, there was a decrease in the level of antiviral antibodies up to undetectable ones. Despite the fact that a significant difference in the frequency of the detection of antibodies to the virus (64.3%, 9/14 and 35.7%, 5/14), (p=0.14) could not be demonstrated, the achieved result can be interpreted as a trend, which needs a further study, since the number of seropositive patients in the comparison group remained unchanged (50%, 8/16). In addition, it should be borne in mind that the mechanism of the action of bacterial lysates and, in particular, ribomunyl is associated with the stimulation of the humoral link in general. This trend also deserves attention because all the children in whom IgG to RSV ceased to be detected as a result of treatment, were moved to a group that did not require a prescription of basic therapy on a regular basis, which is consistent with the data on a close relationship between the RSV infection and BA [3, 4]. Thus, in infants, the RSV infection is associated with a more than 1.5-fold risk of developing BA in the subsequent years, herewith several factors matter. First, the interferon deficiency predisposes to both a more severe course of infection and an overproduction of IgE; and, second, there is a direct damaging effect of the pathogen on the pulmonary parenchyma [23, 27, 28].

Recent studies have also shown the possibility of synthesizing antiviral IgEs, including the ones against RSV [24, 25]. Thus, the damaging role of RSV in young children is significant, especially in conditions of immunodeficiency, which creates a serious problem; it is aggravated by the extremely limited possibilities of antiviral therapy with this type of infection. Thus, palivizumab is used only for seasonal prophylaxis of severe forms in premature infants and children under 2 years of age who have been treated for bronchopulmonary dysplasia, and hemodynamically significant congenital heart defects; ribavirin is toxic and requires a reliable contraception for 7 months after treatment (in this case, when the drug is administered by inhalation, mainly for the staff and parents); type I interferon preparations are ineffective [17, 22].In this regard, immunotropic drugs are of considerable interest, since they can induce various factors of immunity, and these factors are associated with antiviral resistance. Herewith it is impossible to exclude the assumption that the activation of anti-infective defense mechanisms may contribute to the suppression of viral infection up to its eradication, explaining the decrease in serum levels of specific IgGs up to the undetectable ones in some patients.

Table 1 – Clinical efficacy of ribomunyl in children with virus-induced BA

	Group 1 (n=14)		Group 2 (n=16)	
	Before	After	Before	After
Basic treatment of asthma: No	0	50(7/14) *1p=0,0279	0	12.5(2/16)
Step 2	100 (14/14)	50.0(7/14) *2p=0,023	100 (16/16)	75.0(12/16)
Step 3	0	0	0	12.5(2/16)
ARVI episodes/year Me[Q2;Q3]	6 [5–8]	4[3-6] *3p=0,007	6 [5–7]	5[3–6] *3p=0,034
Use of antibiotics in ARVI, % (n1/n)				
No use of antibiotics	21.4(3/14)	57.1(8/14) *p=0,0199	25,0(4/16)	37.5(6/16)
In 50% cases	42.9(6/14)	28.6(4/14)	50.0(8/16)	56.3(9/16)
In 100% cases	35.7(5/14)	14.3(2/14)	25.0(4/16)	6.3(1/16)

*1p=0.0279 vs. outcomes
*2p=0.023 vs. Group 2
*3p=0.007 и *3p=0.034 vs. outcomes
n – number of children in groups
n1 – number of children with effect

Table 2 – Dynamics of immunological parameters of children with VBA against the background of treatment with ribomunil

	Group 1 (n=14)		Group 2 (n=16)	
	IgGк PCB+(n=9)	IgGк PCB-(n=5)	IgGк PCB+(n=8)	IgGк PCB-(n=8)
IgE, ME/ml Me[Q1;Q3]	176[119; 312]	132[87; 460]	112[86; 556]	154[121; 339]
	141[90; 288]*1	107[69; 181]* 2	124[59; 358]	148[109; 411]
ИФНg, pg/ml Me[Q2;Q3]	1.43[0; 3.01]	6.1[3.8; 10.5]	2.4[0; 4.1]	3.2[0; 5.9]
	2.2[1.8; 7.2] * 4	6.2[4.2; 12.9] * 3	3.2[2.1;5.4] * 5	3.9[3.1; 8.7]
IL-4, pg/ml Me[Q2;Q3]	14.4[2.1; 19.0]	8.8[3.6; 98.0]	15.6[0; 44.7]	12.2[6,1; 24.6]
	12.1[0; 17.1] *6	10.4[3.1; 69.1]	17.8[0; 36.5]	10.8[0; 59.5]

* 1 p=0.008 in comparison with initial level
* 2p=0.012 in comparison with initial level
* 3p=0.038 in comparison with initial level
* 4p=0.022 in comparison with initial level
* 5p=0.014 in comparison with initial level
* 6p=0.047 in comparison with initial level
IgG to RSV + – children with detectable level of IgG to RSV
IgG to RSV – –children with undetectable level of IgG to RSV

At the same time, there is evidence that bacterial lysates have not only an immunostimulating effect but can also change the phenotype of the Tr-cell response towards the Th1 variant.

As for the immunological parameters, the serum levels of total IgE, IL-4 and IFNg were assessed in this work (Table 2).

In this study, the integral indicator of atopy – the serum level of total IgE – was, in general, typical for patients with this type of pathology, and its dynamics did not depend on the presence of IgG to RSV, but it was different in the groups with different kinds of treatment. Thus, in the patients of the main group in the combination of BA and RSV infection, the serum level of total IgE decreased from Me176 [Q2; Q3 119; 312] IU / ml to Me141 [Q2; Q3 90; 288] IU / ml (p = 0.008), and among the uninfected participants it decreased from Me132 [Q2; Q3 87; 460] IU / ml to Me107 [Q2; Q3 69; 181] IU / ml (p = 0.012). At the same time, in the group of patients who had not received additional therapy, the dynamics of the total IgE serum level had not been registered by the end of the study (Table 2).

Despite the multiplicity of immunological mechanisms that determine the Th2 phenotype of the response and control allergic inflammation, IL-4 and IFNg are the main regulatory cytokines for IgE.

In general, the first one is the key cytokine produced primarily by CD4+Th2-lymphocytes, mast cells, and basophils [31]. It induces not only the production of IgE, but also the expression of molecules of the main histocompatibility complex of class II, B7 and CD40 receptors, as well as membrane IgM on the surface of B-lymphocytes, thereby increasing the capabilities of antigen-presenting cells. Being one of the regulators of allergic inflammation, IL-4 in asthma is involved in the remodeling of the respiratory tract and the activity stimulation of mucous-producing cells [32]. Hyper-expression of the IL-4 gene in the lung triggers eosinophilic inflammation without developing hyperresponsiveness of the respiratory tract. It is known that an increase in the IL-4 / IFNg ratio in the broncho-alveolar fluid is usually accompanied by an increase in the number of Th2 lymphocytes in the respiratory tract, which is associated with a more severe course of asthma in children [33]. Along with the

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-160-168

level of IL-5, IFNg, GM-CSF (granulocyte-macrophage colony-stimulating factor), IL-4 parameters are significant biomarkers of the severity of allergic diseases, including BA [31].

In the present study, a decrease in the serum IL-4 level (from Me14.4 [Q2; Q3 2.1; 19.0] pg/ml to Me12.1 [Q2; Q3 0; 17.1] pg/ml (p=0.047) was observed only among the children with a combination of BA and RSV infection. At the same time, attention is drawn to the fact that by the end of the study, 3/9 of the participants in this subgroup had undetectable cytokine levels (Table 2).

The second important cytokine that affects the IgE synthesis, but has an effect opposite to IL-4, is IFNg. The main producers of this cytokine are T-lymphocytes (mainly the Th1 subpopulation), natural killers (NKs), natural-killer T-cells (NKT cells) and antigen-presenting cells (macrophages and dendritic ones), as well as B-lymphocytes. Moreover, its origin plays a role in the implementation of various immune responses. Thus, IFNg secreted by NKT cells, is of the greatest importance in the induction of an early protection and autocrine regulation; the T-lymphocytic cytokine is the most important in the implementation of the mechanisms of adaptive immunity [30], in particular, in the eradication of infectious agents and mutated cells, etc. The central effector role of IFNg is determined by its ability to regulate the activity of the T-cell link, which provides multiple anti-infective mechanisms. It is important that an increase in the production of endogenous IFNg promotes the activation of not only antiviral, but also antibacterial and anti-chlamydial defense [30].

Initially, the serum IFNg level in children in the both groups did not differ and, in general, was Me 3.6 [Q2; Q3 1.0-4.1] pg/ml (the results for subgroups are shown in Table 2). Nevertheless, it turned out that the children seropositive for RSV (18/30), had values of this indicator <3.6 pg/ml (72.2% vs 33.3%, p=0.026) significantly more often.

The dynamics of the content of this biomarker in the blood serum of the children who received only standard BA therapy, was observed only in the subgroup of the children with serological markers of infection, in whom, on average, it increased by 8% from Me2.4 [Q2; Q30; 4.1] pg/ml up to Me3.2 [Q2; Q3 2.1; 5.4] pg/ml (p=0.014), while there was no such dynamics in the subgroup of seronegative patients. At the end of the study, the children of the main group showed a significant increase in IFNg both in the group of RSV-seropositive patients and in the absence of serological markers of the infection, respectively, Me1.43 [Q2; Q30; 3.01] pg/ml and Me2.2 [Q2; Q31.8; 7.2] pg/ml (p=0.022) in the first subgroup and Me 6.1 [Q2; Q3 3.8; 10.5] pg / ml and Me6.2 [Q2; Q3 4, 2; 12.9] pg/ml (p=0.014) in the second one (Table 2).

Bacterial lysates are currently considered one of the most promising groups of immunomodulators in sickly children 5]. In addition, it is known that the inclusion of bacterial lysates in the complex therapy of children with moderate asthma, can help restore IFNg production to the level of healthy children and lead to a significant decrease in the total IgE serum level [14]. The ability of bacterial lysates to promote the Th1 phenotype of the immune response, including patients with allergic diseases, has been described by a number of other authors. In particular, a similar immunomodulatory effect has been shown for ribomunyl [12, 13, 15, 16]. In the study by Bystron J., thirteen adult patients with seasonal rhinoconjunctivitis received ribomunil according to the recommended regimen for 3 months (from April to June), after which they were followed up for 2 months. By the 3rd month, in the group who had received the drug, the level of IFNg had significantly increased by 30%, and by the 5th month 5 – by 37%, while the dynamics was more pronounced (65%) among the patients with clinical improvement. An increase in the production of this cytokine correlated with an increase in the serum level of macrophage IL-12. During the study, there was not a single case of deterioration of a patient's condition [15].

The relationship of RSV infection with the IFNg system is also known; in particular, there is evidence that severe forms of RSV infection are associated with impaired IFNg production [3]. This study shows some product differences associated with RSV. Thus, only in the children seropositive for RSV, the serum level of this cytokine significantly increased as a result of treatment (p<0.05), while in uninfected children it remained unchanged. These studies are consistent with the results obtained by other authors for children with atopic pathology and indicate the ability of bacterial lysates to exert pro-Th1 and anti-Th2 effects. Considering that the Th2 phenotype of the immune response is one of the key mechanisms for the development of bronchial hyperreactivity during exacerbation of asthma, the shown effect is important for the patients with this type of pathology.

Despite the fact that the drugs based on IFNg are currently developed, their therapeutic potential, unfortunately, is extremely limited not only by a very high cost of treatment, but also by serious side effects, in particular, influenza-like syndrome, lethargy, cough, depressive conditions, etc. [30]. Today, this makes them unacceptable for use in the children with virus-induced BA and determines the relevance of the search for other directions of therapy. They can be the elimination of the causes leading to the suppression of the synthesis of endogenous interferon, with the aim of their possible elimination, as well as the search for ways to overcome them with the help of drugs having immunomodulatory effects.

During therapy, negative dynamics of the serum IL-4 level also took place only in RSV-positive children. In RSV negative children, as well as in the children of the comparison group, the cytokine level remained at the initial level.

In general, the effect of ribomunyl in viral-induced BA can be represented as follows. The first direction is associated with the antigen-independent activation of differentiation and proliferation of cells of the immune system through the mechanisms of innate immunity. As a mixture of bacterial proteoglycans and ribosomes, the drug reaches the lymphoid cells in Peyer's glands and stimulates the maturation of regional dendritic cells. There are not many data on the effect of the drug on the mechanisms of innate immunity [9,10,29], some of them provide evidence of an increase in the expression of adhesion molecules and phagocytic activity of peripheral blood neutrophils [6].

The dendritic cells activated by the drug, stimulate T-lymphocytes to produce Th1-dependent cytokines, including IFNg, thus enhancing the cytotoxic properties of the body, including the antiviral activity. Moreover, the oral route of the drug administration and the induction of lymphocytic cells of Peyer's glands cause the expansion of B-cells and the production of secretory IgA, as well as serum IgG and IgM, which has been justified for the children with recurrent respiratory infections [16]. In addition, other studies have shown that in volunteers, increased serum IgA levels were associated with decreased adhesion of Streptococcus pneumoniae [16].

The second direction is associated with the induction of acquired antigen-dependent specific immunity. In fact, it is vaccination against pathogens of respiratory infections, the components of which are a part of the drug. It has been shown that their immunogenicity practically does not differ from the antigenic determinants of the native pathogen [29].

It should be borne in mind that many pathogens, including opportunistic ones, have an immunosuppressive effect of their own; therefore, the suppression of their reproduction may have an indirect immunostimulating effect [8].

Within the framework of this study, the microbial composition and microbial-viral associations that led to an exacerbation of asthma in children and necessitated antibiotic therapy were not under discussion, however, it can be assumed that the results obtained are associated with the complex effect of ribomunyl on the immune system.

CONCLUSION

Thus, the inclusion of ribomunyl in the complex therapy of children with virus-induced BA, leads to an improvement in the course of both the underlying disease and a decrease in the frequency and severity of ARVI episodes. The results achieved are consistent with the dynamics of immunological parameters, which turned out to be more pronounced in RS-virus infected children. Considering the fact that bacterial lysates, inducing the synthesis of antibacterial antibodies, do not possess their own antiviral properties, it can be assumed that the suppression of viral infection is associated with the immunomodulatory properties of the drug.

FINANCIAL SUPPORT

This study did not have any financial support from outside organizations

AUTHORS' CONTRIBUTION

All the authors have contributed equally to the research work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. [Federal'nye klinicheskie rekomendacii «Bronhial'naja astma u detej]. Moscow. 2019. 76p. Russian
2. The Global Asthma Report 2018. Available from: http://globalasthmareport.org/Global Asthma Report 2018.pdf (date of the application: 2019 March 22). Russian
3. Le Souëf P. Viral infections in wheezing disorders. European Respiratory Review. 2018;27:170133. DOI: 10.1183/16000617.0133-2017.
4. Mikhail I, Grason M. Asthma and viral infections: An intricate relationship. Annals of Allergy, Asthma & Immunology. 2019;123(4):352–358. DOI: 10.1016/j.anai.2019.06.020.
5. Del-Rio-Navarro BE, Espinosa-Rosales FJ, Flenady V, Sienra-Monge JJL. Cochrane Review: Immunostimulants for preventing respiratory tract infection in children. Evidence-Based Child Health: A Cochrane Review Journal. 2012;7(2):629–717. DOI: 10.1002/ebch.1833.
6. Esposito S, Soto-Martinez EM, Feleszko W, Jones MH, Kun-Ling Shen, Schaad Urs B. Nonspecific immunomodulators for recurrent respiratory tract infections, wheezing and asthma in children: a systematic review of mechanistic and clinical evidence. Current Opinion in Allergy and Clinical Immunology. 2018;18(3):198–209. DOI: 10.1097/ACI.0000000000000433.
7. Esposito S, Bianchini S, Polinori I, Principi N. Impact of OM-85 Given during Two Consecutive Years to Children with a History of Recurrent Respiratory Tract Infections: A Retrospective Study. International Journal of Environmental Research and Public Health. 2019;16(6):1065. DOI: 10.3390/ijerph16061065.
8. Hamill P, Brown K, Jenssen H, Hancock RE. Novel anti-infectives: is host defence the answer? Current Opinion in Biotechnology. 2008;19(6):628–636. DOI: 10.1016/j.copbio.2008.10.006.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-160-168

9. Tejera-Alhambra M, Palomares O, Perez de Diego R, Diaz-Lezcano I, Sanchez-Ramon S. New Biological Insights in the Immunomodulatory Effects of Mucosal Polybacterial Vaccines in Clinical Practice. Current Pharmaceutcal Design. 2016;22(41):6283–6293. DOI: 10.2174/1381612822666160829143129.
10. [PRIMA: pediatricheskie rekomendacii po immunomodulirujushhim preparatam v ambulatornoj praktike: (konsensus) / Pediatr. respirator. o-vo, Ros. assoc. allergologov i klinich. immunologov, Mosk. o-vo det. vrachej, Federacija pediatrov stran SNG [i dr.]. 2-e izd., pererab. i dop.] Moscow.: RG-Press. 2017. 76 p. Russian
11. Herberhold S, Coch C, Zillinger T, Hommertgen B, Busch N, Schuberth C, Hartmann E, Wimmenauer V, Hagmann CA, Lüdenbach B, Schlee M, Bootz F, Hartmann G, Barchet W. Delivery with polycations extends the immunostimulant Ribomunyl into a potent antiviral Toll-like receptor 7/8 agonist. Antiviral Therapy. 2011;16(5):751–758. DOI: 10.3851/IMP1822.
12. Lynch JP, Mazzone SB, Rogers MJ, Arikkatt JJ, Loh Z, Pritchard AL, Upham JW, Phipps S. The plasmacytoid dendritic cell: at the cross-roads in asthma. European Respiratory Journal. 2014;43(1):264–275. DOI: 10.1183/09031936.00203412.
13. Matricardi PM, Bjorksten B, Bonini S, Bousquet J, Djukanovic R, Dreborg S, Gereda J, Malling H.-J, Popov T, Raz E, Renz H. Microbial products in allergy prevention and therapy. Allergy. 2003;58:461–471. DOI: 10.1034/j.1398-9995.2003.00175.x.
14. Prosekova EV, Derkach VV, Shestovskaja TN, Sergienko IS. [Vlijanie bronhomunala na citokinovyj profil' syvorotki krovi detej s bronhial'noj astmoj. Aktual'nye voprosy allergicheskih zabolevanij: sb. tezisov III Regional'noj nauchno-prakticheskoj konferencii]. Vladivostok, 2005:58p. Russian
15. Bystron J, Hermanova Z, Szotkovska J, Heller L, Pazderova D. Effect of Ribosomal Immunotherapy on the Clinical Condition and Plasma Levels of Cytokines IL-4, IL-5, IL-12 and IFNgamma and Total IgE in Patients with Seasonal Allergy during the Pollen Season. Clinical Drug Investigation. 2004;24(12):761–764. DOI: 10.2165/00044011-200424120-00007.
16. Hbabi-Haddioui L, Roques C. Inhibition of Streptococcus pneumonia adhesion by specific salivary IgA after oral immunostimulation with a ribosomal immunostimulant. Drugs. 1997;54:29–32. DOI: 10.2165/00003495-199700541-00008.
17. Barr R, Green CA, Sande CJ, Drysdale SB. Respiratory syncytial virus: diagnosis, prevention and management. Therapeutic Advances in Infectious Disease. 2019;6:2049936119865798. DOI: 10.1177/2049936119865798.
18. Green CA, Yeates D, Goldacre A, Sande C, Parslow RC, McShane P, Pollard AJ, Goldacre MJ. Admission to hospital for bronchiolitis in England: trends over five decades, geographical variation and association with perinatal characteristics and subsequent asthma. Archives of Disease in Childhood. 2016;101(2):140–146. DOI: 10.1136/archdischild-2015-308723.
19. Ralston SL, Lieberthal AS, Meissner HC. et al. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. Pediatrics. 2014;134(5):e1474–e1502. DOI: 10.1542/peds.2014-2742.
20. Bont L, Checchia PA, Fauroux B, Figueras-Aloy J, Manzoni P, Paes B, Simões EA, Carbonell-Estrany X. Defining the Epidemiology and Burden of Severe Respiratory Syncytial Virus Infection Among Infants and Children in Western Countries. Infectious Diseases and Therapy. 2016;5(3):271–298. DOI: 10.1007/s40121-016-0123-0.
21. Shi T, McAllister DA, O'Brien KL. et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet. 2017;390(10098):946–958. DOI: 10.1016/S0140-6736(17)30938-8.
22. Cromer D, van Hoek AJ, Newall AT, Polard AJ, Jit M. Burden of paediatric respiratory syncytial virus disease and potential effect of different immunisation strategies: a modelling and costeffectiveness analysis for England. The Lancet Public Health. 2017;2(8):e367–e374. DOI: 10.1016/S2468-2667(17)30103-2.
23. Rosas-Salazar C, Gebretsadik T, Anderson LJ, Jadhao S, Chappell JD, Larkin EK, Martin L. Moore ML, Peebles RS, Hartert T. Risk or Protective Factor? Mild Respiratory Syncytial Virus Infection in Infancy and the Development of Recurrent Childhood Wheeze. American Journal of Respiratory and Critical Care Medicine. 2020;201:A5028. DOI: 10.1177/2049936119865798.
24. Teach SJ, Gill MA, Togias A, Sorkness CA, Arbes SJ. Jr. et al. Preseasonal treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma exacerbations. The Journal of Allergy and Clinical Immunology. 2015;136(6):1476–1485. DOI: 10.1016/j.jaci.2015.09.008.
25. Tam JS, Jackson WT, Hunter D, Proud D, Grayson MH. Rhinovirus specific IgE can be detected in human sera. Journal of Allergy and Clinical Immunology. 2013;132(5):1241–1243. DOI: 10.1016/j.jaci.2013.07.011.
26. Glaser L, Coulter PJ, Shields M, Touzelet O, Power UF, Broadbent L. Airway Epithelial Derived Cytokines and Chemokines and Their Role in the Immune Response to Respiratory Syncytial Virus Infection. Pathogens. 2019;8(3):106. DOI: 10.3390/pathogens8030106.
27. Bermejo-Martin JF, Garcia-Arevalo MC, De Lejarazu RO, Ardura J, Eiros JM, Alonso A, Matías V, Pino M, Bernardo D, Arranz E, Blanco-Quiros A. Predominance of Th2 cytokines, CXC chemokines and innate immunity mediators at the mucosal level during severe respiratory syncytial virus infection in children. European Cytokine Network. 2007;18(3):162–167. DOI: 10.1684/ecn.2007.0096.
28. Kaneko H, Matsui E, Asano T, Kato Z, Teramoto T, Aoki M, Kawamoto N, Lian LA, Kasahara K, Kondo N. Suppression of IFN-gamma production in atopic group at the acute phase of RSV infection. Pediatriac Allergy and Immunology. 2006;17(5):370–375. DOI: 10.1111/j.1399-3038.2006.00419.x.
29. Zaplatnikov AL, Girina AA, Lepsieva IV, Korovina NA, Cheburkin AA, Svintsitskaya VI, Coroid NV. Ribosomal immunotherapy in children with recurrent infections and chronic respiratory diseases: preventive and economic efficacy. Pediatria. 2017;96(2):151–157.
30. Kak G, Raza M, Tiwari BK. Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases. Biomolecular Concepts. 2018;9(1):64–79. DOI: 10.1515/bmc-2018-0007.
31. Hatami H, Ghaffari N, Ghaffari J, Rafatpanah H. Role of Cy-

tokines and Chemokines in the Outcome of Children with Severe Asthma: Narrative Review. Journal of Pediatrics Review. 2019;7(1):17–28. DOI: 10.32598/jpr.7.1.17.

32. Hussein YM, Alzahrani SS, Alharthi AA, Ghonaim MM, Alhazmi AS, Eed EM, Shalaby SM. Association of serum cytokines levels, interleukin 10 -1082G/A and interferon-γ +874T/A polymorphisms with atopic asthma children from Saudi Arabia. Cellular Immunology. 2014;289(1–2):21–26. DOI: 10.1016/j.cellimm.2014.03.006.

33. Keskin O, Keskin M, Kucukosmanoglu E, Ozkars M.Y, Gogebakan B, Kul S, Bayram H, Coskun Y. Exhaled RANTES and interleukin 4 levels after exercise challenge in children with asthma. Annals of Allergy Asthma & immunology. 2012;109(5):303–308. DOI: 10.1016/j.anai.2012.08.009.

AUTHORS

Eleonora B. Belan – Doctor of Sciences (Medicine), Professor, the Head of the Department of Immunology and Allergology of Volgograd State Medical University. ORCID: 0000-0003-2674-4289. E-mail: belan.eleonora@yandex.ru

Elizaveta M. Nikiforova – Candidate of Sciences (Medicine), Associate Professor, Associate Professor of the Department of Immunology and Allergology of Volgograd State Medical University. ORCID: 0000-0003-1475-9301. E-mail: maior10@yandex.ru

Tatyana E. Zayachnikova – Doctor of Sciences (Medicine), Associate Professor, Professor of the Department of Pediatrics and Neonatology of the Institute of Continuous Medical and Pharmaceutical Education of Volgograd State Medical University. ORCID:0000-0001-6758-4686. E-mail: guz5deti@mail.ru

Ivan N. Shishimorov – Doctor of Sciences (Medicine), Associate Professor, the Head of the Department of Pediatrics and Neonatology of the Institute of Continuous Medical and Pharmaceutical Education of Volgograd State Medical University. ORCID: 0000-0001-6098-7028. E-mail: drshishimorov@gmail.com

Olga V. Magnitskaya – Doctor of Sciences (Medicine), Associate Professor, Professor of the Department of Pediatrics and Neonatology of the Institute of Continuing Medical and Pharmaceutical Education of Volgograd State Medical University. ORCID: 0000-0001-6670-9029. E-mail: magol73@ya.ru

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2020-8-3-169-180

ADDITIVE NEUROPROTECTIVE EFFECT OF 3-HYDROXYPYRIDINE DERIVATIVES AND HUMAN ERYTHROPOETIN ANALOGUE ON A HEMORRHAGIC STROKE MODEL IN RATS

P.D. Kolesnichenko[1], O.V. Scheblykina[1], N.I. Nesterova[1,2], D.V. Scheblykin[1], A.V. Nesterov[1], M.V. Pokrovskiy[1], M.A. Zhuchenko[3], A.V. Tverskoy[1], K.M. Reznikov[4]

[1] Belgorod State National Research University
85, Pobeda St., Belgorod, Russia, 308015
[2] Forensic-histological department of Belgorod Bureau of Forensic Medical Examination
159, Volchanskaya St., Belgorod, Russia, 308017
[3] Sector of development and preclinical research of State Pharmaceutical Foundation of officinal medicines "PHARMAPARK"
8 (Bld. 1), Nauchny proezd, Moscow, Russia, 117246
[4] Voronezh State Medical University n.a. N.N. Burdenko
10, Studencheskaya St., Voronezh, Russia, 394036

E-mail: farpavel@narod.ru

Received 10 December 2019 Review (1) 20 April 2020 Review (2) 15 May 2020 Accepted 05 July 2020

The correction of free radical oxidation processes is one of the most promising strategies of neuroprotection in acute cerebrovascular disorders.

The aim of the study is an experimental study of the neuroprotective effects of 3-hydroxypyridine and erythropoietin derivatives, as well as their combined use.

Materials and methods. The study was performed on 109 male Wistar rats. The neuroprotective effect of the substances was studied on a hemorrhagic stroke model. The study drugs were administered to the animals intraperitoneally. Carbamylated darbepoetin was administered three times in advance at the dose of 100 μg/kg within intervals of 3 days, the last injection took place 1 hour before the operation (the total dose was 300 mg/kg). Etoxidol was administered once 1 hour before the surgery at the dose of 50 mg/kg. The survival rate, behavioral features and the state of the animals on the 1st, 3rd, 7th and 14th days were recorded, and the morphological assessment of the brain was carried out.

Results and discussion. The investigated substances had a positive effect on both the survival rate of the animals during the first day and on the 14th day. The best survival rates on the 14th day were recorded in the group of a combined use of ethoxydol and carbamylated darbepoetin (75%). Thus, in this group of rats, a faster recovery of neurological disorders was already distinguished from the first day on. By the 7th day, more than 50% of the rats receiving the combination of the studied drugs, had had a slight neurological deficit (up to 3 points on the McGrow scale); by the 14th day there had been only minor changes in the neurological status in the rats of this group. A pronounced neuroprotective effect of the combination of 3-hydroxypyridine and erythropoietin derivatives has been confirmed by a histological examination of brain slices – a more rapid decrease in the size of perifocal edema and microcirculation disorders, less damage to neurons and glial elements, and faster processes of resorption and organization of hemorrhage. A macroscopic examination of the brain sections stained with triphenyltetrazolium chloride of the dying rats, showed that perifocal necrosis had been the main cause of high mortality in the control group after the 3rd day.

Conclusion. As a result of the experiment, the nephroprotective effect of the studied derivatives of 3-hydroxypyridine and erythropoietin has been proved. Moreover, the combination of these drugs has shown a greater neuroprotective activity than their isolated use. The additive effect of these drugs was due to their action mechanism resulting from the synergism of various structures and components of the cells.

Keywords: hemorrhagic stroke, 3-hydroxypyridines, carbamylated darbepoetin, neuroprotection

For citation: P.D. Kolesnichenko, O.V. Scheblykina, N.I. Nesterova, D.V. Scheblykin, A.V. Nesterov, M.V. Pokrovskiy, M.A. Zhuchenko, A.V. Tverskoy, K.M. Reznikov. Additive neuroprotective effect of 3-hydroxypyridine derivatives and human erythropoetin analogue on a hemorrhagic stroke model in rats. *Pharmacy & Pharmacology*. 2020;8(3):169-180. **DOI:** 10.19163/2307-9266-2020-8-3-169-180

Для цитирования: П.Д. Колесниченко, О.В. Щеблыкина, Н.И. Нестерова, Д.В. Щеблыкин, А.В. Нестеров, М.В. Покровский, М.А. Жученко, А.В. Тверской, К.М. Резников. Аддитивное нейропротективное действие производных 3-гидроксипиридина и эритропоэтина человека на модели геморрагического инсульта у крыс. *Фармация и фармакология.* 2020;8(3):169-180. **DOI:** 10.19163/2307-9266-2020-8-3-169-180

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

АДДИТИВНОЕ НЕЙРОПРОТЕКТИВНОЕ ДЕЙСТВИЕ ПРОИЗВОДНЫХ 3-ГИДРОКСИПИРИДИНА И ЭРИТРОПОЭТИНА ЧЕЛОВЕКА НА МОДЕЛИ ГЕМОРРАГИЧЕСКОГО ИНСУЛЬТА У КРЫС

П.Д. Колесниченко[1], О.В. Щеблыкина[1], Н.И. Нестерова[1,2], Д.В. Щеблыкин[1], А.В. Нестеров[1], М.В. Покровский[1], М.А. Жученко[3], А.В. Тверской[1], К.М. Резников[4]

[1] ФГАОУ ВО «Белгородский государственный национальный исследовательский университет»
308015, Россия, г. Белгород, ул. Победы, 85
[2] Судебно-гистологическое отделение ОГБУЗ «Белгородское бюро судебно-медицинской экспертизы»
308017, Россия, г. Белгород, ул. Волчанская, 159
[3] Сектор разработки и доклинических исследований ГЛФ ООО «ФАРМАПАРК»
117246, Россия, г. Москва, Научный проезд, д. 8, стр. 1
[4] Федеральное государственное бюджетное образовательное учреждение высшего образования «Воронежский государственный медицинский университет им. Н.Н. Бурденко» Министерства здравоохранения и социального развития Российской Федерации
394036, Россия, г. Воронеж, ул. Студенческая, 10

E-mail: farpavel@narod.ru

Получено 10.12.2019 Рецензия (1) 20.04.2020 Рецензия (2) 15.05.2020 Принята к печати 15.07.2020

Коррекция процессов свободно-радикального окисления является одной из наиболее перспективных стратегий нейропротекции при острых нарушениях мозгового кровообращения.
Цель исследования – экспериментальное изучение нейропротективных эффектов производных 3-гидроксипиридина и эритропоэтина, а также их комбинированного применения.
Материалы и методы. Исследование выполнено на 109 крысах-самцах линии Вистар. Нейропротективное действие субстанций изучалось на модели геморрагического инсульта. Исследуемые препараты вводились животным внутрибрюшинно. Карбамилированный дарбэпоэтин вводился предварительно трехкратно в дозе 100 мкг/кг с интервалом 3 дня, последнее введение за 1 час до операции (суммарная доза – 300 мг/кг). Этоксидол вводился однократно за 1 час до операции в дозе 50 мг/кг. Регистрировали выживаемость, особенности поведения и состояния животных на 1, 3, 7 и 14-е сутки, проводили морфологическую оценку головного мозга.
Результаты. Исследуемые вещества благоприятно влияли как на выживаемость животных в течение первых суток, так и на 14-суточную выживаемость. Наилучшие показатели выживаемости на 14-е сутки зафиксированы в группе комбинированного применения этоксидола и карбамилированного дарбэпоэтина (75%). Так, в этой группе крыс уже с первых суток наблюдалось более быстрое восстановление неврологических нарушений. К 7-м суткам более 50% крыс, получавших комбинацию исследуемых препаратов, имели легкий неврологический дефицит (до 3 баллов по шкале McGrow), к 14-м суткам у крыс этой группы выявлялись лишь незначительные изменения в неврологическом статусе. Выраженный нейропротекторный эффект комбинации производных 3-гидроксипиридина и эритропоэтина подтвержден гистологическим исследованием тканей головного мозга – более быстрое уменьшение перифокального отека и нарушений микроциркуляции, меньшее повреждение нейронов и глиальных элементов и более быстрые процессы резорбции и организации кровоизлияния. При макроскопическом исследовании окрашенных трифенилтетразолием хлористым срезов мозга умирающих крыс установлено, что перифокальный некроз является основной причиной высокой летальности в контрольной группе после 3 суток.
Заключение. В результате эксперимента доказано нейропротективное действие исследуемых производных 3-гидроксипиридина и эритропоэтина. При этом комбинация данных препаратов показала большую нейропротективную активностью, чем изолированное их применение. Аддитивное действие данных препаратов обуславливается их механизмом действия в результате взаимодействия с различными структурами и компонентами клетки.
Ключевые слова: геморрагический инсульт, 3-гидроксипиридины, карбамилированный дарбэпоэтин, нейропротекция

INTRODUCTION

The significance of a stroke as a medical and social problem increases every year around the world. This is associated with an increase in the average age of the population, as well as an increase in the population of people with risk factors for cardiovascular diseases.

Currently, drugs and new compounds of the so-called neuroprotective action, based on a variety of mechanisms, including antioxidant, antihypoxic, anti-apoptotic and other effects, are widely used for the prevention and treatment of cerebrovascular diseases.

In clinical practice, antioxidant drugs based on 3-hydroxypyridine (for example, mexidol, etoxidol, emoxipin), are widely used in the treatment of cerebrovascular diseases, as they inhibit lipid peroxidation processes, increase the activity of antioxidant enzymes, thereby mod-

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-169-180

ulating the activity of receptors and membrane-bound enzymes [1].

However, despite the achievements of modern neuropharmacology, there is an increase in the number of patients with this pathology: their high mortality (50–70%) [2] and disability (approximately 2/3 patients) [3]. The issues of drug support for patients with acute cerebrovascular accidents remain the most important problem of modern pharmacology and neurology.

The experimental data confirming a high neuroprotective potential of erythropoietin, have been accumulated for several decades. The erythropoietin molecule is best known as a positive regulator of erythropoiesis, which is produced primarily in the kidneys in response to a decrease in the partial pressure of oxygen. However, the spectrum of physiological effects of erythropoietin is quite wide and allows us to consider it as an agent with a universal cytoprotective orientation. The metabolic cascades started by them, lead to an increase in the resistance of the cells to damage, and this phenomenon is combined into the concept of "non-hematopoietic effects of erythropoietin" [4]. In ischemic lesions of various organs, erythropoietin causes angiogenic, antioxidant, anti-inflammatory and anti-apoptotic effects [5], which reduce the damage area. At the same time, due to the activation of a large number of secondary mediators, erythropoietin can cause the development of such negative effects as an increase in endothelin production, an increase in tissue renin concentration, a change in the balance of vascular tissue prostaglandins, angiogenesis stimulation and proliferation of vascular smooth muscle cells [6, 7]. Carbamylated darbepoetin is fundamentally different from erythropoietin-based drugs, combining the best qualities of medicinal preparations of the previous generations [8, 9].

THE AIM of the research was an experimental study of the therapeutic efficacy of 3-hydroxypyridine and erythropoietin as well as their combinations, in the simulation of experimental intracerebral posttraumatic hematoma in rats.

MATERIALS AND METHODS

Compliance with the rules of the organization of laboratory research

The study was conducted in accordance with the approved rules of good laboratory practice of the Ministry of Health of the Russian Federation (GOST 51000.3-96 and 51000.4-96) No 267 "On rules of good laboratory practice" dated 19 June 2003.

Study design

Male Wistar rats were divided into several groups: Group 1 – falsely operated rats (10 animals), which were anesthetized, then scalped and trepanned without any destruction of the brain tissue; Group 2 – the animals with a hemorrhagic stroke, not receiving drugs (control group, 23 rats); Group 3 – the animals with a hemorrhagic stroke, which were administered with etoxidol (23 rats); Group 4 – the animals with simulated pathology that were administered with carbamylated darbepoietin (20 rats), Group 5 – the rats with a hemorrhagic stroke, treated with carbamylated darbepoetin and etoxidol (23 rats).

The technique of modeling a hemorrhagic stroke

An acute autohemorrhagic stroke was modelled in the area of the inner capsule of the right hemisphere, according to the methods of Makarenko et al. [10] in the authors' modification [11]. The operation was performed under general anesthesia. After premedication with "Xyla" at the dose of 0.1 ml, chloral hydrate as a basic anesthetic was administered intraperitoneally at the dose of 300 mg/kg. After deep anesthesia, the blood was sampled with a syringe from the rat tail vein. After the treatment of the surgical field, a linear incision of the scalp in the parietal area was made. The incision performed in the frontal plane, was followed by hemostasis. The length of the incision was 1.5 cm. After that, bone skeletonization was performed and the periosteum was separated. With the help of a dental bur, a trephination hole in the right parietal area was superimposed. The diameter of the burr hole was 3 mm. Then, using a device for a stereotactic administration, a puncture needle was inserted in the area of the inner capsule (the coordinates were: H=4 mm, L=3.1 mm, A=1.5 mm from the bregmatic fontanel according to "Atlas of the human brain stem" by G. Paxinos) to a depth of 3 mm. Then the device was fixed, a *mandrel*-wire *knife* was inserted into the needle, the destruction of the brain tissue was carried out (the *mandrel* was turned in three turns clockwise and three turns counterclockwise). The *mandrel* was removed and, under sterile conditions, the autologous blood was taken from the tail vein of the animal and injected into the rat in a volume of 0.11 ml/100 g of weight. The introduction of the blood was carried out by stream infusion. The effectiveness of the introduction was determined by the presence of stem convulsions. After that, the puncture needle was removed, the wound was dried, hemostasis was monitored and the wound was layered. Falsely operated animals underwent scalping and trepanning of the skull.

The study drugs were administered to the animals intraperitoneally. Carbamylated darbepoetin (Pharmstandard LLC, Russia) was administered three times in advance at the dose of 100 μg/kg with an interval of 3 days (the total dose was 300 mkg/kg), the last injection was an hour before the operation. Etoxidol, a 3-hydroxypyridine derivative (Sintez OJSC, Russia) was administered once a day before the operation at the dose of 50 mg/kg (according to the interspecific conversion rate of the human average therapeutic dose). The control animals were injected with saline in an equivalent volume.

Effect of drugs on animals' survival

Observations were carried out for 14 days after the surgery. The registered features of the behavior and the state of the animals on the 1st, 3rd, 7th and 14th days were studied.

Study of neurological status

To assess the behavioral disorders of the animals after a hemorrhagic stroke, a set of traditional methods was used in the experiment. To assess the neurological status, a method of assessing a neurological deficit according to McGrow's CHADS2 in Gannushkina's modification was used [12]. To assess the muscle tone by measuring the strength of the grip of the limbs, a dynamometric software and hardware system had been developed.

In assessing the neurological status according to McGrow's CHADS2 in Gannushkina's modification, the following factors were taken into consideration: mild symptoms (up to 3 points) – lethargy of movements, weakness of limbs, unilateral semidiaphanous, tremor, manoeuvring movements; severe manifestations of neurological disorders (from 3.5 to 10 points) - paresis and paralysis of the limbs, as well as a lateral position and depression of consciousness.

Measurement of the strength of the animals in the grasping reflex was carried out using a dynamometer. As a comparison criterion, the relative value (a specific force) was calculated by dividing the maximum grip force by the rat body weight. In order to assess the orienting-exploratory behavior, the platform was used to study the motor activity of the laboratory animals - ACTI-TRACK (PANLAB HARVARD APPARATS). Testing of the rats was carried out for 5 minutes in the infrared monitor of the activity prior to the creation of pathology, as well as on days 1, 3, 7 and 14 after modeling a hemorrhagic stroke.

Morphological study

For macroscopic confirmation of the repeatability of the results, verification of the localization of the hemorrhagic lesion and the degree of damage in some animals, NADH-dehydrogenase activity was studied on day 4 by the standard method of staining with triphenylterazolium chloride. The animal's brain was extracted, cut into 2 frontal slices through the entry point of the *mandrel* into the brain tissue. Staining was carried out in a 1% solution of triphenyltetrazolium chloride (TTX, Sigma Aldrich) for 30 minutes in a thermostat at 37°C. Then, photographing and a macroscopic evaluation of the slices were carried out.

For a morphological evaluation, the animals were withdrawn from the experiment after 24 hours, 7 and 14 days from the start of the study. The description and assessment of the consequences of a hemorrhagic stroke were carried out according to the recommendations of the atlas of the nervous system histopathology [13]. The rats were decapitated, the brain was collected, fixed in 10% neutral buffered formalin for 24–48 hours, and embedded in paraffin. Frontal histological sections of the brain with a thickness of 7 µm were stained with hematoxylin and eosin. A microscope "MIKMED-6" with a binocular attachment, electric illumination, a digital camera MS-5 and a computer with software "MSview" were used.

Statistical processing of the obtained data was performed using STATISTICA 10.0 and MICROSOFT EXCEL 2016. After estimating the normality of the distribution using the Shapiro-Wilk criterion, the arithmetic mean, confidence intervals (with a parametric distribution) and quartile span (with a nonparametric distribution) were calculated. To assess the reliability of intergroup differences with a normal distribution, the Student's t test was used, with a distribution other than normal – the Mann-Whitney test. To assess the survival analysis, the procedure of constructing survival curves was used; the differences between the groups were considered significant at $p<0.05$.

RESULTS

Effect of drugs on animals' survival analysis

During the operation and for 1 day after it, 50% of rats with a hemorrhagic stroke died in the control group. In the group of the animals administrated with the test substances, the mortality within 1 day was lower than in the control group (Fig.1). In particular, in the groups of rats treated with carbamylated darbepoietin or etoxidol, a daily survival rate was 70%. In the group of the combined use of carbamylated darbepoetin and etoxidol, the daily survival rate was 90%.

The fact that in the groups administrated with the drugs under study there were no deaths of animals after 7 days, is of particular attention.

All the studied substances increased the total (day 14) survival rate by more than 40% compared to the control group. The best survival rates for day 14 were recorded in the group of the combined use of ethoxydol and carbamylateddarbepoetin: the total survival rate of the animals in this group was 75%.

No deaths have been recorded in the group of falsely operated animals during the entire observation period.

Evaluation of neurological deficit according to the McGrow scale

As for the assessment of neurological disorders, on the first day after the operation, almost all the rats with simulated intracerebral hematoma showed pronounced post-stroke changes in the form of cyclic motions, paresis and limb paralysis (Table 1). In the group of falsely operated animals, no severe neurological deficit was observed, and only 30% of the falsely operated rats showed lethargy and slowness of movements.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-169-180

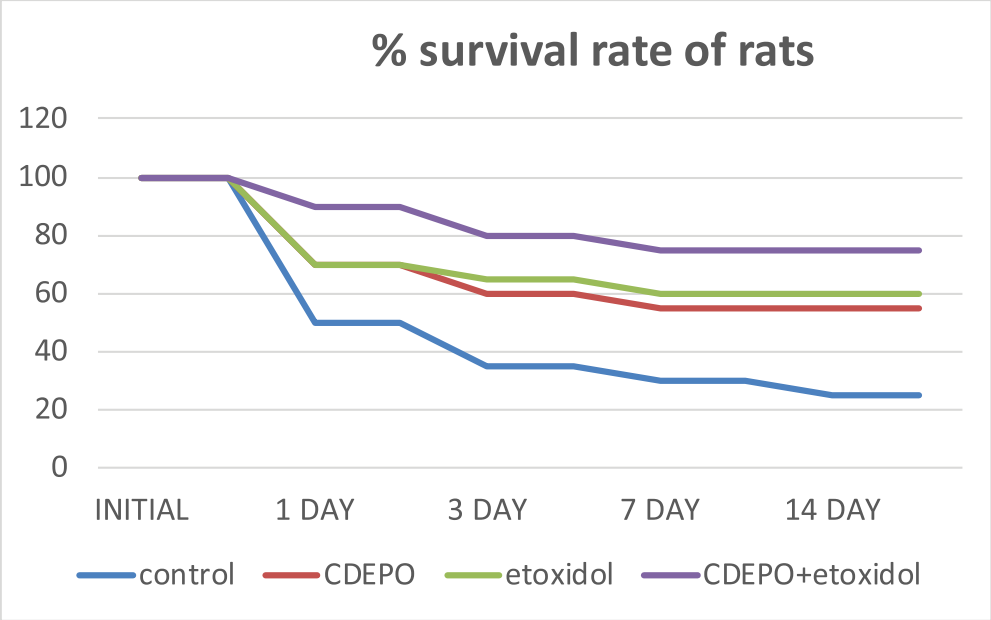

Figure 1 – Effect of ethoxydol, carbamylated darbepoetin and their combined use on the survival analysis of animals on day 1, 3, 7 and 14 after the simulation of hemorrhagic stroke

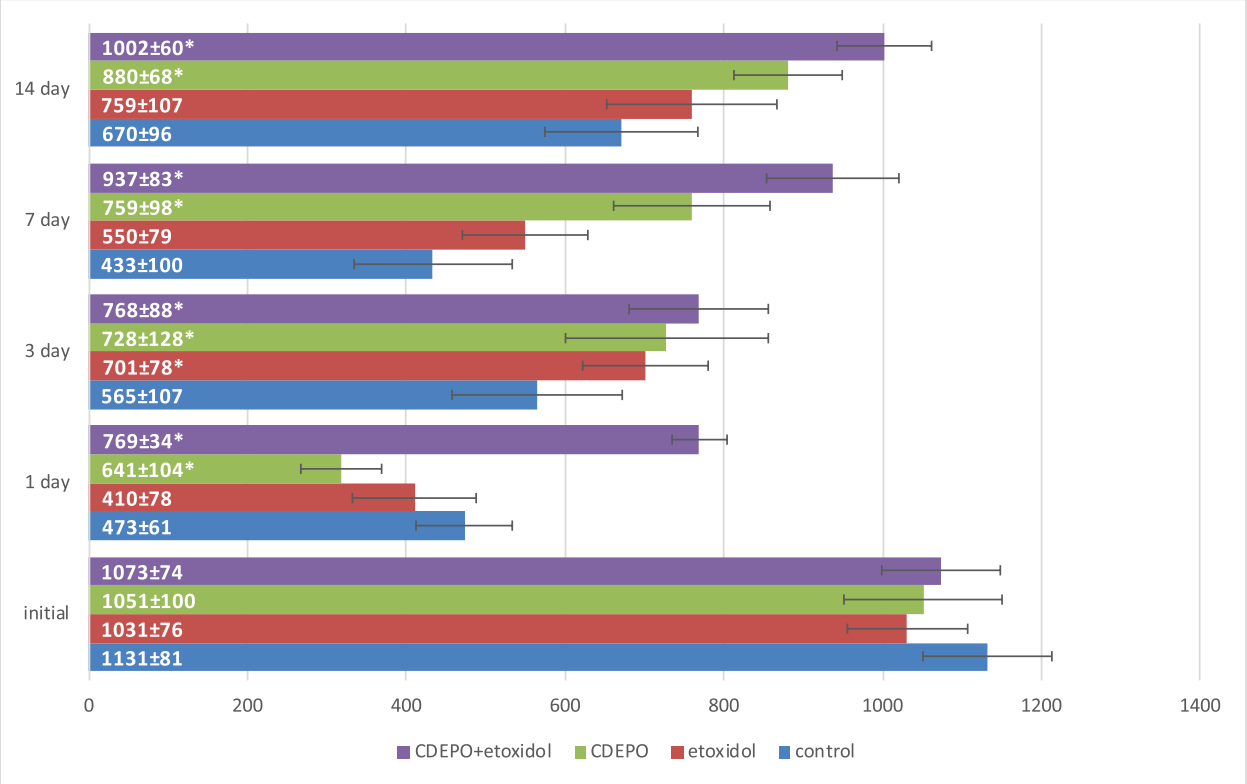

Figure 2 – Effect of etoxidol, carbamylated darbepoetin and their combined use, on total activity indicators calculated by the Acti-Track program on days 1, 3, 7 and 14 after modeling a hemorrhagic stroke

Note: * P<0.05 – the differences are statistically significant when compared with control animals

The rats treated with the test substances, had a statistically significant, less pronounced neurological deficit over the entire observation period compared with the control group.

The group of the rats treated with a combination of etoxidol and carbamylated darbepoetin, had been distinguished by a more rapid recovery of neurological disorders since the first day. By day 7, more than 50% of the rats administrated with the combination of the studied drugs, had had a slight neurological deficit (up to 3 points according to the McGrow scale) in the form of slowness of movements, weakness of limbs, unilateral hemiptosis and manege movements. By day 14, only minor changes in the neurological status had been observed in the rats of this group – 1.1±0.5 according to the McGrow stroke scale.

Study of neurological status

Table 1 – Effect of etoxidol, carbamylated darbepoetin and their combined administration on indicators of neurological status on days 1, 3, 7 and 14 after modeling a hemorrhagic stroke

	Before pathology modeling			
	Control	Etoxidol	CDEPO	CDEPO+etoxidol
Specific force	7.0±0.3	7.5±0.2	7.4±0.3	7.7±0.3
McGrow	0	0	0	0
	Day 1			
	Control	Etoxidol	CDEPO	CDEPO+etoxidol
Specific force	2.7±0.3	3.1±0.2	3.1±0.2*	3.1±0.1*
McGrow	8.1±2.0	6.7±2.4*	7.2±2.0	5.6±1.8*
	Day 3			
	Control	Etoxidol	CDEPO	CDEPO+etoxidol
Specific force	4.0±0.5	4.5±0.5	4.6±0.2*	5.1±0.3*
McGrow	6.4±2.6	4.3±1.8*	4.2±2.5*	3.9±2.4*
	Day 7			
	Control	Etoxidol	CDEPO	CDEPO+ etoxidol
Specific force	4.5±0.3	4.6±0.3	4.8±0.3	4.2±0.5
McGrow	5.4±2.1	3.3±2.1*	3.2±2.2*	2.9±1.9*
	Day 14			
	Control	Etoxidol	CDEPO	CDEPO+etoxidol
Specific force	4.7±0.2	5.0±0.5	4.5±0.4	4.5±0.5*
McGrow	5.0±2.5	3.0±0.5	2.4±0.6*	1.1±0.5*

Note: * P <0.05 – the differences are statistically significant when compared with control animals

Muscle tone studies

The study of the grip strength of rats' paws revealed that on the first day after the stroke, the muscle tone in the control group and in the group receiving carbamylated darbepoetin, did not differ significantly and amounted to 56% on average (Table 1). Against the background of the ethoxidol administration, the decrease in the muscle tone for the 1st day was 38.3%, which was significantly lower than in the control group. The greatest decrease in the muscle tone was observed in the group of the animals administrated with a combination of the studied drugs, and amounted to 27.4%. On day 3, there was an increase in muscle strength in all groups, and an increase in the groups administrated with the test substances, was significantly higher than in the control group. On days 7 and 14, a statistically significant increase in the muscle strength was only in the rats administrated with carbamylated darbepoetin and a combination of carbamylated darbepoetin and etoxidol.

The effect of the studied drugs on the motor activity of the animals with a hemorrhagic stroke was also studied. Within days 1–7, after modeling a hemorrhagic stroke, the indicators of the total activity (Fig. 2) and the total distance (Fig. 3) under the influence of etoxidol and the combination of carbamylated darbepoetin and etoxidol preparations, were significantly higher than in the control group.

On day1, the indicators of the total activity and the distance gone by the rats treated with carbamylateddarbepoietin, were significantly lower than the results of the animals administrated with ethoxydol and the combination of the drugs, and did not have significant differences with the control group. However, by day 3, the activity of the animals treated with carbamylated darbepoietin, had been increasing and it did not have statistically significant differences from the groups of other test substances. By the 14th day, the activity indicators of the animals in this group had even exceeded those in the ethoxidol group, and were slightly inferior to the group of the drug combinations.

Compared with monotherapy, since 1 day, the indicators of the total activity had been increasing most actively and rapidly under the influence of the combination of drugs.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-169-180

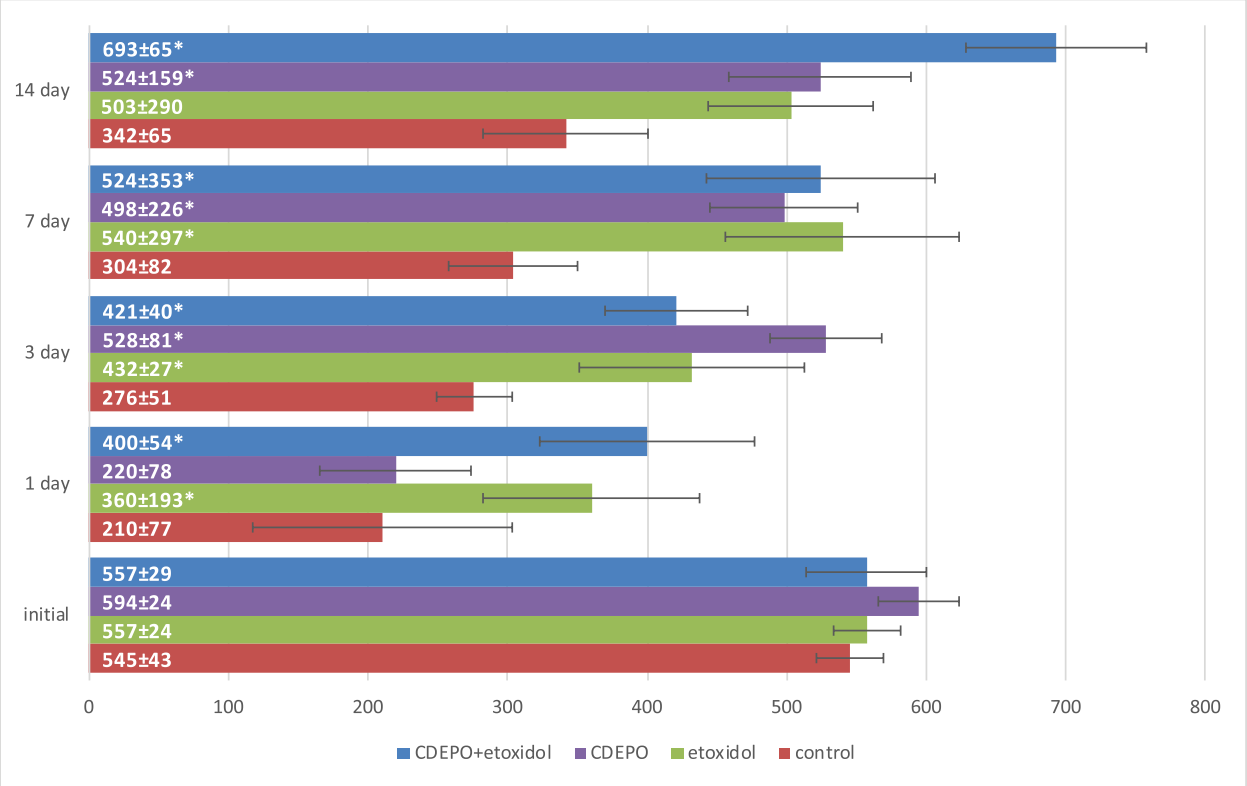

Figure 3 – Effect of etoxidol, carbamylated darbepoetin and their combined use on total distance indicators calculated by the Acti-Track program on days 1, 3, 7 and 14 after modeling a hemorrhagic stroke

Note: * P<0.05 – the differences are statistically significant when compared with control animals

Figure 4 – Efficiency of the additive neuroprotective action of carbamylateddarbepoetin and ethoxidol in modeling a hemorrhagic stroke in rats. Macroscopic view of brain sections stained with triphenyltetrazolium chloride

Note: A. Section of a dying rat's brain from the control group on day 1. B. Section of a dying rat's brain from the control group on day 5. C. Section of a dying rat's brain from the CDEPO+ethoxidol group

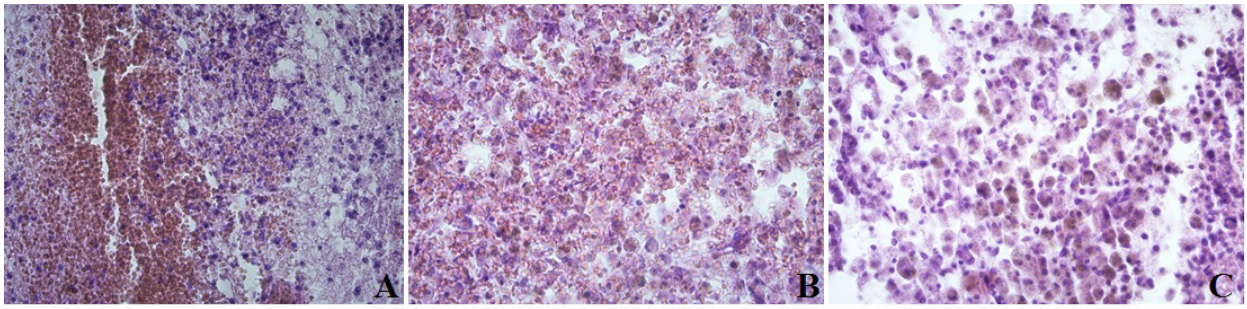

Figure 5 – Rats' brain tissue in the hematoma area

Note: A – control group on the 1st day; B – control group on the 7th day; C – control group on the 14th day. Staining: hematoxylin and eosin; Mag. ×400

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

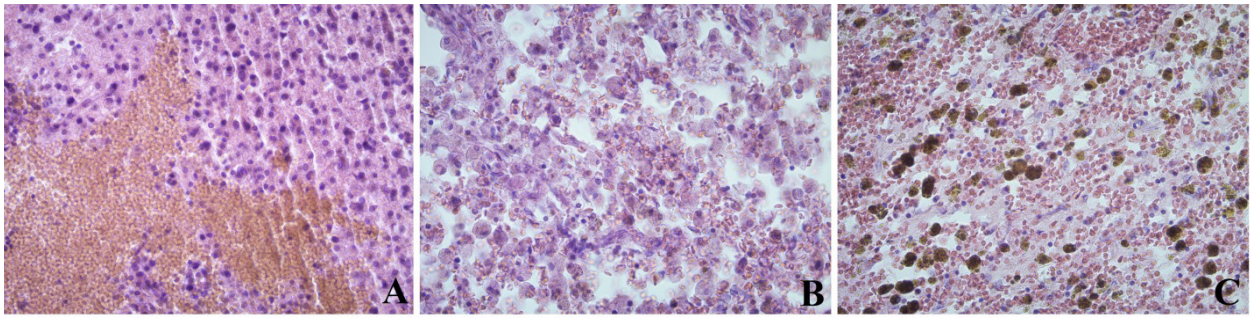

Figure 6 – Brain tissue of rats treated with ethoxidol, in the area of hematoma

Note: A – on the 1st day; B – on the 7th day; C – on the 14th day. Staining: hematoxylin and eosin; Mag. ×400

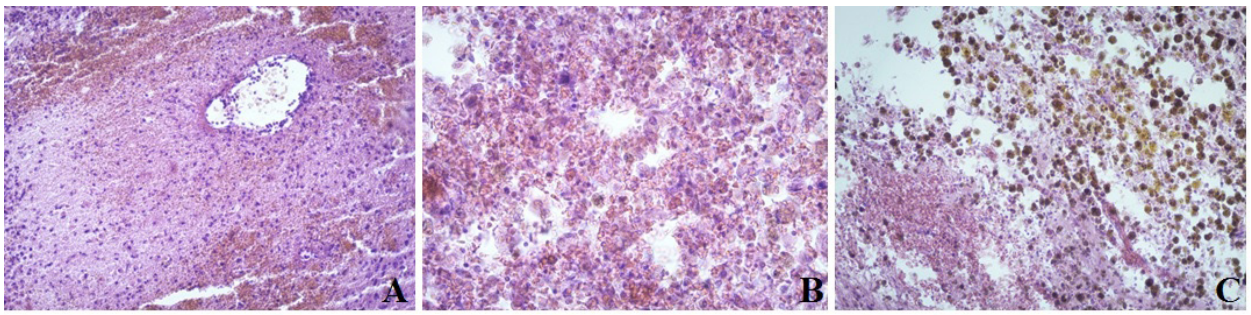

Figure 7 – Brain tissue of rats treated with carbamylated darbepoetin, in the area of hematoma

Note: A – on the 1st day; B – on the 7th day; C – on the 14th day. Staining: hematoxylin and eosin; Mag. ×400

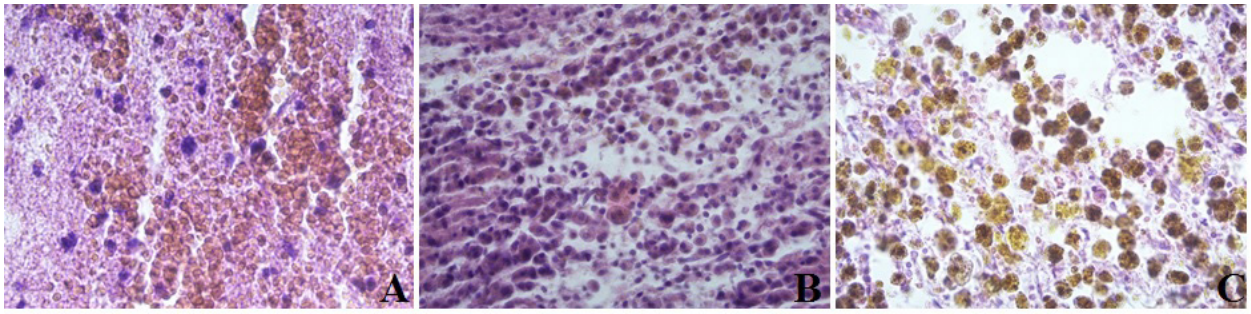

Figure 8 – Brain tissue of rats treated with a combination of carbamylated darbepoetin and ethoxydol, in the area of hematoma

Note: A – on the 1st day; B – on the 7th day; C – on the 14th day. Staining: hematoxylin and eosin; Mag. ×400

High levels of activity of the control group on day 14, can be explained by a high mortality rate in this group and survival of only the strongest individuals with high a regenerative potential.

Morphological study

For a macroscopic confirmation of the results, repeatability and adequacy of the methodology for clinical cases of a hemorrhagic stroke, staining of brain sections with triphenylterazolium chloride was performed (Fig. 4).

A macroscopic evaluation confirmed the adequacy of the use of neuroprotective therapy in the model of a hemorrhagic stroke (Fig. 4). It is obvious that the causes of mortality on the 1st day, were associated both with a direct effect of the blood injected into the brain tissue, and with dislocation complications. However, starting from the 4th day, the mortality was due to massive perifocal necrosis.

In the control group animals, on the first time a day after modeling an intracerebral hemorrhage along the periphery of the hematoma, the following disorders were detected: a pronounced edema; violation of histoarchitectonics of the neuronal layers of the cortex, pronounced ischemic changes, karyolysis of neurons, a moderately pronounced perifocal leukocyte reaction and a less pronounced glial reaction (Fig. 5A).

Polymorphism of neurons was detected: swelling of neurocytons, the nuclei of neurons were deformed and basophilic, the nucleoli were deformed and displaced to the periphery. In places, the nuclei and nucleoli were almost or completely indistinguishable.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-169-180

There was also neuronal karyolysis, a moderate perifocal leukocyte response and a less pronounced glial response (Fig. 5A).

On the seventh day, a moderately pronounced perifocal leukocyte reaction changed to a moderately pronounced glio-macrophage reaction with an admixture of single macrophages with an intracellular accumulation of a blood pigment (hemosiderophage) (Fig. 5B). On day 14, a moderately pronounced glio-macrophage reaction persists, but with an admixture of a few macrophages with intracellular accumulation of a blood pigment (hemosiderophages) (Fig. 5C).

When analyzing the group using etoxidol, it was established that a perifocal edema was less pronounced compared to the control group (Fig. 6A) and the group where carbamylated darbepoetin had been used (Fig. 7A); but it was more pronounced than in the group of a combined use of carbamylated darbepoetin and etoxidol (Fig. 8A). In the animals treated with carbamylated darbepoietin and a combination of drugs, the signs of an inflammatory reaction with the development of leukocyte infiltration were more pronounced. At the same time, its severity did not reach the degree of intensity recorded in the control group. On day 7, a glio-macrophage reaction in the etoxidol groups (Fig.6B) and the carbamylated darbepoetin group (Fig. 7B) was ahead of the control group. In the group of the combined use of carbamylated darbepoietin and etoxidol (Fig. 8B), more pronounced resorption processes in the form of clusters of a few macrophages accumulation of a blood pigment (hemosiderophages), were revealed. On the 14th day, the processes of resorption in the groups of etoxidol (Fig. 6C) and carbamylated darbepoetin (Fig. 7C) were ahead of the control group. But the signs of resorption and organization in the group of a combined use of carbamylated darbepoetin and etoxidol (Fig. 8C), were more pronounced, they were in the form of clusters of numerous macrophages with intracellular and extracellular accumulations of a blood pigment (hemosiderophages).

Thus, according to the histological studies, a simultaneous administration of a carbamylated darbepoetin and etoxidol combination is accompanied by a more rapid decrease in perifocaledema and microcirculation disorders, less damage to neurons and glial elements, and faster processes of resorption and organization of the hemorrhage focus.

DISCUSSION

The results of the study confirm the presence of neuroprotective properties in all the studied substances. However, the neuroprotective activity of carbamylated darbepoetin had been developing more slowly - by day 3, in contrast to etoxidol and the combination of etoxidol and carbamylated darbepoietin, in which cerebroprotective properties were observed already on the 1st day (a less prounced severity of neurological disorders and a greater activity of the animals in these groups in the infrared monitor).

The combination of etoxidol and carbamylated darbepoetin has more pronounced neuroprotective properties than when used in isolation. This is manifested by higher survival rates of animals of this group, a significant decrease in the severity of post-stroke disorders from the very first day, as well as in histological examination of brain sections.

The additive effect of these drugs is due to their action mechanism. Hypothetical synergism is achieved by affecting various structures and components of the cell.

Etoxidol is among the inhibitors of free radical processes. The presence of 3-hydroxypyridine in the structure of etoxidol, provides a complex of its antioxidant and membranotropic effects, the ability to reduce glutamate excitotoxicity, to modulate functioning of the receptors. The 3-hydroxypyridine residue affects the activity of membrane-bound enzymes (phosphodiesterase, adenylatecyclase), inhibits free radical stages of the synthesis of prostaglandins, catalyzed by cyclooxygenase and lipooxygenase, changes the ratio of simple cyclin / thromboxane A2 and inhibits the formation of leukotrienes [14].

Malate, which is a part of etoxidol, easily penetrates the blood-brain barrier. During hypoxia, malate undergoes metabolism with the formation of adenosine triphosphate. Depending on the degree of hypoxia, malate is reversibly rebuilt, due to which the cell continues to receive energy even in the absence of oxygen. Malate has the advantage of being able to turn into fumarate and even succinate.

Depending on the degree of ischemia and the cell's energy requirements, malate can be oxidized with the release of ATP with enough oxygen in the mitochondria and even with insufficient oxygen in the cytoplasm, it can also be restored to succinate. It has been established that the cell does not expend the ATP energy for the transfer of malate to mitochondria. For this, there is a special malate-aspartate shuttle. The ability of malate to increase the respiratory control coefficient of mitochondria, to restore cytochrome b5 in the presence of nicotinamide adenine dinucleotide, a coenzyme participating in redox reactions, has been proved and shown off [15–17].

The hematopoietic functions of erythropoietin are due to their effect on the central nervous system. Erythropoietin receptors are expressed on the surface of neurons [18]. At various CNS injuries, astrocyte synthesis of erythropoietin having a neuroprotective effect, is observed [19–21]; it inhibits apoptosis, stimulates neuronal proliferation and angiogenesis.

Carbamylated darbepoetin, a hyperglycosylated

variant of human recombinant erythropoietin,' has less pronounced hematopoietic properties than the basic molecule due to the heterodimeric receptor EpoR / CD131 than to the homodimeric receptor EpoR / EpoR [22]. When the carbamylated darbepoietin molecule, as well as erythropoietin, binds to the EpoR receptor, a cascade of phosphorylation reactions of key proteins, such as Ras-mitogen-activating protein kinase, Janus tyrosine kinase-2, etc., is launched. They, in turn, activate the expression of the bcl-xl genes and the synthesis of anti-apoptotic proteins that suppress apoptotic cell deaths [23, 24].

However, due to the carbamylation of primary protein amines and amino acid lysine residues of the protein in the N-terminal region, without affecting the glycosylation profile of the whole molecule, carbamylated darbepoietin does not interact with the classical erythropoietin receptor (does not stimulate the proliferation of TF1 cell line), and does not have a number of undesirable side effects, such as an increased blood pressure and the risk of blood clots, which, in the case of cerebrovascular diseases, is absolutely contraindicated. [25–27].

The results obtained, determine the possibility of further studying the neuroprotective effects of a combination of etoxidol and carbamylated darbepoetin, and the possible future of its introduction into clinical practice for the treatment and prevention of cerebrovascular diseases.

CONCLUSION

The investigated derivatives of 3-hydroxypyridine and human erythropoietin have a neuroprotective effect, which is manifested in the smallest severity of neurological disorders and a more rapid decrease in signs of neurodegeneration, accelerated processes of hemorrhage.

The neuroprotective activity of carbamylated darbepoetin had been developing more slowly – by day 3, in contrast to ethoxydol and the combination of ethoxydol and carbamylated darbepoietin, in which cerebroprotective properties had been notified by already day 1.

The combination of ethoxydol and carbamylated-darbepoetin has a more pronounced neuroprotective properties which are manifested by a significant decrease in the severity of post-stroke disorders. These differences are already noticeable by day 1 of the disease, as evidenced by higher survival rates of the animals in this group, the animals' activity in this group; as well as by a histological examination of brain slices – a faster reduction of perifocaledema and microcirculation disorders, less damage to neurons and glial elements, and more rapid processes of resorption and organization of hemorrhage.

FINANCIAL SUPPORT

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

AUTHOR'S CONTRIBUTION

Kolesnichenko PD – working out the conceptual foundation and design of the study, implemention of the experiment, processing the results, writing the text; **Shcheblykina OV** – statistical processing of the results, editing; **Nesterova NI** – collecting and processing of the morphological materials, carrying out a semi-quantitative morphological analysis; **Shcheblykin DV** – collecting and processing of the materias, implementation of the experiment; **Nesterov AV** – refinement of the experimental model, working out the conceptual foundation and design of the study, implemention of the experiment, carrying out a morphological study; **Pokrovsky MV** – working at the conceptual foundation and design of the study, administrative organization of the experimental work; **Zhuchenko MA** – working at the conceptual foundation, production of the carbamylated form of darbepoetin; **Tverskoy AV** – editing a morphological study of the experimental material; **Reznikov KM** – approval of the final version of the article, responsibility for the integrity of all the parts of the article.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Kesarev OG, Danilenko LM, Pokrovskii MV, Timokhina AS, Khavanskii AV. Study of dose-dependent effect of 2-ethyl-6-methyl-3 hydroxypyridine succinate on the contractile function of isolated rat heat. Research Result in Pharmacology. 2017;3(1):3–9. DOI: 10.18413/2500-235X-2017-3-1-3-9.
2. Skvorcova VI, Chazova IE, Stahovskaja LV. [Vtorichnaja profilaktika insul'ta]. M.: PAGRI. 2002: 120p. Russian
3. Karpov SM, Dolgova IN, Vishlova IA. The main issues of topical diagnosis of nervous system diseases. Stavropol. Stavropol State Medical University. 2015:120p.
4. Reznikov KM, Gorbunova NS, Kolesnichenko PD, Tverskoy AV, Kostina DA, Bashkatova DA, Nikitina VA. Search of new pharmaceuticals on the basis of darbepoetin in the treatment of ischemic stroke (review of literature). Research Result: Pharmacology and Clinical Pharmacology. 2017;3(1):125–136. DOI: 10.18413/2500-235X-2017-3-1-125-136
5. Zhu L, Bai X, Wang S, Hu Y, Wang T, Qian L, Jiang L. Recombinant human erythropoietin augments angiogenic responses in a neonatal rat model of cerebral unilateral

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-169-180

hypoxia-ischemia. Neonatology. 2014;106(2):143–148. DOI: 10.1159/000362262.

6. Fisher JW. Erythropoietin: physiology and pharmacology update. Experimental Biology and Medicine (Maywood). 2003;228(1):1–14. DOI: 10.1177/153537020322800101.
7. Shabelnikova AS. Correction of ischemic damage to the retina on application of pharmacological preconditioning of recombinant erythropoietin. Research Result in Pharmacology. 2016;2(2):67–90. DOI: 10.18413/2313-8971-2016-2-2-67-90.
8. Catlin DH, A. Breidbach, Elliott S, Glaspy J. Comparison of darbepoetin alfa, recombinant human erythropoietin, and endogenous erythropoietin from human urine. Clinical Chemistry. 2002;48(11):2057–2059. DOI: 10.1093/clinchem/48.11.2057.
9. Alehin SA, Kolmykov DI, Pokrovskii MV. Human recombinant erythropoietin gradient dosage in-fluence on ischemic and reperfusion liver injury. Research Result in Pharmacology. 2015;1(1):9–12. DOI: 10.18413/2500-235X-2015-1-4-9-14.
10. Makarenko AN, Kositsin NS, Pasikova NV, Svinov VV. Simulation of local cerebral hemorrhage in different brain structures of experimental animals. Journal of Higher Nervous Activity. 2002;52(6):765–768.
11. [Patent 2721289. Rossijskaja Federacija, MPK G09B 23/28. Sposob modelirovanija gemorragicheskogo insul'ta u krys / Nesterov A.V., Kolesnichenko P.D., Pokrovskij M.V., Nesterova N.I., Markovskaja V.A., Ivanova M.I., Karagodina A.Ju., Saparboeva N.M., Murashev B.V., Proshin A.Ju., Patrahanov E.A., Arhipov I.S., Pokrovskij V.M.; zajavitel' i patentoobladatel' federal'noe gosudarstvennoe avtonomnoe obrazovatel'noe uchrezhdenie vysshego obrazovanija "Belgorodskij gosudarstvennyj nacional'nyj issledovatel'skij universitet" (NIU "BelGU") – № 2019134892; zajavl. 30.10.2019; Published: 2020 May 18]. Russian
12. Gannushkina IV. The pathophysiological mechanisms of disorders of cerebral circulation and the new directions in their prevention and treatment. Journal. Neuropatol. and psychiatrist. 1996. 1:14–18.
13. Ermohin PN. Gistopatologija central'noj nervnoj sistemy: atlas mikrofotografij. PN. Ermohin; pod red. A.P. Avcyna. – Moskva: Medicina, 1969:243. Russian
14. Djumaev KM, Voronina TA, Smirnov LD. Antioksidanty v profilaktike i terapii patologij CNS. – M.: Medicina, 1995:65. Russian
15. Peresypkina A, Pazhinsky A, Pokrovskii M, Beskhmelnitsyna E, Pobeda A, Korokin M. Correction of experimental retinal ischemia by l-isomer of ethylmethylhydroxypyridine malate. Antioxidants. 2019;8(2):34. DOI: 10.3390/antiox8020034. Russian
16. Kolesnichenko PD, Reznikov KM, Zhhernakova NI, Stepchenko AA, Popova IA. The Value Changes Redox System the Body Fluid Media for Life Processes and the Action of Drugs. Journal of International Pharmaceutical. 2018; 45:440–444. Russian
17. Livanov GV, Aleksandrov MV, Vasilyev SA, Batotsyrenova KV, Batotsyrenov BV, Lodyagin AN, Lutsyk MA, Nosov AV. Metabolic Desynchronization in Critical Conditions: Experimental Study. General Reanimatology. 2006;2(1):42–46. DOI: 10.15360/1813-9779-2006-1-42-46.
18. Sanchis-Gomar F, Perez-Quilis C, Lippi G. Erythropoietin Receptor (EpoR) Agonism Is Used to Treat a Wide Range of Disease. Molecular Medicine. 2013;19(1):62–64. DOI: 10.2119/molmed.2013.00025.
19. Tjurenkov IN, Kurkin DV, Bakulin DA, Volotova EV. Studying the Neuroprotective Effect of the Novel Glutamic Acid Derivative Neiroglutam on Focal Cerebral Ischemia in Rats Russian Journal of Experimental and Clinical Pharmacology. 2014;77(9):8–12. DOI: 10.30906/0869-2092-2014-77-9-8-12.
20. Celik M, Gokmen N, Erbayraktar S, Akhisaroglu M, Konakc S, Ulukus C, Genc S, Genc K, Sagiroglu E, Cerami A, Brines M. Erythropoietin prevents motor neuron apoptosis and neurologic disability in experimental spinal cord ischemic injury. PNAS. 2002;99(4):2258–2263. DOI: 10.1073/pnas.042693799.
21. Basov AA, Elkina AA, Samkov AA, Volchenko NN, Moiseev AV, Fedulova LV, Baryshev MG, Dzhimak SS. Influence of deuterium-depleted water on the isotope D/H composition of liver tissue and morphological development of rats at different periods of ontogenesis. Iranian Biomedical Journal. 2019;23(2):129–141. DOI: 10.29252/.23.2.129.
22. Tverskoy AV, Kolesnichenko PD, Shcheblykina OV, Gorbunova NS, Morozov VN, Mukhina TS. Morphology of the rat's brain in four vessels model of ischemic stroke after administration of carbamylated darbepoetin. Drug Invention Today. 2018;10(5):3897–3900.
23. Middleton SA, Barbone FP, Johnson DL, Thurmond RL, You Y, McMahon FJ, Jin R, Livnah O, Tullai J, Farrell FX, Goldsmith MA, Wilson IA, Jolliffe LK. Shared and unique determinants of the erythropoietin (EPO) receptor are important for binding EPO and EPO mimetic peptide. Journal of Biological Chemistry. 1999;274(20):14163–14169.
24. Kolesnichenko PD, Dolzhikov AA, Zhernakova NI, Shaposhnikov AA, Stepchenko AA, Batishcheva GA, Reznikov KM. Preclinical study of the allergenic properties of carbamylated darbepoetin. Indo American Journal of Pharmaceutical Sciences. 2017;4(10):3798–3802.
25. Gaudard A, Varlet-Marie E, Bressolle F, Audran M. Drugs for Increasing Oxygen Transport and Their Potential Use in Doping. Sports Medicine. 2003;33(3):187–212.
26. Pozdniakova NV, Turobov V, Garanina EE, Ryabaya OA. Temporal dynamics of cytokines in the blood of rats with experimentally induced autoimmune encephalomyelitis. Bulletin of RSMU. 2017; 6:69–77. DOI: 10.24075/brsmu.2017-06-12.
27. Basov AA, Kozin SV, Bikov IM, Popov KA, Moissev AV, Elkina AA, Dzhimak SS. Changes in Prooxidant-Antioxidant System Indices in the Blood and Brain of Rats with Modelled Acute Hypoxia Which Consumed a Deuterium-Depleted Drinking Diet. Biology Bulletin. 2019;46(6):531–535. DOI: 10.1134/S1062359019060049.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

AUTHORS

Pavel D. Kolesnichenko – Candidate of Sciences (Medicine), Associate Professor of the Department of Pharmacology and Clinical Pharmacology of Belgorod State National Research University. ORCID 0000-0002-2434-994X. E-mail: farpavel@yandex.ru

Olesya V. Shcheblykina – postgraduate student of the Department of Pharmacology and Clinical Pharmacology of Belgorod State National Research University. E-mail: sheolvi31@gmail.com

Natalya I. Nesterova – postgraduate student of the Department of Pharmacology and Clinical Pharmacology of Belgorod State National Research University, legal physician of the forensic histological department of Belgorod Bureau of Forensic Medical Examination. E-mail: nesterova@mail.ru

Dmitry V. Shcheblykin – postgraduate student of the Department of Pharmacology and Clinical Pharmacology of Belgorod State National Research University. ORCID 0000-0002-2420-2243. E-mail: dmitryshch1@gmail.com

Arkady V. Nesterov – Candidate of Sciences (Medicine), Associate Professor of the Department of Pathology of Belgorod State National Research University. E-mail: nesterov_a@bsu.edu.ru

Mikhail V. Pokrovsky – Doctor of Sciences (Medicine), Professor of the Department of Pharmacology and Clinical Pharmacology, the Head of Research Institute of Pharmacology of Living Systems of Belgorod State National Research University. ORCID 0000-0002-2761-6249. E-mail: mpokrovsky@yandex.ru

Maxim A. Zhuchenko -Candidate of Sciences (Biology), the head of the sector of development and preclinical research of officinal medicines (OOO “FARMAPARK”). E-mail: maksim.zhuchenko@pharmapark.ru

Aleksey V. Tverskoy – Candidate of Sciences (Medicine), the head of the Department of Human Anatomy and Histology of Belgorod State National Research University. ORCID 0000-0003-1537-65-64. E-mail: tverskoy@bsu.edu.ru

Konstantin M. Reznikov – Doctor of Sciences (Medicine), Professor of the Department of Pharmacology of Voronezh State Medical University n. a. Burdenko. E-mail: vrkmf@yandex.ru

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2020-8-3-181-194

ANTIBACTERIAL AND IMMUNOTROPIC PROPERTIES OF ISOLIQUIRITIGENIN IN GENERALIZED STAPHYLOCOCCAL INFECTION IN MICE

E.A. Solyonova, S.I. Pavlova

Chuvash State University n.a. I.N. Ulyanov
15, Moskovsky prospect, Cheboksary, Chuvash Republic, Russia, 428015

E-mail: elensoul@mail.ru

Received 10 February 2020 Review (1) 10 April 2020 Review (2) 20 April 2020 Accepted 28 April 2020

The article is devoted to the study of the effects of isoliquiritigenin in generalized bacterial infections.
The aim is to study antibacterial and immunotropic mechanisms and effects of isoliquiritigenin in generalized staphylococcal infections in a mouse model.
Materials and methods. To assess the survival rate of Balb/C mice, a generalized infection model caused by *Staphylococcus aureus J49 ATCC 25923* with Kaplan-Meier curves was used. The degree of bacteremia during the development of infection was determined by the method of sector crops. The minimum inhibitory concentration of isoliquiritigenin against *Staphylococcus aureus J49 ATCC 25923* was determined by serial dilutions methods. To study an antibiofilm activity, the MTT test and atomic force microscopy were used. Immunotropic effects were studied by assessing peptone-induced migration of phagocytes into the abdominal cavity, proliferation of mitogen-activated lymphocytes in the MTT test and their cytokine secretion using the MILLIPLEX MAP kit on a Magpix multiplex analyzer.
Results. It has been established that a preliminary intraperitoneal administration of isoliquiritigenin (30 mg/kg) increases the survival rate of Balb/C mice in case of generalized staphylococcal infections. Isoliquiritigenin has antibacterial (MOC = 64 μg/ml) and antibiofilm (4–32 μg/ml) activities against *S. aureus J49 ATCC 25923,* does not inhibit the migration of phagocytes in the abdominal cavity, dose-dependently inhibits the proliferation and secretion of cytokines by mitogen-activated T-lymphocytes and modulates the production of cytokines (IL-2, IL-12p70, IFNg, TNFα, IL-6, IL-22, IL-23, IL-17A, IL-17F, IL-17E/IL-25, GM-CSF, MIP – 3a/CCL20, IL-10) by the cells of inguinal lymph nodes and splenocytes in the early stages of generalized staphylococcal infections.
Conclusion. A preliminary administration of isoliquiritigenin increases the survival rate of mice with generalized staphylococcal infections, which may be associated with both antimicrobial (antistaphylococcal, antibiofilm) and immunotropic mechanisms. The obtained data on the pharmacodynamics of isoliquiritigenin deserve attention from the point of view of the prospects of the new drugs creation that reduce mortality in staphylococcal sepsis.
Keywords: antimicrobial activity, biofilms, isoliquiritigenin, immunity, Balb/C mice, *S. aureus*
Abbreviations: MHB – Mueller-Hinton Broth; DMSO – dimethyl sulfoxide; ISL – isoliquiritigenin; SI – stimulation index; CFU – colony forming unit; ConA – concanavalin A; MIC – minimal inhibitory concentration; PBS – phosphate buffered saline; GM-CSF – colony stimulating factor 2 (granulocyte-macrophage); IFNg – interferon-gamma; IL – interleukin; MIP-3a/CCL20 – Macrophage Inflammatory Protein-3/Chemokine (C-C motif) ligand 20; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; OD – optical density; S. aureus – *Staphylococcus aureus;* SD – standard deviation; Th – T-helper cell; TNFα – tumor necrosis factor alpha.

АНТИБАКТЕРИАЛЬНЫЕ И ИММУНОТРОПНЫЕ СВОЙСТВА ИЗОЛИКВИРИТИГЕНИНА ПРИ ГЕНЕРАЛИЗОВАННОЙ СТАФИЛОКОККОВОЙ ИНФЕКЦИИ У МЫШЕЙ

Е.А. Солёнова, С.И. Павлова

Федеральное государственное бюджетное образовательное учреждение высшего образования
«Чувашский государственный университет им. И.Н. Ульянова»
428015, Российская Федерация, Чувашская Республика, г. Чебоксары, пр. Московский, д. 15

E-mail: elensoul@mail.ru

Получено 10.02.2020 Рецензия (1) 10.04.2020 Рецензия (2) 20.04.2020 Принята к печати 28.04.2020

For citation: E.A. Solyonova, S.I. Pavlova. Antibacterial and immunotropic properties of isoliquiritigenin in generalized staphylococcal infection in mice. *Pharmacy & Pharmacology*. 2020;8(3):181-194. **DOI:** 10.19163/2307-9266-2020-8-3-181-194

Для цитирования: Е.А. Солёнова, С.И. Павлова. Антибактериальные и иммунотропные свойства изоликвиритигенина при генерализованной стафилококковой инфекции у мышей. *Фармация и фармакология.* 2020;8(3):181-194. **DOI:** 10.19163/2307-9266-2020-8-3-181-194

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Статья посвящена изучению эффектов изоликвиритигенина при генерализованной бактериальной инфекции.
Цель: изучение антибактериальных и иммунотропных механизмов и эффектов изоликвиритигенина при генерализованной стафилококковой инфекции в мышиной модели.
Материалы и методы. Для оценки выживаемости мышей линии Balb/C использовали модель генерализованной инфекции, вызванной *Staphylococcus aureus J49 ATCC 25923* с построением кривых Каплан-Мейера. Степень бактериемии при развитии инфекции определяли методом секторных посевов. Минимальную подавляющую концентрацию изоликвиритигенина в отношении *Staphylococcus aureus J49 ATCC 25923* определяли методом серийных разведений. Для исследования антибиопленочной активности использовали МТТ-тест и атомно-силовую микроскопию. Иммунотропные эффекты изучали, оценивая пептон-индуцированную миграцию фагоцитов в брюшную полость, пролиферацию митоген-активированных лимфоцитов в МТТ-тесте и секрецию ими цитокинов с помощью набора MILLIPLEX MAP на мультиплексном анализаторе Magpix.
Результаты. Установлено, что предварительное внутрибрюшинное введение изоликвиритигенина (30 мг/кг) увеличивает выживаемость мышей Balb/C при генерализованной стафилококковой инфекции. Изоликвиритигенин обладает антибактериальной (МПК = 64 мкг/мл) и антибиопленочной (4–32 мкг/мл) активностью в отношении *S. aureus J49 ATCC 25923,* не ингибирует миграцию фагоцитов брюшную полость, дозозависимо подавляет пролиферацию и секрецию цитокинов митоген-активированными Т-лимфоцитами и модулирует выработку цитокинов (IL-2, IL-12p70, IFNg, TNFα, IL-6, IL-22, IL-23, IL-17A, IL-17F, IL-17E/IL- 25, GM-CSF, MIP-3a/CCL20, IL-10) клетками паховых лимфатических узлов и спленоцитов на ранних стадиях генерализованной стафилококковой инфекции.
Заключение. Предварительное введение изоликвиритигенина повышает выживаемость мышей при генерализованной стафилококковой инфекции, что может быть связано как с антимикробными (антистафилококковым, антибиопленочным действием), так и иммунотропными механизмами. Полученные данные о фармакодинамике изоликвиритигенина заслуживают внимания с точки зрения перспективы создания новых лекарственных препаратов, снижающих летальность при стафилококковом сепсисе.
Ключевые слова: антимикробная активность, биопленки, изоликвиритигенин, иммунитет, мыши, *S. aureus*
Сокращения: БМХ – бульон Мюллера-Хинтона; ДМСО – диметилсульфоксид; ИЛГ – изоликвиритигенин; ИС – индекс стимуляции; КОЕ – колониеобразующие единицы; КонА – конканавалин А; МПК – минимальная подавляющая концентрация; ФСБ – фосфатно-солевой буфер; GM-CSF – гранулоцитарно-макрофагальный колониестимулирующий фактор; IFNg – интерферон-гамма; IL – интерлейкин; MIP-3a/CCL20 – макрофагальный белок воспаления-3а/хемокиновый лиганд (CC) 20; МТТ – 3-(4,5-диметилтиазол-2-ил)-2,5-дифенил-тетразолиум бромид; OD – оптическая плотность; *S. aureus* – *Staphylococcus aureus*; SD – стандартное отклонение; Th – Т-хелперы; TNFα – фактор невроза опухоли альфа.

INTRODUCTION

Staphylococcus aureus (S. aureus) is a pathogen that causes severe generalized infections in humans. Among the infections caused by gram-positive bacteria, *S. aureus* infection is characterized by high mortality due to the development of sepsis and septic shock [1]. A septic process is known to be accompanied by a "cytokine storm" leading to a multiple organ failure. At the same time, an early prescription of antibacterial drugs is not always effective due to the development of the uncontrolled systemic inflammation, as well as the resistance of *S. aureus* to antibiotics [2]. Currently, low doses of corticosteroids which have undesirable immunosuppressive effects, are recommended to reduce mortality in septic shock in this situation [3]. Thus, many aspects of the treatment of sepsis remain controversial and require an in-depth fundamental study.

In case of massive generalization of infections, the reaction of the immune system is known to take on the features of systemic inflammation with a multiple organ failure, the main pathogenetic factor of which is the production of pro-inflammatory cytokines that trigger the generation of free radicals [4]. *S. aureus* can produce a toxic shock syndrome toxin [5], which acts as a superantigen able of inducing cytokine release at low concentrations, triggering the development of a "cytokine storm".

Recent studies have shown that licorice root flavonoids increase the secretion of IL-17 by activated T cells *in vitro* [6], and also lead to a switch of the immune response with differentiation of IL-17-producing cells in a contact sensitivity model [7]. Moreover, in the model of generalized staphylococcal infections, a preliminary administration of the sum of licorice flavonoids increased the survival rate of the laboratory animals [8].

Isoliquiritigenin (ISL) is one of the main flavonoids of licorice roots, which has various types of pharmacological activity: antitumor [9], antimicrobial [6], as well as anti-inflammatory and immunomodulatory [9–11]. These properties make it relevant for studying licorice roots as an agent in generalized infectious and inflammatory processes. In this study, an attempt was made to experimentally substantiate the use of chalcone isoliquiritigenin (ISL) in generalized infections (in the mice), caused by *S. aureus.*

THE AIM of this work was to study antibacterial and immune mechanisms of ISL in sepsis in mice, caused by an intraperitoneal administration of *S. aureus J49 ATCC25923* strain.

MATERIALS AND METHODS

Bacterial strain and conditions for its cultivation

S. aureus J49 ATCC25923 strain, obtained from the Federal State Budgetary Institution "Scientific Center for Expertise of Medicinal Products" of the Ministry of Health of Russia (Moscow, Russia), was grown in Mueller-Hinton broth (MHB, Medica plus LLS, Russia) at 37 °C in glass vials with aeration. For experimental purposes, a medium log phase bacterial culture was used, which had been cultured in 96-well flat-bottomed plates. (Corning Costar, USA). The calculation of colony forming units (CFUs) was carried out by measuring the optical

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-181-194

density (OD) of the bacterial suspension at 630 nm using a microplate photometer (ImmunoChem 2100, USA) based on the following ratio: 1 optical unit OD630 = 8.5×10^8 CFUs/ml.

Isolation of mice's mononuclear cells and their cultivation conditions

The isolation of mononuclear cells from inguinal lymph nodes or mice's spleen, was performed by gentle homogenization in RPMI-1640 (Thermo Fisher Scientific, USA) with osmotic lysis of erythrocytes in a 0.15 M ammonium chloride solution. The isolated lymphoid cells were cultured at 37 °C, 100% humidity, and 5% CO_2 in RPMI-1640 supplemented with 10% inactivated fetal calf serum (Thermo Fisher Scientific, USA), penicillin (100 U/ml), streptomycin (100 µg/ml) ("a complete medium") in 96-well round-bottom cell culture plates (Corning Costar, USA). Concanavalin A (ConA, PanEco LLC, Russia) at the final concentration of 15 µg/ml was used to activate T cells.

Test agent

ISL (98% purity, Xi'An Yiyang Bio-Tech Co., China) was dissolved in dimethyl sulfoxide (DMSO, Panreac, Spain). In the experiments *in vitro*, ISL was tested in the concentration range so that the final concentration of DMSO in the test samples did not exceed 1%. In the control samples, the corresponding volumes of DMSO were added instead of ISL. In the experiments on the animals, ISL was injected intraperitoneally three times with an interval of 4 h in a single dose of 10 mg/kg in 0.5 ml of phosphate-buffered saline at pH 7.4 (PBS, PanEco LLC, Russia).

Experimental animals

Balb/C mice (males, 20–22 g, 6–8 weeks old) were obtained from the Research and Production Enterprise "Nursery for Laboratory Animals" of the Institute of Biology, the Russian Academy of Sciences (Pushchino, Russia). The animals were cared for and handled in accordance with the ARRIVE principles [12]. The animals were kept with a free access to water and food. For the experiments, the mice were randomly assigned to groups of 8 animals. Withdrawal from the experiment was carried out by decapitation or cervical dislocation.

When performing the experiments, the provisions of the Helsinki Declaration (Brazil, 2013) were observed, the protocol of these experiments was approved by the ethical committee of Chuvash State University n. a. I.N. Ulyanov" (Protocol No 20-04 dated 17 April, 2020).

Determination of antimicrobial activity

The antimicrobial activity was determined by the dilutions method in Mueller-Hinton broth in 96-well flat-bottomed plates [13]. The serial two-fold dilutions of ISL (with the final concentration range of 0.1–128 µg/ml) were added to the bacterial suspension of *S. aureus* ($5x10^5$ CFUs/ml) and incubated at 37 °C for 24 hours. The minimum inhibitory concentration (MIC) of ISL was considered the lowest concentration with no visible bacterial growth after the incubation time.

Assessment of the dynamics of bacterial growth

To assess a bacterial growth, the method described by Wang [14], was used with minor modifications. ISL was added to the bacterial suspension (5×10^5 CFUs/ml) so that the final concentrations of ISL in the samples were 1/8 MPK, 1/4 MPK, 1/2 MPK, MPK. To assess bacterial growth in the samples, OD was measured after 4, 8, 12, 24 hours at 630 nm using a microplate photometer.

MTT test for bacterial biofilm formation

To study the formation of bacterial biofilms, the method described by Grela [15] was used. Bacteria (5×10^5 CFUs/ml) were inoculated into 96-well flat-bottomed cell culture plates and cultured for 24 hours. 2 hours before the end of the cultivation, then the bacterial suspension was removed, the wells were washed three times with PBS and a 1% solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-dislenyltetrazolium bromide (MTT, eBioscience, USA) was added into PBS and incubated for 2 hours at 37 °C. After that, to dissolve the formazan particles, the MTT solution was replaced with DMSO and incubated for 15 min at 20 °C, then the OD was measured at 492 nm using a microplate photometer.

Model of Systemic S. aureus Infection in Mice

S. aureus suspension was injected in PBS intraperitoneally: 5×10^8 CFUs/mouse, 1.5×10^9 CFUs/mouse. The infecting day was considered zero. The survival was assessed every 6 hr on the first day, then every day for 24 days. The experimental animals were injected with ISL before infecting (the total dose was 30 mg/kg, intraperitoneally, three times every 4 hours). The control animals were administrated with 5% DMSO.

The mice infected with a sublethal dose of *S. aureus* (5×10^8 CFUs/mouse) were withdrawn from the experiment every day for 7 days, to collect blood from large vessels (determination of bacteremia) and determine the excretions of the spleen and inguinal lymph nodes.

Determination of bacteremia

Bacteremia was determined by methods of sector inoculations методом секторных посевов on Petri dishes [16] with blood agar. The cups were incubated at 37 °C for 24 hours, then CFUs per 1 ml were calculated.

Peptone-induced phagocyte migration

The migration of phagocytes into the abdominal cavity was assessed according to the method proposed by Miyazaki [17], with minor changes. For this, group 1 of the negative control was injected three times with sterile PBS (0.5 ml, intraperitoneally); group 2 was injected with a sterile solution of peptone in PBS (3% –3 ml, intraperitoneally); group 3 was injected three times

with DMSO (5% – 0.5 ml, intraperitoneally), then with a sterile solution of peptone in PBS (3% – 3 ml, intraperitoneally). The mice of group 4 were injected with ISL three times, then with a sterile peptone solution in PBS (3% – 3 ml, intraperitoneally). After 24 hours and 72 hours, the animals withdrawn from the experiment, were injected intraperitoneally sequentially with 20 ml of PBS. After the palpation of the abdomen, the resulting washings were taken into plastic tubes and centrifuged. The number of cells was counted by a light microscopy using a Goryaev camera. The stimulation index (SI) was calculated using the following formula: SI = A/B, where A is the number of cells in the groups receiving peptone, B is the number of cells in the negative control group.

Determination of cytokines

On days 4 and 5 after infecting, the cells of the spleen and inguinal lymph nodes (5×10^6 cells/ml) of infected (5×10^8 CFUs/mouse) or intact mice were cultured for 24 or 48 hours at 37 °C in 100% humidity and 5% CO_2 in "a complete medium" with the addition of ConA. The supernatants had been collected and stored at – 70 °C till the analysis with a reagent kit for the determination of Mouse Th17 cytokines – MILLIPLEX MAP, Mouse Th17 MAGNETIC BEAD PANEL KIT 96-Well Plate Assay (USA); then they were analyzed using a multiplex analyzer (Magpix, USA) to determine the concentration of cytokines IL-2, IL-12p70, interferon gamma (IFNg), a necrosis factor of alpha tumor (TNFα), IL-6, IL-22, IL-23, IL-17A, IL-17F, IL- 17E/IL-25, a granulocyte macrophage colony stimulating factor (GM-CSF), macrophage inflammatory protein-3 (MIP-3a/CCL20), IL-10.

Statistical analysis

All the experiments have been performed in at least three series. The data obtained during them, were statistically processed using the GraphPadPrism 8.4.0 software. To assess the dynamics of the mice's deaths, Kaplan-Meier curves were constructed. The results obtained, complied with the law of normal distribution, were processed by the methods of variation statistics, and were presented as the arithmetic mean (M) ± standard error of the mean (SEM). The significance of differences between the groups in the experiments was determined by the Student's test. The differences were considered significant at $p<0.05$, where p is the level of significance.

RESULTS

Effect of ISL on the survival of Balb/C mice infected with *S. aureus J49 ATCC 25923*

Infecting with $5x10^8$ CFUs/mouse did not lead to deaths in the control group, although the animals showed such symptoms as a decreased activity and appetite, tousled coats, diarrhea. The survival rate of the healthy animals, which had been injected three times intraperitoneally with 5% DMSO, was 100%.

Intraperitoneal injection of 1.5×10^9 CFUs/mouse caused the deaths of 100% of the animals in the control group within 48–72 hours. A preliminary administration of ISL significantly reduced the mortality: 62.5±12.5% of mice died after 2 days, 12.5±6.3% of mice survived by day 24 (Fig. 1).

Autopsy made it possible to establish that 48–72 after infecting the mice treated with ISL, there was a less pronounced injection of mesenteric vessels and intestinal distention. In the ISL group, 14 days later, a pronounced adhesive process was observed, and 24 days later, retroperitoneal abscesses with dense capsules and adhesions were notified.

Antimicrobial activity of ISL

To determine the MIC against *S. aureus J49 ATCC 25923,* ISL was tested in the concentration range of 0.1 – 128 μg/ml. After 24 hours, the samples with ISL at the concentrations of 128 μg/ml and 64 μg/ml were completely transparent, like the negative control (sterile MHB). The bacterial suspension with ISL at the concentration of 32 μg/ml was opalescent. In the rest of the samples with ISL (0.1–16 μg/ml), an intensive bacterial growth was observed, as in the positive control (bacterial suspension without ISL). Thus, the MIC of ISL against *S. aureus J49 ATCC 25923* was 64 μg/ml.

As Fig. 2 shows, ISL dose-dependently suppressed the growth of the studied strain of *S. aureus* at the concentrations of MIC – MIC/8. In the first 4 hours of the observations, the optical density of the samples did not differ significantly, while after 8 h the differences in OD630 between the positive control and the samples with ISL increased. By the end of the incubation (24 hours), OD630 for ISL in MIC was 0.1±0.0 ($p<0.05$), MIC/2 was 0.3±0.1 ($p<0.05$), MIC/4 was 0.4±0.1 ($p<0.05$), MIC/8 was 0.5±0.3 ($p<0.05$).

Effect of ISL on biofilm formation of *S. aureus J49 ATCC 25923*

Using the MTT test, it was found out that ISL dose-dependently reduces the ability of *S. aureus J49 ATCC 25923* to adhere to plastic (Fig. 3). The removal of the bacterial suspension with repeated washings of the wells of the culture plate after 24 hours of incubation showed that the optical density of washings from the plastic surface in the control samples was significantly higher than in the wells with ISL: 0.8±0.1 *vs.* 0.4±0.0 (MIC, $p<0.05$), 0.8±0.1 *vs*. 0.5±0.1 (MIC/2, $p<0.05$), 0.8±0.1 *vs.* 0.5±0.2 (MIC/4, $p<0.05$), 0.8±0.1 *vs.* 0.5±0.1 (MIC/8, $p<0.05$). The OD492 value in the wells with ISL MIC/16 also tended to decrease: 0.7±0.1 ($p>0.05$).

Using the atomic force microscopy, it was found out that the number of bacteria in the field of view in the samples with MIC ISL (8.0±2.0 bacteria in the field of view, 300×, $p<0.05$), MIC/2 (30.0±7, 0 bacteria in the field of view, 300×, $p<0.05$) was fewer than in the control samples with massive bacterial conglomerates (83.0±13.0 bacteria in the field of view, 300×) (Fig. 3).

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-181-194

Effect of ISL on peptone-induced phagocyte migration in mice

The migration of phagocytes into the abdominal cavity was assessed by calculating the stimulation index (SI) – the number of cells stimulated by intraperitoneal injection of peptone relative to PBS. The SI in mice treated with ISL, and the control animals stimulated with peptone, did not differ from each other significantly. After 24 hours, the SI in the control group was 2.4±0.1 *vs*. 2.0±0.1 of the group administrated with ISL; after 72, the SI values were characterized by values 1.6±0.1 (control group) compared with 1.8±0.1 (the group administrated with ISL).

Dynamics of splenocytes and cells of inguinal lymph nodes in generalized staphylococcal infection in Balb/C mice

The number of the cells was counted every day after the intraperitoneal infection with a sublethal concentration of bacteria (5×10^8 CFUs/mouse) for 2 weeks. The dynamics of the number of the cells is shown in Fig. 5. On the 1st day after infecting, the number of the cells in the inguinal lymph nodes in the mice treated with ISL ($0.4\pm0.2\times10^6$ cells/mouse) and control mice ($0.9\pm0.4\times10^6$ cells/mouse), decreased compared with intact mice ($2.3\pm1.1\times10^6$ cells/mouse), and only after the 3rd day it gradually increased in both groups, reaching maximum values on the 7th day ($9.3\pm0.5\times10^6$ cells/mouse) or on the 10th day 10 ($10.6\pm0.5\times10^6$ cells/mouse, the ISL group). After reaching a peak on days 9–10 of the development of the infection, the number of lymph node cells gradually decreased, reaching their normal values (in intact animals) in both groups by the 16th day.

In the first days after infecting, the number of splenocytes in both groups was comparable and fewer than in the uninfected mice ($363.0\pm125.4\times10^6$ cells/mouse), up to the 6th day. From 3 to 9 days, the number of splenocytes gradually increased in both groups. The number of splenocytes in the mice treated with ISL reached a maximum by day 10 ($3328.0\pm166.4\times10^6$ cells/mouse). The control group had similar dynamics with a maximum on day 10, but with a lower peak value ($1488.0\pm74.4\times10^6$ cells/mouse).

Effect of ISL on proliferation of splenocytes and their cytokine secretion in vitro

According to the results of the MTT test with the use of the T-cell mitogen ConA, it was found out that ISL at the concentrations of 4–64 μg/ml dose-dependently suppresses the proliferation of activated lymphocytes. Thus, ISL at the concentrations of 16–64 μg/ml almost completely suppressed the cell proliferation. In the presence of 8 μg/ml of ISL, the cell viability decreased more than twice (50.0±7.5%, $p<0.05$) in comparison with the control and had a tendency to decrease upon the exposure to ISL at the concentration of 4 μg/ml (88.0±22.0%).

In the samples with ISL, even at the concentration of 4 μg/ml (Table 1), after 24-48 hours of incubation, the level of almost all the studied cytokines was lower than in the control samples.

Effect of ISL on bacteremia

To detect bacteremia, the blood of the infected mice (5×10^8 CFUs/mouse) was inoculated on the blood agar. A significant bacterial growth was observed one day later after infecting the control mice (10^5 CFUs/ml) compared with the mice treated with ISL (no growth was observed). On days 4-5 of the infection in the group administrated with ISL, there was no marked bacterial growth in the blood samples ($<10^3$ CFUs/ml). In the other samples of the control and experimental groups, no growth was observed, either.

Effect of ISL on cytokine production by cells of inguinal lymph nodes in generalized staphylococcal infections in Balb/C mice

The secretion of cytokines by the cells of the inguinal lymph nodes was determined on the 4th and 5th days after infecting (5×10^8 CFUs/mouse), by incubating the cells for 24–48 hours *in vitro*. As Fig. 5 and 6 show, the production of many cytokines detected on the 4th day of the infection was higher compared to the 5th day after infecting.

By 48 h of the incubation, the levels of IL-2, IFNg, IL-6, GM-CSF and, in particular, IL-17A, had been characterized by rather high values in the infected control mice. The intraperitoneal administration of ISL prior to the infection in the mice, significantly reduced the production of such cytokines as IL-2 (5524.3±669.8 pg/ml *vs*. 1265.0±94.8 pg/ml, $p<0.05$), IFNg (3936.3±567.8 pg/ml *vs*. 587.6±20.9 pg/ml, $p<0.05$), IL-6 (4861.3±361.8 pg/ml *vs*. 412.3±11,8 pg/ml, $p<0.05$), GM-CSF (553.3±64.6 pg/ml *vs.* 80.3±6.3 pg/ml, $p<0.05$), and IL-17A (6804.0± 754.9 pg/ml *vs.* 1129.0±31.1 pg/ml, $p<0.05$).

Effect of ISL on cytokines produced by splenocytes during staphylococcal infection in Balb/C mice

The secretion of cytokines by splenocytes was determined on days 4 and 5 after infecting (5×10^8 CFUs/mouse) by incubating the cells for 24–48 *in vitro*. The administration of ISL to mice led to a gradual increase in the secretion of cytokines from 4 to 5 days after infecting. As Fig. 7 and 8 show, on the 4th day after infecting, the GM-CSF values (586.7±95.5 pg/ml *vs*. 306.5±11.4 pg/ml, $p<0.05$) were significantly higher in the mice treated with ISL than in the control group.

On the 5th day after infecting, in the group administrated with ISL, the secretion level of a lot of the studied cytokines (at the time of the incubation for 24 hours and/or 48 hours) was significantly higher than in the control mice (Table 2):

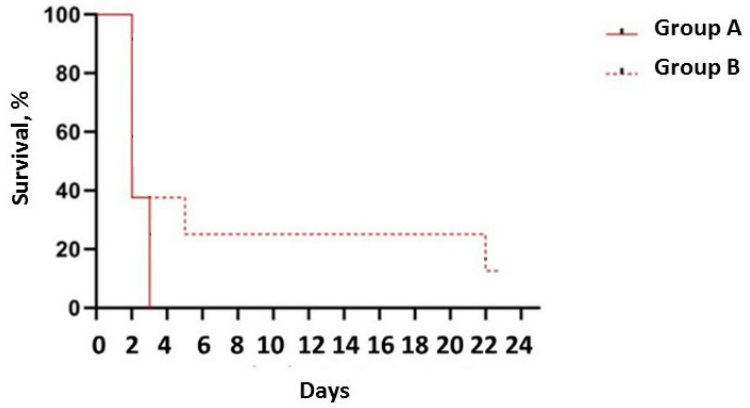

Figure 1 – Survival of Balb/C mice (males) infected with *S. aureus J49 ATCC 25923*

Note: Group A – control, 1.5–10^9 CFUs/mouse. Group B – a preliminary administration of ISL (30 mg/kg), 1.5×10^9 CFUs/mouse

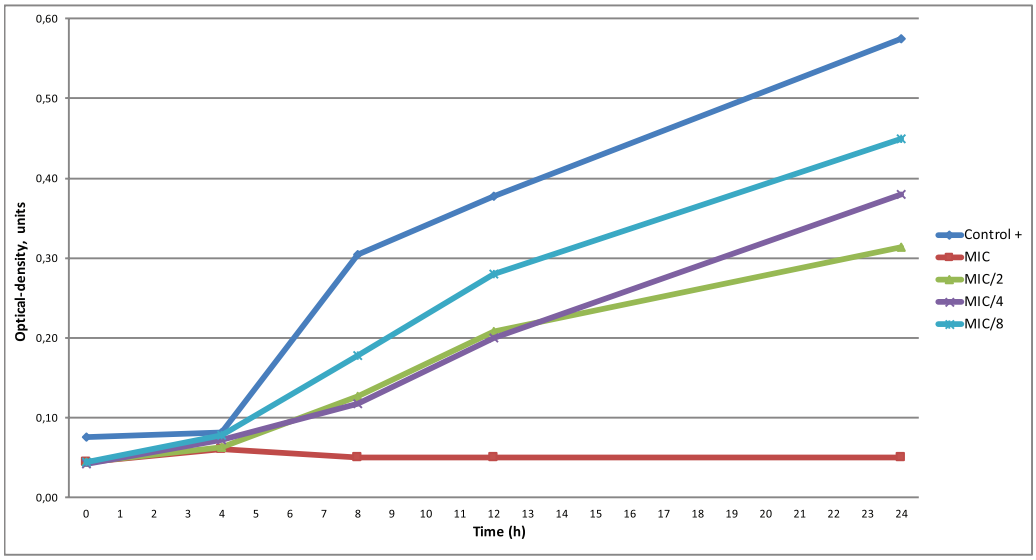

Figure 2 – Effect of ISL on the growth of *S. aureus J49 ATCC 25923*

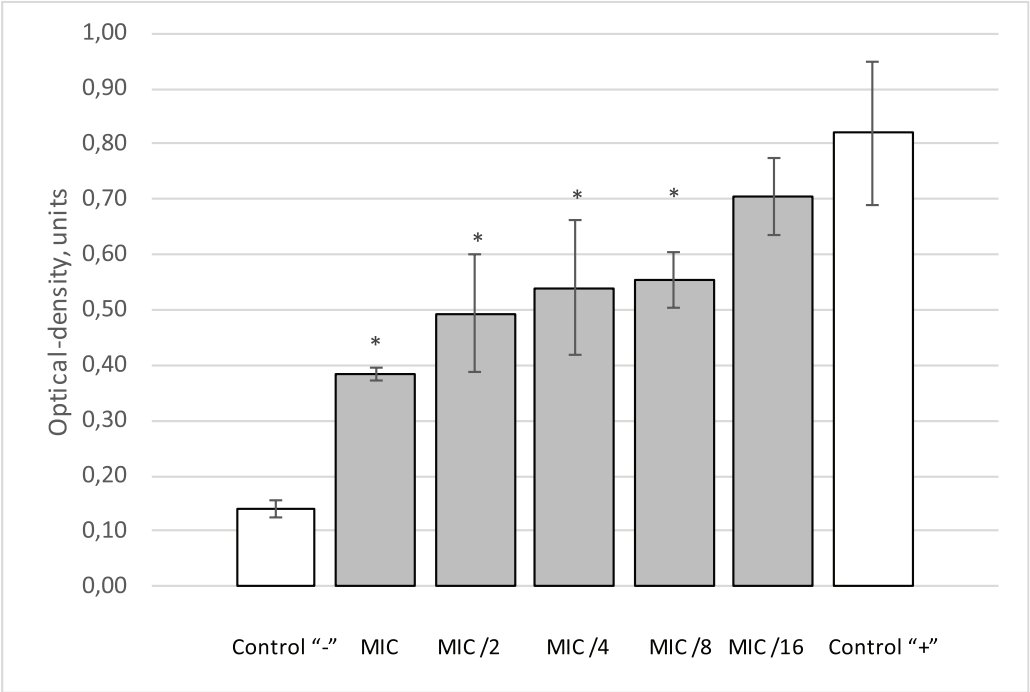

Figure 3 – Effect of ISL on biofilm formation of *S. aureus J49 ATCC 25923*

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-181-194

DISCUSSION

The investigation is devoted to the study of the effects of the chalcone flavonoid ISL in the model of generalized staphylococcal infections. The aim of the paper was to study antibacterial and immunotropic mechanisms of ISL.

Despite the fact that mice's models of *S. aureus* infection weakly correlate with staphylococcal infection in humans [18], they are widely used in experimental medicine and pharmacology. It is known that very high inoculums are required to reproduce *S. aureus* infection in animals.

Thus, in the present work, generalized infections were reproduced in Balb/C mice by intraperitoneal injection of *S. aureus J49 ATCC 25923* suspension in the quantity of 5×10^8–1.5×10^9 CFUs/mouse. The generalization of the infection (sepsis) was confirmed by the presence of bacteremia, abscesses in internal organs even in the case of a bacterial load of 5×10^8 CFUs/mouse, which practically did not cause death of mice.

Mass mortality of the experimental animals was observed only at infecting 1.5×10^9 CFUs/mouse or more. Moreover, most of the mice died in the early stages of the infection (on days 2–3), which could indicate the development of a septic shock [19].

It was found out that the administration of ISL 1 hour before the bacterial infection significantly increased the survival rate of mice. In this case, the protective effect of ISL in staphylococcal sepsis could be realized by restraining the symptoms of a toxic shock, which are largely the result of the overproduction of cytokines (IL-2, INF-γ and TNF-α) [20] by T-lymphocytes activated by a superantigen [21].

In the culture of ConA-activated splenocytes, ISL dose-dependently inhibited the production of cytokines even at the concentrations which did not cause a decrease in proliferation. Thus, in the presence of ISL (4 μg/ml), the secretion of the entire spectrum of cytokines under study (IL-2, IL-12p70, IFNg, TNFα, IL-6, IL-22, IL-23, IL-17A, IL-17F, IL-17E/IL-25, GM-CSF, MIP-3a/CCL20, IL-10) was below the control values. When ISL was added to the lymphoid cell culture at the concentrations of 16-64 μg/ml, most cytokines (IL-2, IL-12p70, IFNg, TNFα, IL-23, IL-17A, IL-17F, IL-17E/IL-25, GM-CSF, MIP-3a/CCL20) were not detected.

Table 1 – Effect of ISL on cytokine secretion by splenocytes *in vitro*

	A, pg/ml	B, pg/ml
IL-2	<4.0	2020.0±115.7
IL-12p70	<7.9	23.8±2.6
IFNg	<4.5	6253.0±157
TNFa *	25.0±0.2	213.2± 16.1
IL-6 *	27.6±1.4	2249.0±132.0
IL-22*	13.2±0.4	1276.0±71.2
IL-23	<123.7	223.3±1.4
IL-17A	<20.4	724.5±76.9
IL-17F	<6.2.	968.4±107.4
IL-17E / IL-25	<377.5	527.6±25.4
GM-CSF	<22.5	310.8±32.6
MIP-3A / CCl20	<35.6	355.8±3.1
IL-10 *	32.8±0.1	424.3± 24.3

Note: A – the level of cytokines in the samples incubated with ISL (4 μg/ml); B – the level of cytokines in control samples. * – p <0.05

Table 2 – Effect of ISL on cytokines produced by splenocytes during staphylococcal infection in Balb/C mice, on the 5th day of incubation

	A, pg/ml	B, pg/ml
IL-2*	3818.0±265.9	2158.5±140.7
IL-12p70*	100.6±2.6	31.8±4.6
IFNg*	7191.0±0.0	2356.0±179.6
IL-22*	1028.5±33.2	604.4±45.4
IL-23*	374.0±17.8	186.3±0.0
IL-17A*	3094.5±95.5	756.9±20.3
L-17F*	1223.5±79.9	865.45±39.4
MIP-3a/CCL20*	428.8±16.7	302.0±0.8
IL-10*	664.0±23.7	56.0±2.7

Note: A – the group administrated with ISL (preliminary intraperitoneal injection, 10 mg/kg, three times); B – control group. * – p<0.05

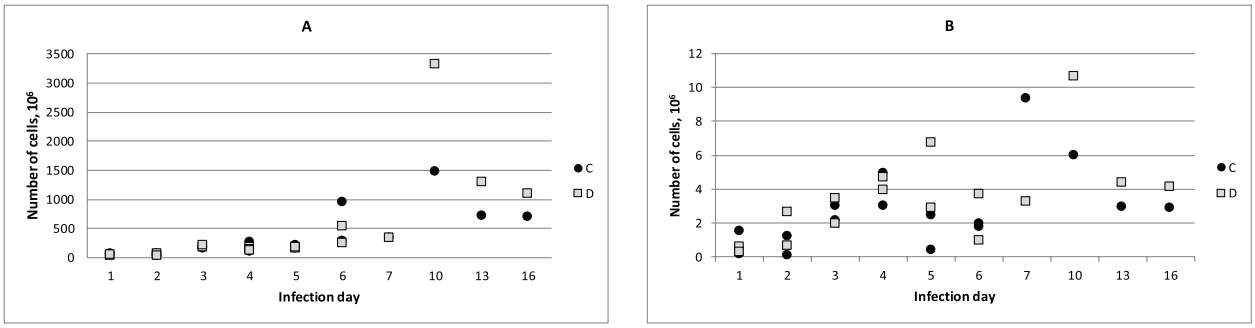

Figure 4 – Dynamics of splenocytes (A) and inguinal lymph node cells (B) in the model of Balb/C mice staphylococcal infection

Note: (C) Control group, 5×10^8 CFUs/mouse. (D) Pre-treatment with ISL (30 mg/kg), 5×10^8 CFUs/mouse. Intact mice: the number of splenocytes = $363.0\pm125.4\times10^6$ cells/mouse, the number of inguinal lymph node cells = $2.3\pm1.1\times10^6$ cells/mouse

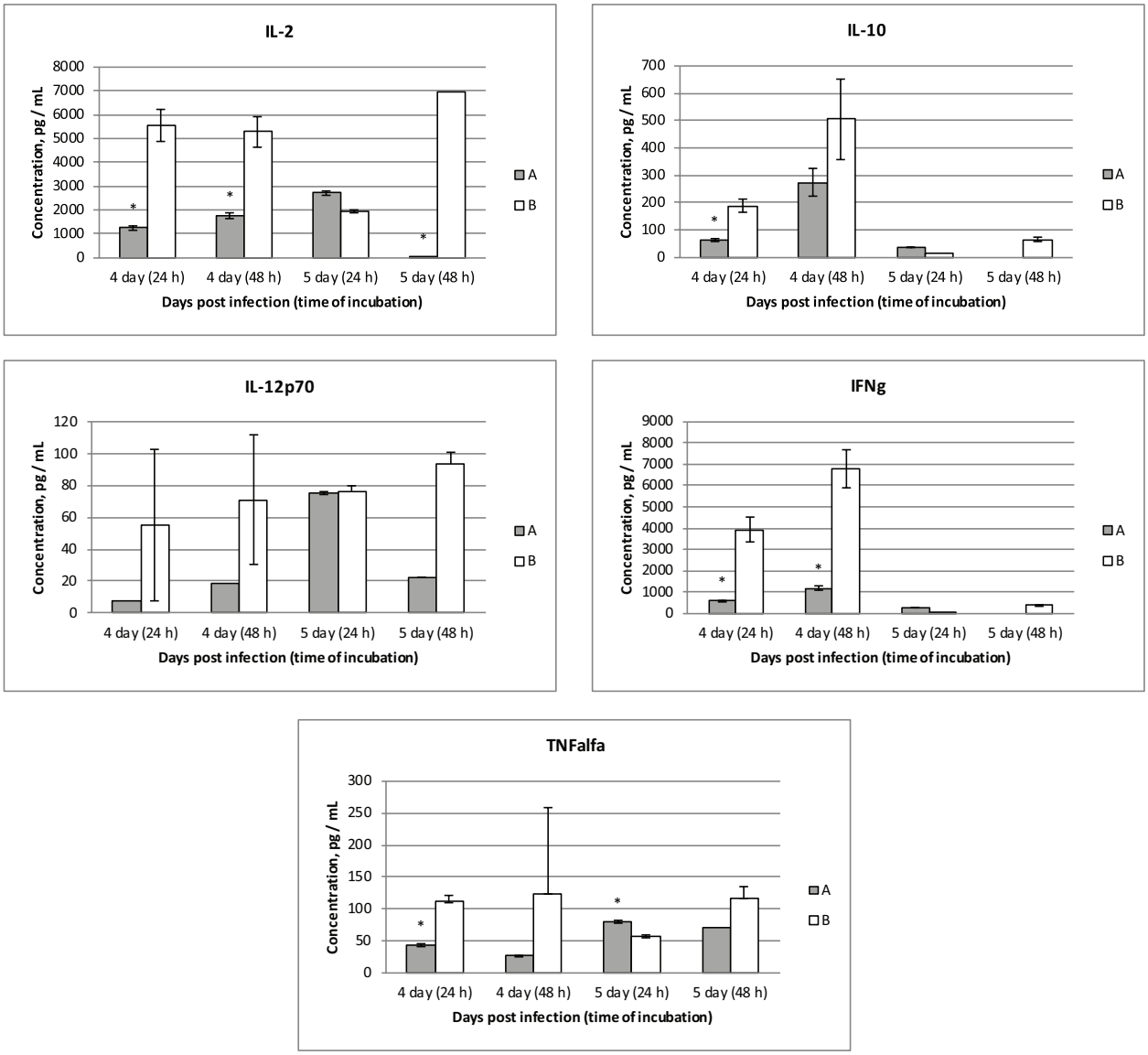

Figure 5 – Effect of preliminary ISL administration on the levels of cytokines (groups Th-1 and IL-10) produced by the cells of the inguinal lymph nodes of Balb/C mice infected with *S. aureus J49 ATCC 25923* (5×10^8 CFUs/mouse) (* $p<0.05$)

Note: (A) Preliminary ISL administration (30 mg/kg). (B) control group (* $p<0.05$)

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-181-194

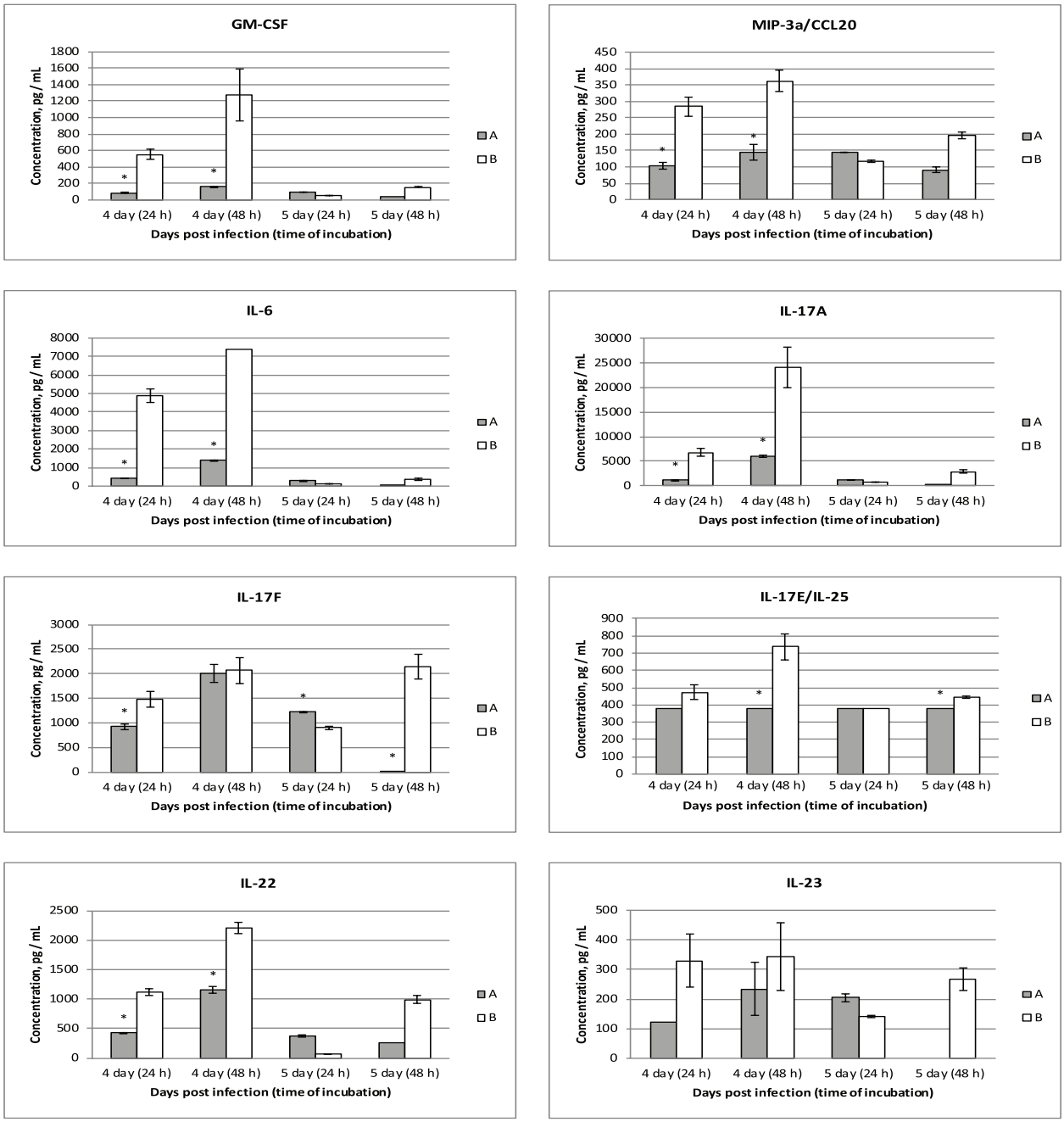

Figure 6 – Effect of preliminary ISL administration on the levels of cytokines (Th-17 group) produced by the cells of the inguinal lymph nodes of Balb/C mice infected with *S. aureus J49 ATCC 25923* (5×10^8 CFUs/mouse) (* p<0.05)

Note: (A) The group administrated with ISL (30 mg/kg). (B) Control group (* p<0.05)

Other researchers have also shown the effectiveness of ISL in the sepsis caused by other mechanisms. Thus, in the model of the sepsis caused by ligation and puncture of the cecum, ISL reduced the concentration of proinflammatory cytokines in the blood serum, the activity of NO-synthase, cyclooxygenase-2 [22], and also had antioxidant and anti-inflammatory effects [23].

In the early stages of the infection, the inflammatory response is mediated by the involvement of factors of innate, but not adaptive immunity. It is innate immune responses that are most significant for preventing generalization and limiting the purulent-inflammatory processes. According to the authors' opinions, it is positive that there is no suppressive effect of ISL on the migration of phagocytes to the focus of the pathogen introduction. In the present study, ISL did not reduce the number of cells after 24 h (chemotaxis of neutrophils) and 72 h (chemotaxis of macrophages) in response to the introduction of the inducer of migration, peptone. At

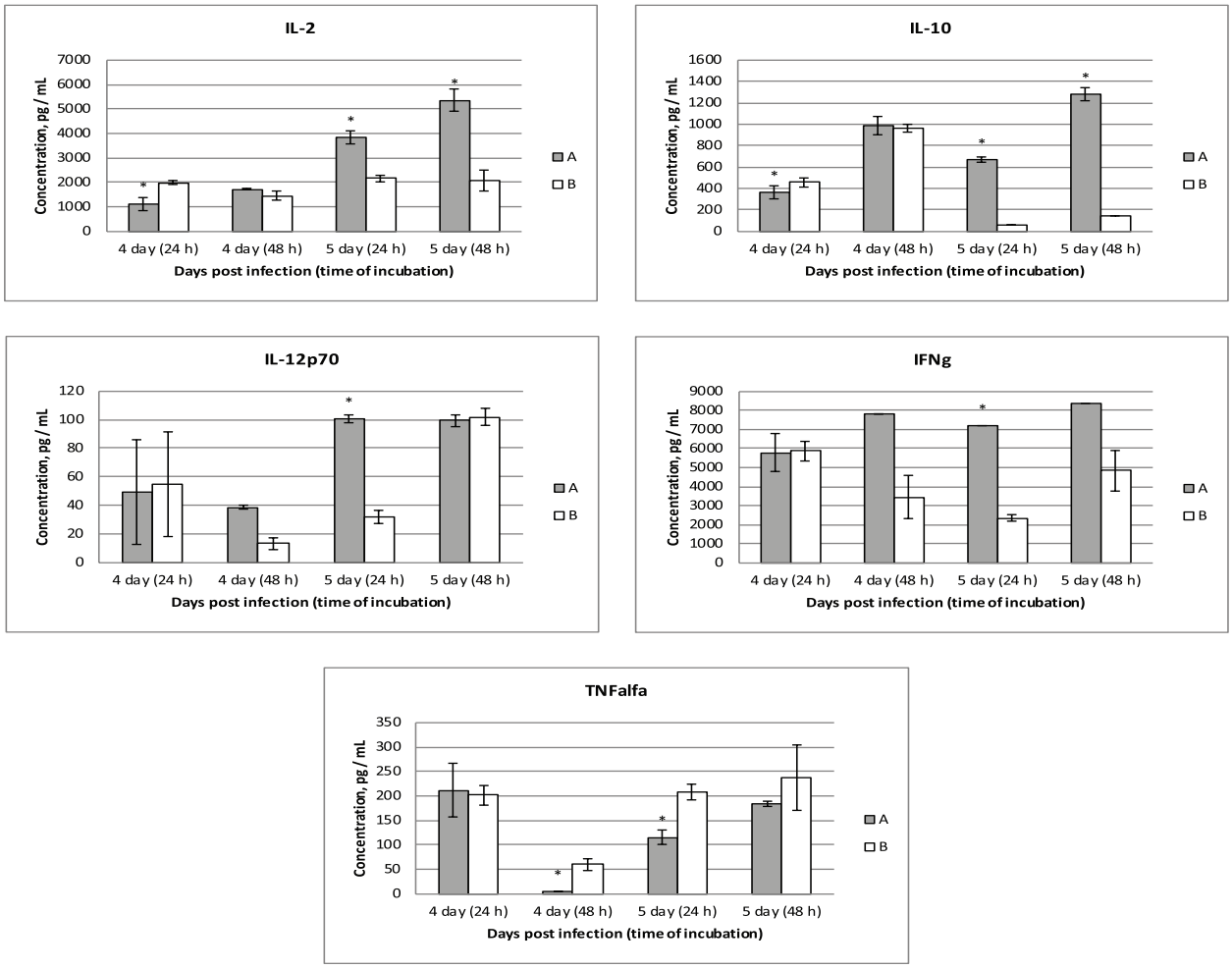

Figure 7 – Effect of preliminary ISL administration on cytokine levels (groups Th-1 and IL-10) produced by splenocytes of Balb/C mice infected with *S. aureus J49 ATCC 25923* (5×10^8 CFUs/mouse, intraperitoneal) (* p<0.05)

Note: (A) – the group administrated with ISL (30 mg/kg). (B) Control group (* p<0.05)

the same time, a visual assessment during autopsy of mice receiving ISL showed that, in comparison with the control group, a more pronounced adhesive process was observed with the formation of abscesses with dense capsules.

Based on the findings, as well as taking into account a rather short half-life of ISL [24], the authors can consider such substances being potentially of interest as an alternative to corticosteroids, preventing lethality in systemic inflammation.

In addition, ISL, in comparison with corticosteroids, has a direct antistaphylococcal effect. Under the conditions of the carried out experiment, the MIC of ISL against *S. aureus J49 ATCC 25923* was 64 μg/ml, which was comparable with the data of some authors who had studied the effect of ISL on other bacteria of the Staphylococcus genus [6]. Despite the fact that the antistaphylococcal activity was not high, ISL dose-dependently inhibited the suspension growth of *S. aureus J49 ATCC 25923* at the concentrations less than the MIC (8–32 μg/ml). It suggests that the antibacterial activity of ISL also played a role in increasing the survival of animals.

Staphylococcus bacteria are able to form biofilms on various surfaces. This ability of *S. aureus* was found out in both collection strains [25] and clinical isolates of *MSSA* and *MRSA* [26]. Using an atomic force microscopy and the MTT test, *S. aureus J49 ATCC 25923* strain was found out to be able to form biofilms on the plastic surface, and the addition of ISL at the concentrations lower than the MIC, inhibits the formation of bacterial biofilms. The antibiofilm effect of ISL was dose-dependent (4–32 μg/ml) and correlated with the severity of inhibition of the suspension growth of bacteria, which may indirectly indicate that this effect was a consequence of a direct antibacterial effect of ISL. The antibiofilm activity of ISL has been demonstrated by other researchers against *S. xylosus* [7]; however, the mechanisms of the ISL effect on the biofilms of the Staphylococcus genus bacteria have not been thoroughly studied, although there is information about other flavonoids that affect the *quorum sensing system* of *S. aureus.*

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-181-194

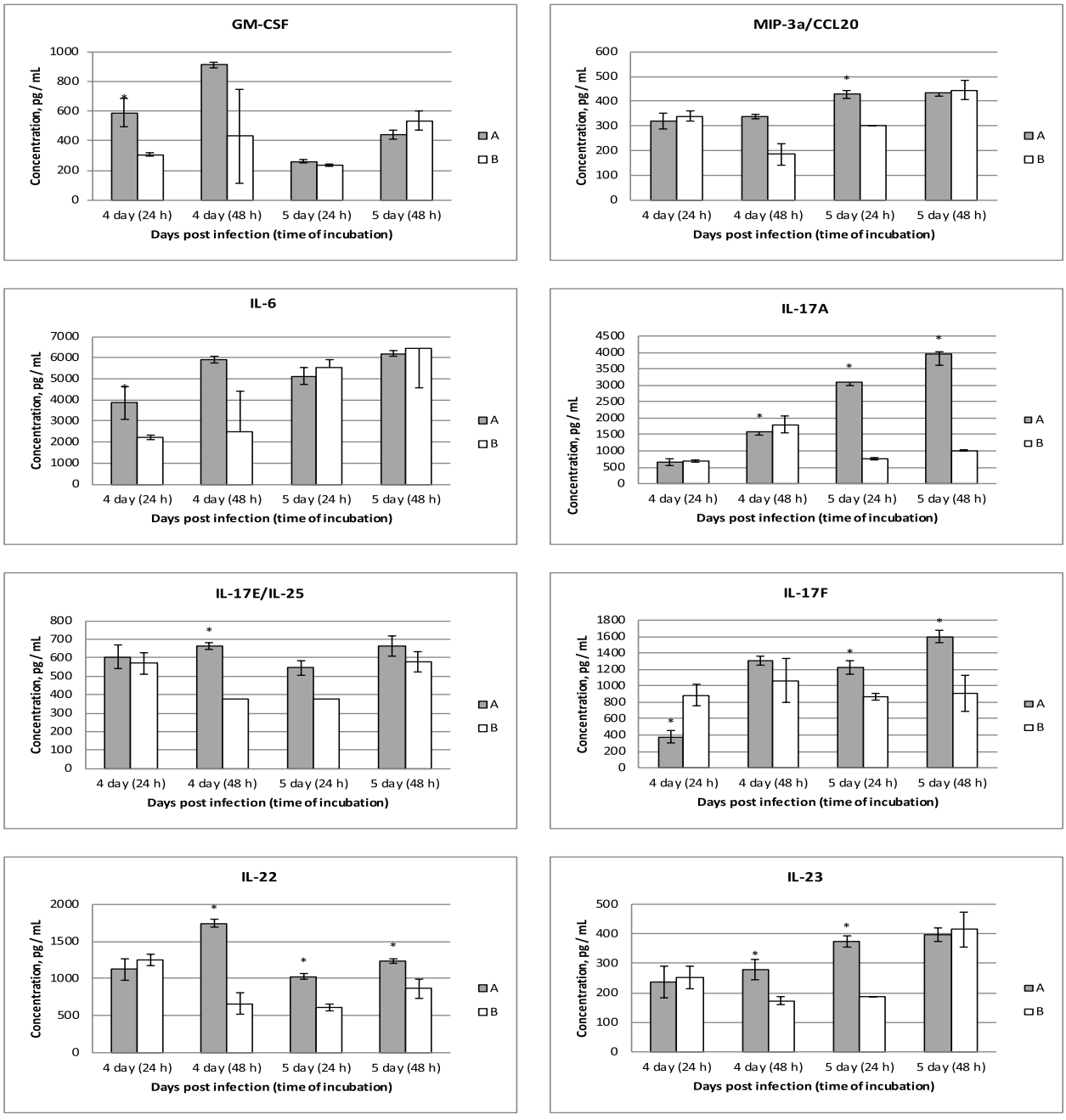

Figure 8 – Effect of preliminary ISL administration on cytokine levels (group Th-17), produced by splenocytes of Balb/C mice infected with *S. aureus J49 ATCC 25923* (5×10^8 CFUs/mouse, intraperitoneally) (*p<0.05)

Note: (A) – preliminary ISL administration (30 mg/kg). (B) Control group (* p<0.05)

In recent years, some publications have appeared about *S. aureus* actively avoiding immune surveillance, "turning off" various mechanisms of the adaptive immune response in the host organism [27]; it allows bacteria to persist even in the process of the development of an antigen-specific response.

To study the effect of ISL on the immune response, a mouse model of generalized infections was used, and no death of the animals was observed in the inoculation (5x10^8 CFUs/mouse). The dynamics of the number of cells of the inguinal lymph nodes and spleen, as well as their production of cytokines after infecting, was assessed.

Attention was drawn to the fact that in the first days after infecting the animals, the number of cells in the regional lymph nodes and spleen was significantly fewer than in the intact animals. Apparently, a massive bacterial invasion led to the development of a nonspecific response — a stress response, as a result of which the increased secretion of corticosteroids in the first days of the septic process, could be the cause of the lymphocytic effect [28]. Only after 3 days of infecting, there

was a gradual increase in the number of lymphocytes in the regional lymph nodes, reaching maximum values on day 7 (in the control group) and on day 10 (in the group administrated with ISL). To study the secretion of cytokines, regional lymph nodes and spleen from mice were removed on the 4th and 5th days after infecting (the period of increasing "cellularity").

When lymphocytes are activated, first IL-2 is produced, and then other cytokines necessary for the differentiation of various Th-subpopulations. The functional state of Th-subpopulations is usually judged by the production of a characteristic spectrum of cytokines by immunocompetent cells: IFNg is a Th1 marker, IL-4 is a Th2 marker, and IL-17A is the main Th17 cytokine.

From the point of view of modern concepts, Th17 cells are involved in antistaphylococcal immunity, enhancing the effector function of neutrophils [29] and, thus, acting as the most important protective population. However, taking into consideration the plasticity of Th-subpopulations in a dynamically changing microenvironment *in vivo*, cytokines of Th17-dependent effectors such as GM-CSF, MIP-3a/CCL20, are also evaluated.

The study of supernatants of the cells of the inguinal lymph nodes of the infected control mice, revealed the predominance of such cytokines as IL-12p70, IFNg, IL-6, IL-22, IL-23, IL-17A. IL-17F, IL-17E/IL-25. This could indicate the differentiation of activated $CD4^+$-cells into Th1 and Th17. The differentiation hypothesis is also supported by an increase in serum concentrations of GM-CSF, as well as the antibacterial chemokine MIP-3a/CCL20. The soluble components of *S. aureus* contribute to the induction of Foxp3+Treg, and in addition to an increase in the secretion of proinflammatory cytokines in the control group, an increase in the secretion of IL-10 was noted. It was probably produced by T-regulatory cells that suppress excessive inflammation by suppressing Th1 and Th17. In the group of mice treated with ISL, the suppression of the secretion of both pro-inflammatory (IL-2, IFNg, IL-6, IL-17A, GM-CSF) cytokines and IL-10 by cells of the inguinal lymph nodes was revealed, the concentration of which was less than the control values by more than twice.

In the present experiment, ISL, like many other flavonoids [30], exhibited immunosuppressive properties. In the culture of mononuclear cells activated by the T-cell mitogen KonA, ISL dose-dependently inhibited proliferation: in the concentration of 8 μg/ml, the proliferation was suppressed by about twice, while in the concentrations above 16 μg/ml, the proliferative response was almost completely absent, and it reduced the cytokine production not only *in vitro,* but also by inguinal lymph node cells in the infected mice. Thus, despite the presence of antibacterial mechanisms, ISL could potentially provoke the generalization of the infection. However, when blood was inoculated on the first day of infecting, bacteremia in the group of the control mice was significantly higher and reached 10^5 CFUs/ml, while against the background of the introduction of ISL on a dense medium, single colonies grew, indicating bacteremia $<10^3$ CFUs/ml, on the following days, for a week, as shown by the blood culture, bacteremia was not detected in all animals in both groups at low levels ($<10^3$ CFUs/ml).

Considering that the spleen plays an important role in curbing the hematogenous spread of infection [31], the production of cytokines by splenocytes was investigated 4-5 days after infecting, where unexpected results were obtained. In the spleens of the both groups, the maximum "cellularity" was observed on the 10th day of infecting, but in the group administrated with ISL, their number was twice higher than the control values. Splenocytes of mice, which were injected with ISL before infecting, significantly increased the production of cytokines that activate the immune response in the Th-1 type (IL12p70, TNFα, IFNg) and Th-17 (IL22, IL23, IL6, IL17). It has been suggested that the expansion of a large number of T-cells after the stimulation with a superantigen, can deplete IL-2, thereby limiting the development of a protective T-cell response [32]. It is possible that the inhibition of the cytokines secretion (in particular, IL-2) and the lower bacterial load against the background of the ISL administration promoted a more effective participation of splenocytes in the immune response.

Against the background of the ISL administration, attention is drawn to a more effective Th-17 response with an increase in cytokines of innate immunity effectors (GM-CSF, MIP-3a/CCL20). It has been found that chemokines such as MIP-3a/CCL20, have a pronounced antibacterial activity against both gram-positive and gram-negative bacteria [33]. It is assumed that pro-inflammatory Th17 cells of the first wave actively secrete IL-17 in the target tissues; it induces the secretion of the antibacterial peptide MIP-3a/CCL20 by a variety of cells. It is likely that the insufficient activity of MIP-3a/CCL20 can lead to a decrease in the T-cell-mediated control of bacterial pathogen eradication.

CONCLUSION

Thus, in the course of the study, it was revealed that the preliminary administration of ISL increases the survival rate of the mice in the generalized infection caused by *S. aureus J49 ATCC 25923.* This protective effect of ISL is based on both antimicrobial (moderate direct antistaphylococcal with MIC = 64 μg/ml, antibiofilm in the concentrations below MIC), and immunomodulatory defense mechanisms. All these factors deserve attention in order to create new drugs that reduce mortality in sepsis.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-181-194

ACKNOWLEDGEMENT

The authors express their gratitude to Professor Sidorenko (FSBI "Children's Scientific and Clinical Center for Infectious Diseases" FMBA of Russia) and A.N. Shkoporov (MD, PhD, University College Cork, Ireland) for consultations and valuable advice on writing the manuscript.

FINANCIAL SUPPORT

The study has been carried out with the financial support of the Russian Foundation for Basic Research within the framework of Scientific project No 19-315-90085.

AUTHORS' CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Ani C, Farshidpanah S, Stewart AB, Nguyen HB. Variations in organism-specific severe sepsis mortality in the United States: 1999–2008. Critical Care Medicine. 2015;3(1):65–77. DOI: 10.1097/CCM.0000000000000555.
2. Narita K, Hu DL, Mori F, Wakabayashi K, Iwakura Y, Nakane A. Role of Interleukin-17A in Cell-Mediated Protection against Staphylococcus aureus Infection in Mice Immunized with the Fibrinogen-Binding Domain of Clumping Factor A. Infection and Immunity. 2010;78(10):4234–4242. DOI: 10.1128/IAI.00447-10.
3. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R. Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Medicine. 2017; 43(3):304–377. DOI: 10.1007/s00134-017-4683-6.
4. Kumar S, Gupta E, Kaushik S, Kumar Srivastava V, Mehta SK, Jyoti A. Evaluation of Oxidative Stress and Antioxidant Status: Correlation With the Severity of Sepsis. Scandinavian Journal of Immunology. 2018;87(4):e12653. DOI: 10.1111/sji.12653.
5. Fraser JD. Clarifying the Mechanism of Superantigen Toxicity. PLoS Biology. 2011;9(9):e1001145. DOI: 10.1371/journal.pbio.1001145.
6. Qu Q, Wang J, Cui W. In vitro activity and in vivo efficacy of Isoliquiritigenin against Staphylococcus xylosus ATCC 700404 by IGPD target. PLoS One. 2019;14(12):e0226260. DOI: 10.1371/journal.pone.0226260.
7. Park SJ, Song HY, Youn HS. Suppression of the TRIF-dependent signaling pathway of toll-like receptors by isoliquiritigenin in RAW264.7 macrophages. Molecules and Cells. 2009;28(4):365–368. DOI: 10.1007/s10059-009-0130-z.
8. Chen X, Cai X, Le R, Zhang M, Gu X, Shen F, Hong G, Chen Z. Isoliquiritigenin Protects Against Sepsis-Induced Lung and Liver Injury by Reducing Inflammatory Responses // Biochemical and Biophysical Research Communications. 2018;496(2):245–252. DOI: 10.1016/j.bbrc.2017.11.159.
9. Pavlova SI Immunosupressivnye i protivoopuholevye farmakodinamicheskie jeffekty flavonoidov kornej solodki [dissertacija na soiskanie uchenoj stepeni doktora medicinskih nauk]. Rossijskij nacional'nyj issledovatel'skij medicinskij universitet imeni N.I. Pirogova. Moskva. 2012.
10. Pavlova SI, Albegova DZ, Dmitrieva NV, Dibirova GO, Kozlov IG. Licorice root flavonoids effect the functions of mouse and human activated T-lymphocytes. Russian Journal of immunology. 2011;5(14):62–68.
11. Pavlova SI, Albegova DZ, Kjagova AA, Kozlov IG. Mechanisms of immunosuppressive action of licorice root flavonoids in contact sensitivity in mice: inhibition of T lymphocyte effector function mediated by non-effector cells. Medical Immunology. 2010;12(6):503–510. DOI: 10.15789/1563-0625-2010-6-503-510.
12. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biology. 2010;8(6):e1000412. DOI: 10.1371/journal.pbio.1000412.
13. CLSI. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts, Approved Guideline. 2004. URL: https://www.semanticscholar.org/paper/Method-for-antifungal-disk-diffusionsusceptibility-Rex-Clinical/65de3bf5c2b026c9e3b0995780e7fa790e7c0295 (дата обращения 06.08.2020).
14. Wang C, Chang T, Yang H, Cui M. Antibacterial mechanism of lactic acid on physiological and morphological properties of Salmonella Enteritidis, Escherichia coli and Listeria monocytogenes. Food Control. 2015;47:231–236. DOI: 10.1016/j.foodcont.2014.06.034.
15. Grela E, Kozłowska J, Grabowiecka A. Current methodology of MTT assay in bacteria – A review. Acta Histochemica. 120;4:303–311. DOI: 10.1016/j.acthis.2018.03.007.
16. Sharma-Chawla N, Stegemann-Koniszewski S, Christen H. In vivo Neutralization of Proinflammatory Cytokines During Secondary Streptococcus pneumoniae Infection Post Influenza A Virus Infection. Frontiers in immunology. 2019;10:1864. DOI: 10.3389/fimmu.2019.01864.
17. Miyazaki S, Ishikawa F, Fujikawa T, Nagata S, Yamaguchi K. Intraperitoneal Injection of Lipopolysaccharide Induces Dynamic Migration of Gr-1^{high} Polymorphonuclear Neutrophils in the Murine Abdominal Cavity. Clinical Diagn Lab Immunol. 2004;11(3):452–457. DOI: 10.1128/CDLI.11.3.452-457.2004.
18. Thomsen IP, Liu JY. Targeting Fundamental Pathways to Disrupt Staphylococcus Aureus Survival: Clinical Implications of Recent Discoveries. JCI Insight. 2018;3(5):e98216. DOI:10.1172/jci.insight.98216.

19. Magrone T, Jirillo E. Sepsis: From Historical Aspects to Novel Vistas. Pathogenic and Therapeutic Considerations. Endocrine, Metabolic & Immune Disorders – Drug Targets. 2019;19(4):90–502. DOI: 10.2174/1871530319666181129112708.

20. Chen P, Stanojcic M, Jeschke MG. Differences Between Murine and Human Sepsis. Surgical Clinics of North America. 2014;94(6):1135-1149. DOI: 10.1016/j.suc.2014.08.00.

21. Chesney PJ, Bergdoll MS, Davis JP, Vergeront JM. The disease spectrum, epidemiology, and etiology of toxic-shock syndrome. Annual Review of Microbiology. 1984;38:315–338. DOI:10.1146/annurev.mi.38.100184.001531.

22. Zou P, Ji HM, Zhao JW, Ding X, Zhen Z, Zhang X, Nie X.-Q., Xue L.-X. Protective effect of isoliquiritigenin against cerebral injury in septic mice via attenuation of NF-κB. Inflammopharmacology. 2019;27(4):809–816. DOI: 10.1007/s10787-018-0503-z.

23. Kumar S, Sharma A, Madan B, Singhal V, Ghosh B. Isoliquiritigenin inhibits IkappaB kinase activity and ROS generation to block TNF-alpha induced expression of cell adhesion molecules on human endothelial cells. Biochemical Pharmacology. 2007;73(10):1602–1612. DOI: 10.1016/j.bcp.2007.01.015.

24. Qiao H, Zhang X, Wang T, Liang L, Chang W, Xia H. Pharmacokinetics, Biodistribution and Bioavailability of Isoliquiritigenin After Intravenous and Oral Administration. Pharmaceutical Biology. 2014;52(2):228–236. DOI: 10.3109/13880209.2013.832334.

25. Hiltunen AK, Savijoki K, Nyman TA, Miettinen I, Ihalainen P, Peltonen J, Fallarero A. Structural and Functional Dynamics of Staphylococcus aureus Biofilms and Biofilm Matrix Proteins on Different Clinical Materials. Microorganisms. 2019;7(12):584. DOI: 10.3390/microorganisms7120584.

26. McCarthy H, Rudkin JK, Black NS, Gallagher L, O'Neill E, O'Gara JP. Methicillin resistance and the biofilm phenotype in Staphylococcus aureus. Fronties in Cellular and Infection Microbiology. 2015;5(1). DOI: 10.3389/fcimb.2015.00001.

27. Goldmann O, Medina E. Staphylococcus Aureus Strategies to Evade the Host Acquired Immune Response. International Journal of Medical Microbiology. 2017;308(6):625–630. DOI: 10.1016/j.ijmm.2017.09.013.

28. Cortes-Puch I, Hicks CW, Sun J, Solomon SB, Eichacker PQ, Sweeney DA, Nieman LK, Whitley EM, Behrend EN, Natanson C, Danner RL. Hypothalamic-pituitary-adrenal axis in lethal canine Staphylococcus aureus pneumonia. Am J Physiol Endocrinol Metab. 2014;307(11):E994–E1008. DOI: 10.1152/ajpendo.00345.2014.

29. Kojima H, Takeda Y, Muromoto R, Takahashi M, Hirao T, Takeuchi S. Jetten A.M., Matsuda T. Isoflavones Enhance interleukin-17 Gene Expression via Retinoic Acid Receptor-Related Orphan Receptors α and γ. Toxicology. 2015;329:32–39. DOI: 10.1016/j.tox.2015.01.007.

30. Pavlova SI, Albegova DZ, Vorob'eva JuS, Laptev OS, Kozlov IG. Flavonoids as Potential Immunosuppressive Agents Affecting Intracellular Signaling Pathways (A Review). Pharmaceutical Chemistry Journal. 2015;49(10):3–10. DOI: 10.30906/0023-1134-2015-49-10-3-10.

31. Wang L, Yang R, Yuan B, Liu Y, Liu C. Antiviral and Antimicrobial Activities of Licorice, a Widely-Used Chinese Herb. Acta Pharmaceutica Sinica B. 2015;5(4):310–315. DOI: 10.1016/j.apsb.2015.05.005.

32. Llewelyn M, Cohen J. Superantigens: microbial agents that corrupt immunity. The Lancet Infectious Diseases. 2002;2(3):156–162. DOI: 10.1016/s1473-3099(02)00222-0.

33. Sadowska B, Więckowska-Szakiel M, Paszkiewicz M, Różalska B. The Immunomodulatory Activity of Staphylococcus Aureus Products Derived From Biofilm and Planktonic Cultures. Archivum Immunologiae et Therapiae Experimentalis (Warsz). 2013;61(5):413–420. DOI: 10.1007/s00005-013-0240-3.

AUTHORS

Elena A. Solyonova – Junior Researcher, Department of Pharmacology, Clinical Pharmacology and Biochemistry of Chuvash State University n.a. I.N. Ulyanov ORCID 0000-0001-6104-0864. E-mail: elensoul@mail.ru

Svetlana I. Pavlova – Doctor of Sciences (Medicine), the Head of the Department of Pharmacology, Clinical Pharmacology and Biochemistry of Chuvash State University n.a. I.N. Ulyanov. ORCID:0000-0001-9976-7866. E-mail: flavonoid@yandex.ru

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2020-8-3-195-204

CONCEPTUALLY-THEORETICAL JUSTIFICATION AND UPDATING OF THE PREVENTIVE APPROACH IN THE IMPLEMENTATION OF A PHARMACIST'S INFORMATION CONSULTANCY SERVICES IN THE PUBLIC HEALTH SYSTEM

I.A. Kirshchina, A.V. Soloninina, V.N. Michailova

Perm State Pharmaceutical Academy
2, Polevaya St., Perm, Russia 614990

E-mail: irina.kirshina@mail.ru

Received 22 October 2020 Review (1) 05 May 2020 Review (2) 30 August 2020 Accepted 06 October 2020

Public health protection is the most important law of the development of a civilized society. As participants in the health care system, pharmaceutical specialists must take upon themselves certain preventive tasks aimed at strengthening and maintaining the health of the population.

The aim of the work was to substantiate and develop a methodology for implementing the professional role of pharmaceutical specialists in public health protection.

Materials and methods. The search for information was carried out using the methods of address, thematic and factual search in the Scopus, The Cochrane Library, Pubmed, eLibrary databases. The analyzed body of information included systematic reviews, retrospective and randomized studies, and other applied developments on the topic of publication. The depth of the bibliographic search for scientific publications is about 20 years (2000-2019). The following keywords were used for the search: "a pharmacist in health protection", "prevention of adverse drug reactions", "functions of a pharmacist", "pharmaceutical care", "sanitary competence", "a pharmacist's role in the prevention of diseases", "pharmacy services", "patient compliance", "collaboration in health care", etc.

Results. On the basis of the analysis, the demand for pharmacists in the health protection activities has been substantiated, the scientific and applied methodology of using pharmaceutical knowledge in the protection of public health has been updated and the necessary professional competencies (PC) of a pharmacist have been proposed for their implementation. The relevance of the preventive approach in the implementation of information and consultancy activities of a pharmacist has been substantiated, the essence of which is to prevent undesirable events associated with the use of medicinal preparations (MPs) and preventive health care in the society. The proposed methodology is based on the concept of "a pharmaceutical vigilance", the practical implementation of which is proposed in the publication. The need for pharmacists to perform certain professional functions aimed at preserving and strengthening the health of the population has been updated, the functions have been proposed and the list of possible pharmaceutical services for the practical implementation of the proposed functions in the health care system has been provided.

Conclusion. The following pharmaceutical services have been identified as relevant: pharmaceutical enlightenment of the population, patient-oriented pharmaceutical counseling and patronage of patients, pharmaceutical informing of medical specialists about drugs and social prevention of the problems affecting the public health. The definitions of pharmaceutical services have been specified, the methodology for providing the services has been substantiated, the efficiency of their provision has been updated and the professional competencies of pharmacists for their implementation in practical healthcare have been formed.

Keywords: review, health care, pharmacist, pharmaceutical services, professional competence

For citation: I.A. Kirshchina, A.V. Soloninina, V.N. Michailova. Conceptually-theoretical justification and updating of the preventive approach in the implementation of a pharmacist's information consultancy services in the public health system. *Pharmacy & Pharmacology*. 2020;8(3): 195-204. **DOI:** 10.19163/2307-9266-2020-8-3-195-204

Для цитирования: И.А. Кирщина, А.В. Солонинина, В.Н. Михайлова. Концептуально-теоретическое обоснование и актуализация превентивного подхода при осуществлении информационно-консультационной деятельности провизора в системе общественного здоровья. *Фармация и фармакология.* 2020;8(3):195-204. **DOI:** 10.19163/2307-9266-2020-8-3-195-204

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

КОНЦЕПТУАЛЬНО-ТЕОРЕТИЧЕСКОЕ ОБОСНОВАНИЕ И АКТУАЛИЗАЦИЯ ПРЕВЕНТИВНОГО ПОДХОДА ПРИ ОСУЩЕСТВЛЕНИИ ИНФОРМАЦИОННО-КОНСУЛЬТАЦИОННОЙ ДЕЯТЕЛЬНОСТИ ПРОВИЗОРА В СИСТЕМЕ ОБЩЕСТВЕННОГО ЗДОРОВЬЯ

И.А. Кирщина, А.В. Солонинина, В.Н. Михайлова

Федеральное государственное бюджетное образовательное учреждение высшего образования
«Пермская государственная фармацевтическая академия»
Министерства здравоохранения Российской Федерации
Российская Федерация, 614990, г. Пермь, ул. Полевая, д. 2

E-mail: irina.kirshina@mail.ru

Получено 22.10.2019 Рецензия (1) 05.05.2020 Рецензия (2) 30.08.2020 Принята к печати 06.10.2020

Охрана здоровья населения – важнейший закон развития цивилизованного общества. Специалисты фармацевтического профиля, как участники системы здравоохранения, должны взять на себя определенные профилактические задачи, направленные на укрепление и сохранение здоровья населения.

Цель работы заключалась в обосновании и разработке методологии по реализации профессиональной роли фармацевтических специалистов в охране здоровья населения.

Материалы и методы. Поиск информации осуществлялся методами адресного, семантического и фактографического поиска по базам данных Scopus, The Cochrane Library, Pubmed, eLibrary. В анализируемый массив информации включались систематические обзоры, ретроспективные и рандомизированные исследования и другие прикладные разработки по теме публикации. Глубина библиографического поиска научных публикаций порядка 20 лет (2000–2019), в качестве ключевых слов для поиска использовались: «фармацевтический работник в охране здоровья», «предупреждение нежелательных лекарственных реакций», «функции фармацевтического работника», «фармацевтическая помощь», «санитарная грамотность», «роль фармацевта в предупреждении заболеваний», «услуги аптеки», «пациентский комплаенс», «коллаборация в здравоохранении» и др.

Результаты. На основе проведенного анализа аргументирована востребованность фармацевтических специалистов в здоровьесберегающей деятельности, актуализирована научно-прикладная методология использования фармацевтического знания в охране здоровья населения и предложены необходимые профессиональные компетенции (ПК) специалиста фармацевтического профиля для ее реализации. Обоснована актуальность превентивного подхода при осуществлении информационно-консультационной деятельности провизора, суть которого заключается в предупреждении нежелательных событий, связных с использованием лекарственных препаратов (ЛП), и профилактике заболеваемости в обществе. Предлагаемая методология базируется на понятии «фармацевтическая бдительность», практическая реализация которой предложена в публикации. Актуализирована необходимость осуществления специалистами фармацевтического профиля определенных профессиональных функций, направленных на сохранение и укрепление здоровья населения, предложены функции и приведен перечень возможных фармацевтических услуг для практической реализации предлагаемых функций в системе здравоохранения.

Заключение. В качестве релевантных фармацевтических услуг определены: фармацевтическое просвещение населения, пациентоориентированное фармацевтическое консультирование и патронаж пациентов, фармацевтическое информирование медицинских специалистов о лекарственных препаратах и социальная профилактика проблем, влияющих на здоровье населения. Конкретизированы определения фармацевтических услуг, обоснована методология предоставления услуг, актуализирована эффективность их предоставления и сформированы профессиональные компетенции специалистов фармацевтического профиля для их реализации в практическом здравоохранении.

Ключевые слова: обзор, охрана здоровья, провизор, фармацевтические услуги, профессиональные компетенции

INTRODUCTION

Throughout the development of the human society, the main task of medicine has been human health maintenance. As an integral part of the institute of health, pharmaceutical science is a key link in providing health care, because according to statistics, 95% of medical prescriptions account for pharmacotherapy[1] [1]. “If we consider drugs in the broad sense of the word – as any substances that effect on living organisms – only a few of these substances will be outside the competence of pharmacology”.[2] In the conditions of the rapidly developing market of medicinal preparations (MPs), modern demands of the national policy in the field of health care, the following norms are required from a pharmaceutical specialist for the organization of pharmacy practice: adequate specialized training, professional mobility and quick adaptation in the context of constant updating of knowledge in the professional field.

THE AIM of the work was to substantiate the role

[1] Order of the Ministry of Education and Science of the Russian Federation No. 219 dated 27.03.2018 "On approval of the state educational standard of higher education-specialist’s program with a specialization in 33.05.01 "Pharmacy". URL: http://www.consultant.ru/cons/cgi/online.cgi?req=doc&ts=104919653604168107263790574&cacheid=9C443E5F58B8734C3DFEF56B2244D60D&mode=splus&base=LAW&n=296116&rnd=DE4CA02918499814E30E01A8BEABE09D#9p93zns8tu/ (accessed 14.06.2019).

[2] A. Goodman, L. Gilman, 1941

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-195-204

of pharmaceutical knowledge in health promotion of the population and to determine the professional competencies of pharmaceutical specialists aimed at the implementation of information and consultance services from the standpoint of public health care maintaining.

MATERIALS AND METHODS

For the information search, the following methods were used: address, semantic and factographic ones. The information was looked up in Scopus, The Cochrane, Pubmed, eLibrary databases. The analyzed array of information included systematic reviews, retrospective and randoamized studies, and other applied developments on the publication topic. The depth of the bibliographic search of scientific publications was about 20 years (2000–2019); the keywords for the search were: “pharmaceutical specialist in health care”, “prevention of adverse drug reactions”, “functions of a pharmaceutical specialist”, “pharmaceutical care”, “health literacy”, “a pharmacist’s role in the prevention of diseases”, “pharmacy services “, “patient compliance”, “collaboration in health care “, etc.

RESULTS AND DISCUSSION

The list of pharmacists’ professional competencies (PCs) is not determined in the requirements of the Federal State Educational Standard for Higher Education in the specialist’s program with a specialization in 33.05.01 “Pharmacy”. When forming mandatory and recommended PCs, the standard prescribes to be guided by the established professional standards that correspond to the professional activities of graduates, and by the demand for specialists in the labor market.[3] In this regard, it becomes necessary to work out a list pharmacists’ professional competencies, based on the employment functions of pharmacy specialists, prescribed in professional standards.

At the same time, it is advisable to take into account the results of the analysis of homeland and foreign practices of pharmaceutical specialists’ professional implementation in the healthcare system. The analysis of the standardized requirements to the professional skills of a specialist with a higher pharmaceutical education showed that the employment functions of a pharmacist include, among others, providing information consultancy services in choosing over-the-counter drugs, drugs compatibility and their interaction. The professional “Pharmacist” standard prescribes the necessity for pharmacists to have professional knowledge in the range of drugs and their characteristics, the basics of responsible self-medication, the principles of pharmacotherapy, taking into account pharmacokinetics and pharmacodynamics of drugs, the basics of clinical pharmacology and the rules of drugs rational use. The standard requires the necessity for a pharmacist to have communicative skills dealing with patients.[4]

The relevance of the formation and implementation of professional information consultancy competencies of a pharmacy specialist from the standpoint of personal responsibility, has also been highlighted by a number of homeland researchers [2–5].

The main thesis of these scientific publications is that a drug should be considered as a drug plus information about it, because the informing procedure is just as important and obligatory as the drug itself in pharmacotherapy [6]. The analysis of foreign publications confirms that qualified advice from a pharmaceutical specialist can reduce risks and increase the effectiveness of pharmacotherapy. Thus, scientific publications of Ukrainian colleagues have substantiated the expediency of interaction between pharmaceutical and medical specialists in organizing rational pharmacotherapy [7, 8]. Scientists from Australia have confirmed the importance of pharmaceutical consulting in self-medication [9]. The scientific materials of the colleagues from the Italian University of Catanzaro have proved the need for special knowledge about drugs when choosing their trade names. Certain pharmaceutical aspects of generics (reproduced drugs) in relation to the original drugs by the example of specific clinical cases have been illustrated, and arguments for the relativity of their use in therapeutic practice have been provided [10]. Colleagues from Turkey [11] and Japan [12] prove the need for pharmaceutical knowledge in the prevention of problems of pharmacotherapy in geriatrics. American colleagues substantiate the importance of pharmaceutical information in child health care and the choice of safe pharmacotherapy in pediatric practice [13, 14]. Scientists from the University of Aston, Birmingham, (UK) in their works actualize the need for pharmaceutical, medical and pedagogical cooperation in order to achieve maximum therapeutic benefit in pediatric patients with chronic diseases [15]. Colleagues from Switzerland [16] and America [17] prove the role of pharmaceutical informing of medical specialists in the achievements of personalized medicine, emphasizing the importance of pharmaceutical information in the prevention of undesirable and dangerous drug reactions when choosing pharmacotherapy.

The review of scientific publications can be continued, but the position of scientists regarding the issue under study, will remain unchanged – the role of pharmaceutical knowledge in the prevention of dangerous

[3] Order of the Ministry of Education and Science of the Russian Federation No. 219 dated 27.03.2018 "On approval of the state educational standard of higher education-specialist’s program with a specialization in 33.05.01 "Pharmacy". URL: http://www.consultant.ru/cons/cgi/online.cgi?req=doc&ts=104919653604168107263790574&cacheid=9C443E5F58B8734C3DFEF56B2244D60D&mode=splus&base=LAW&n=296116&rnd=DE4CA02918499814E30E01A8BEABE09D#9p93zns8tu/ (accessed 14.06.2019)

[4] Приказ Order of the Ministry of Labor of Russia No. 91n dated 03.09.2016 "On the approval of the professional standard" of Pharmacist ".URL: http: //www.consultant.ru/cons/cgi/online.cgi?Req = doc & ts = 11274201530948461943212058 2 & cacheid = 3C043F863335363DBDA01171 & mode 196697 & rnd = DE4CA02918499814E30E01A8BEABE09D # 1hsop1e9sbr / (access date 10/14/2019)

events associated with the use of drugs, is extremely significant. The foregoing makes it possible to assert that the primary importance in the implementation of professional information consultancy competencies of a pharmacy specialist, acquires a preventive component of a specialist's professional activity.

By the "preventive approach in the organization of pharmaceutical services", a complex of professional information and recommendations given by a pharmacy specialist focused on preventing the risks of pharmacotherapy associated with pharmaceutical and medico-biological aspects of drugs and the formation of health-preserving principles of behavior in society in order to preserve and strengthen public health", is meant. This thesis serves to achieve the aim set by the authors. To concretize the proposed definition, it should be notified that well-known characteristics of drugs and the risks of pharmacotherapy associated with them, have been taken by the authors as the basis for the concepts of "pharmaceutical and medico-biological aspects". [18]. The ideology of the preventive approach lies in the oldest principle of medical ethics and deontology "primum non nocere",[5] the identical concept and methodology of which is reflected in the concept of "pharmaceutical vigilance".

"Pharmaceutical vigilance is adequate alertness of pharmacy specialists in their professional activities, aimed at reducing the risks of adverse drug reactions, optimizing a rational choice and consulting support of pharmacotherapy, as well as implementing an active professional position in the prevention of diseases and other activities focused on human health maintenance.

As it follows from the definition, the practical significance of the proposed methodology is not limited to the pharmacotherapeutic aspects of the professional activities of a pharmacist, but it is also focused on maintenance and promotion of personal and public health. It should be notified that health is the most important individual and social resource, but the concepts of personal and public kinds of health are not identical. According to the scientists in the field of health care, public health cannot be restricted to the corpus of indicators of the health of individuals [20]. According to the World Health Organization's Constitution, adopted in 1946, health is defined as "a state of complete physical, mental and social well-being, and not just the absence of physical defects and diseases." However, this definition is not to be used to assess health at the individual and population levels.

Taking the thesis "public health is health for all"[6] and his own criterion for assessing the health of the society as "a productive way of life" as a basis, Professor Lisitsyn Yu.P. proposed a challenging definition of the term "public health" – this is the quality of society that provides conditions for the lifestyle of people who are not burdened with diseases, physical and mental disorders, i.e. the condition in which the formation of a healthy lifestyle is ensured. It is common practice to measure public health in statistical indicators: morbidity, mortality, average life expectancy, etc. At the same time, it is advisable to assess individual health by personal well-being, work qualification, personal perception of well-being, joy of life, etc." [20].

It is important to note that in the modern ideology of Russian healthcare system, the issues of health protection at the individual and population levels are extremely important. The key thesis on which the state policy in the field of health protection is based, can be defined as follows: health is the highest value of a person and society as a whole, and the most important principle of the country's health care system development is maintenance and promotion of personal and public health. The main documents defining the concept of the state policy in the field of health care,[7,8,9,10] are focused on the maintenance of public health through the implementation of the state programs aimed at the formation of the active motivation of the individual and society as a whole. It should be emphasized that the concept of public health, considered from the standpoint of conditions and lifestyle, is directly related to strategies and social policies in the field of maintenance and promotion of health of the Russian Federation population. In this regard, there is a need for search and updating the conceptual directions of the professional activities of a pharmacy specialist from the standpoint of a preventive approach and the development of organizational technologies for strategic management of a pharmacist's information consultancy services, taking into account the implementation of the state tasks concerning public health maintenance.

The implementation of the following information consultancy services of a pharmacy specialist in the form of certain professional functions is proposed by

[5] First of all, do no harm".
[6] Halfday T. Mahler, WHO, 1976.

[7] The Constitution of the Russian Federation (adopted by a nationwide vote on 12.12.1993 with amendments approved during the all-Russian vote on 01.07.2020). URL:http://www.consultant.ru/cons/cgi/online.cgi?req=doc&ts=315831219053797560923230 8&cacheid=57F1054E5D1306E48974280A63B9D093&mode=splus&base=LAW&n=2875&dst=100067&rnd=0.4768493268810434#1wkaai07uy6/ (accessed 14.06.2019).
[8] Federal Law of the Russian Federation No. 323-FZ dated 21.11.2011. "On the basics of health protection of citizens in the Russian Federation" URL: http://www.consultant.ru/document/cons_doc_LAW_121895/ (accessed 12.14.2020).
[9] Resolution of the Government of the Russian Federation No. 1640 dated 26.12.2017 " On Approval of the State Program of the Russian Federation "Development of Healthcare" URL:http://www.consultant.ru/cons/cgi/online.cgi?req=doc&ts=108420909106947179518728 93&cacheid=A8DB92BD22089A186A084A5DC5417A0F&mode=splus&base=LAW&n=360632&rnd=0.3187056380265709#1x83q3vw692/ (accessed 14.10.19).
[10] Passport of the national project " Demography, approved by the Presidium of the Presidential Council for Strategic Development and National Projects, Protocol No. 16 dated 24.12.2018.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-195-204

the authors: educational, consultancy, informational, accompanying, social prevention. Their implementation will help with maintenance and promotion of personal health and improving the public health indicators in the future. To implement in practice the above listed functions, a list of pharmaceutical services has been developed, and professional competencies of pharmacy specialists have been formed (Fig. 1).

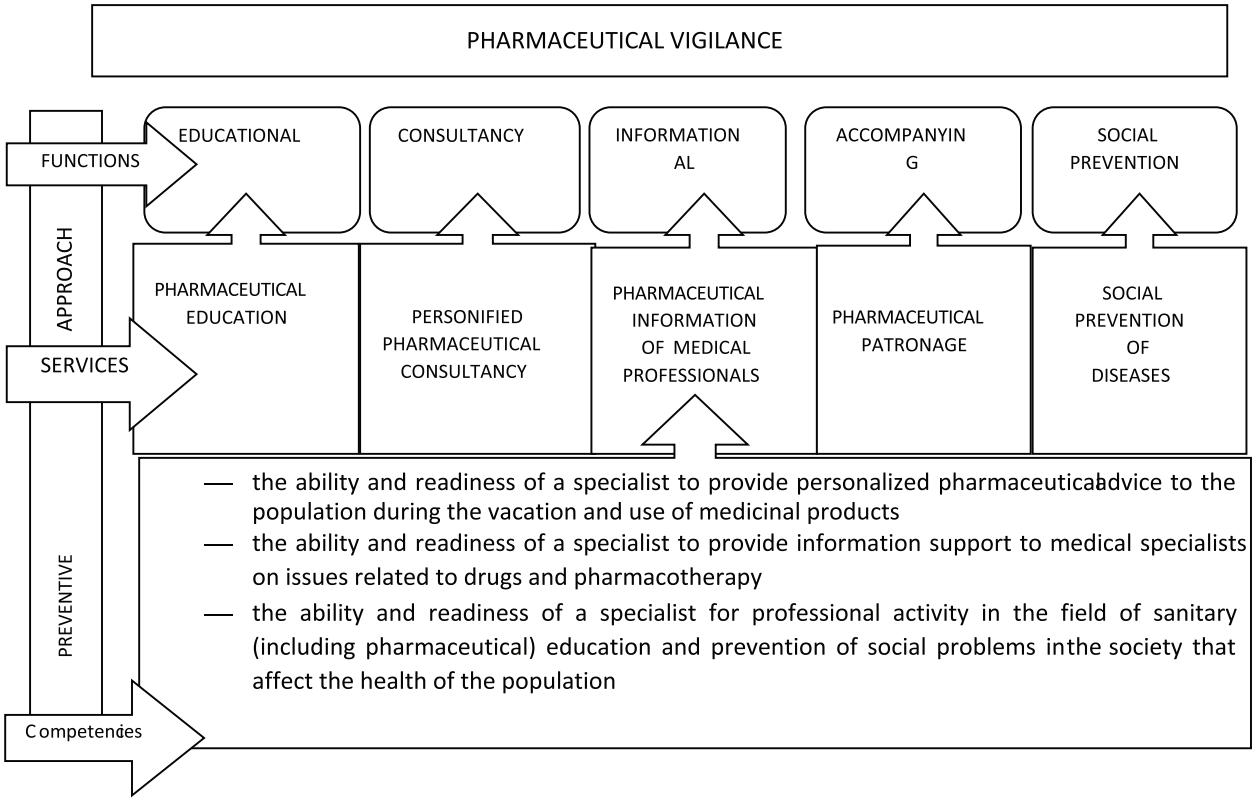

Figure 1 – Functional-applied model of the formation of information consultancy competencies of pharmacy specialists from the standpoint of a preventive approach

Fig. 1 shows that pharmaceutical services are defined as follows: pharmaceutical education; personified pharmaceutical consultancy; pharmaceutical information of medical professionals; pharmaceutical patronage; social prevention of diseases. The definitions of the services under consideration have been proposed, and methodological approaches for their implementation in the healthcare system have been developed.

Pharmaceutical education is a professional activity of a pharmacy specialist aimed at increasing the pharmaceutical literacy of potential drug users and preventing health risks from their use. The methodology of pharmaceutical education is based on the principles of medicinal education presented in the WHO reference paper on the topical issues of sanitary education in the world community. In this document, the WHO experts convincingly argue that "the key determinant of health is the level of health literacy of the population; the degree of education being the most accurate parameter to predict the health of the society in the future. Limited health literacy of citizens is associated with a lower level of their participation in health promotion and disease detection, reduced ability to self-manage chronic diseases, lack of adherence to pharmacotherapy, higher hospitalization rates, increased rates of morbidity and premature mortality[11] Pharmaceutical education, as a significant component of medicinal literacy, deserves a separate study, since a significant part of medical issues, is connected with the use of drugs. A methodological approach to the professional implementation of pharmaceutical education is the promotion of pharmaceutical literacy, which should be understood as the level of education of the population, which is necessary to obtain, understand, evaluate and use pharmaceutical information. All these make it possible to solve standard everyday tasks, using drugs from the standpoint of minimizing health risks, preventing diseases and health promotion, to maintain or improve the quality of life at all its stages.

Health literacy problems are regularly reported internationally [21–25]. The main conceptual ideas of scientific publications can be summarized in the following theses: sanitary education is a special medical

[11] Health literac. The solid facts. – Copenhagen, WHO, 2013. 86p. URL:https://www.euro.who.int/ru/publications/abstracts/health-literacy.-the-solid-facts

and pedagogical activity aimed at improving the culture of public health and achieving a healthy lifestyle, which cannot be limited by the framework of one sphere; interdisciplinary cooperation in sanitary education is carried out through the participation of individuals, groups, populations and organizations in health care, with health professionals playing a leading role.

Patient-oriented pharmaceutical consultancy (PPC) is a professional activity of a pharmacy specialist aimed at minimizing possible undesirable consequences of the use of drugs. Herewith, the patient's concomitant diseases, drugs taken, and / or the patient's belonging to a group of increased risk of developing adverse reactions from the use of drugs, should be taken into account [26]. The PPC methodology is based on the theory of an individual approach to the patient when choosing pharmacotherapy: "Different patients react differently to the same drug, therefore, each prescription should be considered as an experiment, during which the hypothesis about the individual efficacy and safety of the drug should be tested"[12].

Scientists pharmacologists note that an individual approach to pharmacotherapy is of particular importance in the organization of medical care. It is based on the peculiarities of the relationship between the clinical effects of drugs and pharmacodynamic and pharmacological processes [27], and the patient's adherence to medical prescriptions ("Medicines will not work if they are not taken"[13]). At the same time, the organizers of the pharmaceutical business consider pharmaceutical consultancy as an integral part of pharmaceutical and medical care, the purpose of which is human health maintenance by satisfying the needs for rational pharmacotherapy and adherence to the rules for using drugs. A special form of pharmaceutical patient care based on an individual (exclusive for a particular patient) approach has been proposed. A Procedure for patient-oriented pharmaceutical consultancy in the organization of pharmaceutical advisory care from the standpoint of critical assessment, prevention and minimization of undesirable risks of pharmacotherapy for a patient and a personal responsibility of a pharmaceutical specialist for possible events associated with taking drugs, has been developed. It is known that, along with functional and physiological factors affecting the body's reactions to drug intake (gender, age, individual sensitivity, etc.), significant aspects of the safety of pharmacotherapy are the risks associated with unreasonable and / or forced polypharmacy, which is usually caused by polymorbidity, undesirable drug-drug interactions and insufficient patient adherence to the prescribed treatment [28–33]. In this regard, comprehensive PPC can influence the safety of pharmacotherapy and is associated as an integral component of public health care maintaining. Hereby, potential and real factors should be taken into account.

Pharmaceutical informing of medical specialists is a professional activity of a pharmacy specialist aimed at optimizing information support for the rational choice of safe and effective pharmacotherapy. The pharmaceutical communication methodology, is based on international principles of rational use of medicines, the WHO concept of the importance of patient compliance and the implementation of the role of the pharmacist in the public health system [35–40]. The proposed conception is based on the principle of being "necessary and sufficient". Its main idea includes providing medical specialists with independent, comprehensive and timely information about the pharmaceutical and medico-biological aspects of drugs and the rules for their safe use from the standpoint of the priority of monotherapy, or prescribing as few drugs as possible with the purpose of preventing polypharmacy and undesirable drug-drug interactions. Scientific publications describe modern methods of management and prevention of polypharmacy [29–33,38–40], which are advisable to use in the development of organizational technologies for the selection of rational drug combinations in polymorbid and / or comorbid conditions. Timely pharmaceutical informing of medical specialists about the existing approaches to the choice of drug therapy from the standpoint of scientific evidence and personification, the joint participation of medical and pharmacy specialists in the development and implementation of pharmacotherapeutic programs, will help reduce the risks of adverse events associated with the prescription and use of drugs, and increase patient compliance during the period of its receipt.

Pharmaceutical patronage (support) is a professional activity carried out by specialists of the health care system based on an interdisciplinary approach in order to solve medical and pharmaceutical problems of the patient; it is aimed at improving certain aspects of the quality of life and maintaining human health. The methodology of the service is based on the principles of interdisciplinary interaction in the field of public health, the basics of medical prevention and the role of a pharmacy specialist in the health care system [34, 36, 40–44]. According to the concept proposed by Professor Kasavin I.T., "interdisciplinary interaction (which does not imply rigid boundaries of each discipline involved) is a natural state of science, the limiting case of which is relatively strict disciplinary structures, the boundaries of which are set not so much by knowledge systems as by institutional forms" [45]. Relevance, effectiveness, versatility and significance of interdisciplinary interaction for the optimization of medical and pharmaceutical care has been confirmed and re-

[12] A. Goodman, L. Gilman, 1941.
[13] WHO, 2003, New-York.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-195-204

peatedly proven by scientists in experimental studies [44, 46–53]. The methodological approach to the professional implementation of pharmaceutical patronage consists in medical and pharmaceutical support, training, control and management of the process of adaptation of a person to physiological characteristics and (or) illness, the ability to perform his usual functions corresponding to his socio-economic status in order to preserve the quality of life associated with his health (health-related quality of life). The term "health-related quality of life" refers to the assessment of parameters associated and not associated with a disease, allowing to differentiate the impact of disease and treatment on the psychological, emotional state of the patient and his social status [54]. Regular monitoring of the patient's quality of life, systematic consulting support of pharmacotherapy and other aspects that affect the subjective assessment of a person's health in the framework of interdisciplinary medico-pharmaceutical content with a patient, will make it possible to return to the most important principle of medical care: "treat not a disease, but a patient".[14]

Social prevention of diseases is a conscious, organized activity of a pharmacy specialist aimed at social orientations, habits and worldview of people, the formation of medical and social activities and health-saving principles of behavior in the society in order to improve public health indicators. The methodology of social prevention is based on international principles of pharmacy practice organization, the role of a pharmacy specialist in the health care system, the principles of medical prevention and the priority of preventive activities in the public health system ("A disease is easier to prevent than to cure"[15]) [34, 36, 40–44].

As a basis for the implementation of the preventive function of a pharmaceutical specialist, the principle of medical practice was taken. In the 19th century it was voiced by Professor Pirogov N.I.: "Teaching people to be healthy and to heal those who could not be taught this."

Updating the conceptual directions of social prevention in the framework of the professional activity of pharmacy specialists, includes the promotion of the basic postulates of a healthy lifestyle throughout the entire life cycle, including prevention of bad habits; development of healthy eating habits; compliance with treatment, work and rest regimens; sleep hygiene; regular and adequate physical activities and walks in the open air; planned trips to dispensary examinations; prevention of psychological health and the formation of healthy relationships in the society, etc.

The development and implementation of the proposed conception is associated with the important mission of a pharmacy specialist in the health care system – promotion of a healthy lifestyle, prevention of diseases and maintenance and promotion of public health in the longer term.

CONCLUSION

The practical result of the conducted research, can be considered the professional competencies, offered by the authors to pharmacy specialists within the framework of their mastering the specialist's program with a specialization in 33.05.01 "Pharmacy".

So, as a mandatory professional competence, it is advisable to consolidate "the ability and readiness of a specialist for personalized pharmaceutical consultancy of the population when dispensing and using drugs, as well as information support of medical specialists on the issues related to drugs and pharmacotherapy".

It is rational to refer "the ability and readiness of a specialist for professional activities in the field of sanitary (including pharmaceutical) education and prevention of social problems in the society that affect public health" to the recommended pharmacists' professional competencies.

As an epilogue to the scientific review, it is relevant to recall the maxim "Medica mente, non medicamentis",[16] and notify that the solid ground and centuries-old constants of the pharmacist's activity and an indicator of his professional maturity as a specialist in the public health system, are permanent professional development, adherence to the norms of medical and human ethics , high social responsibility, full awareness of their mission in the chosen profession and absolute love for pharmacy.

FINANCIAL SUPPORT

This study did not have any support from third-party organizations.

AUTHOR'S CONTRIBUTION

All authors have equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

[14] Mudrykh M.YA. (1776–1831).
[15] Hippocrates.
[16] «Treat with the mind, not with medicine».

REFERENCES

1. Vovk EI. [Bazovye principy farmakoterapii.] Russian pharmacies; 2003. 7–8: 47–51. Russian.
2. Dremova NB., Ovod AI., Korzhavyh EA. Osnovy farmacevticheskoj pomoshchi v zdravoohranenii. Kursk State Medical University; 2009:412p. Russian.
3. Fedina EA. Osnovy sistemy kachestva farmacevticheskih informacionno- konsul'tacionnyh uslug. Novaya apteka. Effektivnoe upravlenie. 2008;8:74–79. Russian.
4. Kononova SV, Petrova SV, Dadus NN, CHesnokova NN, ZHukova EV. [Pharmaceutical consuting: efficacy and safety]. Remedium. 2019;(11):40–46. DOI: 10.21518/1561-5936-2019-11-40-46.
5. Maskaeva AR, Glembockaya GT. Integraciya deyatel'nosti provizora i vracha v obespechenie effektivnosti i bezopasnosti lekarstvennoj terapii. Pharmateka. 2001;4:24–31. Russian.
6. Glembockaya GT. Mnogogrannost', specifichnost', otvetstvennost' raboty provizora – specialista novogo tysyacheletiya. Vestnik voronezhskogo gosudarstvennogo universiteta. Seriya: Himiya. Biologiya. Pharmaciya. 2004;2:175–178. Russian.
7. Zupanec IA, CHernyh VP, Popov SB. Farmacevticheskaya opeka: atlas. Kiev: Farmacevt Praktik. 2007:146p. Ukrainian.
8. Zupanec IA, CHernyh VP, Popov SB, Bezdetko NV. Farmacevticheskaya opeka – vazhnejshij aspekt klinicheskoj farmacii. Provizor (Har'kov). 2000;11:6–9. Ukrainian
9. Seubert LJ, Whitelaw K, Hattingh L, Watson MC, Clifford RM. Development of a Theory-Based Intervention to Enhance Information Exchange during Over-The-Counter Consultations in Community Pharmacy. Pharmacy (Basel). 2018;6(4):117. DOI: 10.3390/pharmacy6040117.
10. Gallelli L, Palleria C, De Vuono A, et al. Safety and efficacy of generic drugs with respect to brand formulation. J Pharmacol Pharmacother. 2013;4(1):S110–S114. DOI:10.4103/0976-500X.120972.
11. Ertuna E, Arun MZ, Ay S, Koçak FÖK, Gökdemir B, İspirli G. Evaluation of pharmacist interventions and commonly used medications in the geriatric ward of a teaching hospital in Turkey: a retrospective study. Clin Interv Aging. 2019;14:587–600. DOI: 10.2147/CIA.S201039.
12. Hashimoto R, Fujii K,Yoshida K, Shimoji S, Masaki H, Kadoyama K, Nakamura T, Onda M. Outcomes of Pharmacists' Involvement with Residents of Special Nursing Homes for the Elderly. Yakugaku Zasshi. 2018;138:1217–1225. DOI: 10.1248/yakushi.18-00065.
13. Parrish II RH, Casher D, van den Anker J, Benavides S. Creating a Pharmacotherapy Collaborative Practice Network to Manage Medications for Children and Youth: A Population Health Perspective. Children (Basel). 2019;6(4):58. DOI: 10.3390/children6040058.
14. Rieder M. Adverse Drug Reactions in Children: Pediatric Pharmacy and Drug Safety. Pediatr Pharmacol Ther. 2019;24(1):4–9. DOI: 10.5863/1551-6776-24.1.4.
15. Aston J, Wilson KA, Terry DRP. The treatment-related experiences of parents, children and young people with regular prescribed medication. Int J Clin Pharm. 2019;41(1):113–121. DOI: 10.1007/s11096-018-0756-z.
16. Loustalot MC, Berdot S, Sabatier P, et al. The impact of interventions by pharmacists collected in a computerised physician order entry context: a prospective observational study with a 10-year reassessment. Swiss Medical Weekly. 2019;149:w20015. DOI: 10.4414/smw.2019.20015.
17. Kennedy MJ. Personalized medicines – are pharmacists ready for the challenge? Integr Pharm Res Pract. 2018;7:113–123. DOI: 10.2147/IPRP.S133083
18. Percev IM, Zupanuc IA. Biofarmaciya i effektivnost' lekarstv. Provizor. 2001;4: 25-28. Russian.
19. SHabunova AA, Kalachikova ON, SHabunova AA, Kalashnikov KN. Obshchestvennoe zdorov'e i zdravoohranenie territorij. Vologda: «ISERT RAN». 2010:211. Russian
20. Lisicyn YUP. Obshchestvennoe zdorov'e i zdravoohranenie. Moscow: GEOTAR-Media.2009:512p. Russian.
21. Mononen N, Airaksinen MSA, Hämeen-Anttila K, Helakorpi S, Pohjanoksa-Mäntylä M. Trends in the receipt of medicines information among Finnish adults in 1999-2014: a nationwide repeated cross-sectional survey. BMJ Open. 2019;9(6):e026377. DOI: 10.1136/bmjopen-2018-026377.
22. Garov S. [Sanitary literacy – determining health factor]. Nacional'naya Associaciya Uchenyh. 2018;37:12–15. Russian.
23. Popov T, Garov S. [About nature and priorities of sanitary education]. Vestnik Vostochno-Sibirskoj Otkrytoj Akademii. 2018; 27:14p. Russian.
24. Nutbeam D. Health literacy as a public health goal: a challenge for contemporary health education and communication strategies into the 21st century. Health Promotion International. 2000;15(3):259–267. doi.org/10.1093/heapro/15.3.259.
25. Amlaev KR, Dakhkilgova KhT. Health literacy matters: concept, classification, methods of assessment. Vrach. 2018; 29(6):83–86. Russian.
26. Gabdrafikova YuS, Kirshchina IA, Soloninina AV. [Farmaceutical help for geriatric patients: vital problems and possible solutions]. Pharmacy. 2018; 5: 35–41. DOI. org/10.29296/25419218-2018-05-07. Russian.
27. Abdulhabirova FM, et al. Obshchaya vrachebnaya praktika. Moscow: "GEOTAR-Media". 2020:1024p. Russian.
28. Sychev DA, Sosnovskij EE, Orekhov RE, Bordovskij SP. Contemporary methods of dealing.With polypharmacy in elderly and senile patients. Siberian medical review. 2016;2:13–21. Russian.
29. Sychev D.A. Polipragmaziya i bezopasnost' pacientov. Medical alphabet. 2015; 2(9):52. Russian.
30. Adherence to Long-Term Therapies: Evidence for Action. [Internet]. WHO; 2003:16p. Available from: https://www.who.int/chp/knowledge/publications/adherence_report/en/
31. Vol'skaya EA. Patient compliance. Overview of research trends. Remedium. 2013;11:6–15. DOI:10.21518/1561-5936-2013-11-6-15.
32. Kluchnikov S.O. Polypharmacy: response. Children infections. 2014;13(4):36–41. DOI:10.22627/2072-8107-2014-13-4-36-4. Russian.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-195-204

33. The Pursuit of Responsible Use of Medicines:Sharing and Learning from Country Experiences. [Internet]. WHO; 2012:78p. Available from: https://www.who.int/medicines/publications/responsible_use/en/. Russian.
34. Developing pharmacy practice. A focus on patient care. Geneva. [Internet]. WHO; 2003:97p. Available from: https://www.who.int/medicines/publications/WHO_PSM_PAR_2006.5.pdf. Russian.
35. The Role of Education in the Rational Use of Medicines. [Internet]. WHO. 2006:99p. Available from::https://digicollections.net/medicinedocs/documents/s16792e/s16792e.pdf. Russian.
36. The legal and regulatory framework for community pharmacies in the WHO European Region. [Internet]. World Health Organization. Regional Office for Europe 2020:112p. Available from: https://apps.who.int/iris/bitstream/handle/10665/331232/9789289054591-rus.pdf. Russian.
37. Létinier L, Cossin S, Mansiaux Y, Arnaud M, Salvo F, Bezin J, Thiessard F, Pariente A. Risk of Drug-Drug Interactions in Out-Hospital Drug Dispensings in France: Results From the DRUG-Drug Interaction Prevalence Study. Front Pharmacol. 2019;10:265. DOI: 10.3389/fphar.2019.00265.
38. Molokhia M, Majeed A. Current and future perspectives on the management of polypharmacy. BMC Fam Pract. 2017;18(1):70. DOI: 10.1186/s12875-017-0642-0.
39. Garzón González G, Montero Morales L, de Miguel García S, Jiménez Domínguez C, Domínguez Pérez N, Mediavilla Herrera I. Análisis descriptivo de los errores de medicación notificados en atención primaria: aprendiendo de nuestros errores [Descriptive analysis of medication errors notified by Primary Health Care: Learning from errors]. Aten Primaria. 2020;52(4):233–239. Spanish. DOI: 10.1016/j.aprim.2019.01.006.
40. HEALTH21: the health for all policy framework for the WHO European Region. [Internet]. Copenhagen, WHO. 1999:314p. Available from: https://www.euro.who.int/ru/publications/abstracts/health21-the-health-for-all-policy-framework-for-the-who-european-region. Russian.
41. European Action Plan for Strengthening Public Health Capacities and Services. [Internet]. WHO. 2012:52p. Available from: https://www.euro.who.int/ru/health-topics/Health-systems/public-health-services/publications/2012/european-action-plan-for-strengthening-public-health-capacities-and-services. Russian.
42. Strengthening people-centred health systems in the WHO European Region: framework for action on integrated health services delivery. WHO. 2016:56p. Available from: https://www.euro.who.int/ru/health-topics/Health-systems/pages/publications/2016/eurrc6615-strengthening-people-centred-health-systems-in-the-who-european-region-framework-for-action-on-integrated-health-services-delivery. Russian.
43. Gaining health. The European Strategy for the Prevention and Control of Noncommunicable Diseases. [Internet]. Denmark, WHO. 2006:66p. Available from: https://www.euro.who.int/ru/publications/abstracts/gaining-health.-the-european-strategy-for-the-prevention-and-control-of-noncommunicable-diseases. Russian.
44. Smolina VA, Novokreshchenova IG. INTERDISCIPLINARY APPROACH TO THE STUDY OF PHARMACEUTICAL CARE IN THE PROBLEM FIELD OF SOCIOLOGY OF MEDICINE (REVIEW). Saratov Journal of Medical ScientificResearch. 2017;13(2):295–299. Russian
45. Kasavin IT. Filosofiya poznaniya i ideya mezhdisciplinarnosti. Epistemology & Philosophy of Science. 2004: 2(2):5–13. Russian.
46. Cavanaugh JJ, Lindsey KN, Shilliday BB, Ratner SP. Pharmacist-coordinated multidisciplinary hospital follow-up visits improve patient outcomes. J Manag Care Spec Pharm. 2015;21(3):256–60. DOI: 10.18553/jmcp.2015.21.3.256.
47. Rojas E, Gerber BS, Tilton J, Rapacki L, Sharp LK. Pharmacists' perspectives on collaborating with community health workers in diabetes care. J Am Pharm Assoc (2003). 2015;55(4):429–33. DOI: 10.1331/JAPhA.2015.14123.
48. Colla CH, Lewis VA, Beaulieu-Jones BR, Morden NE. Role of pharmacy services in accountable care organizations. J Manag Care Spec Pharm. 2015;21(4):338–44. DOI: 10.18553/jmcp.2015.21.4.338.
49. Kennedy MJ. Personalized medicines – are pharmacists ready for the challenge? Integr Pharm Res Pract. 2018;7:113–123. DOI: 10.2147/IPRP.S133083.
50. Johansen JS, Havnes K, Halvorsen KH, et al. Interdisciplinary collaboration across secondary and primary care to improve medication safety in the elderly (IMMENSE study): study protocol for a randomised controlled trial. BMJ Open. 2018;8(1):e020106. doi:10.1136/bmjopen-2017-020106.
51. Ensing HT, Vervloet M, van Dooren AA, Bouvy ML, Koster ES. Patient-pharmacist communication during a post-discharge pharmacist home visit. Int J Clin Pharm. 2018;40(3):712–720. DOI: 10.1007/s11096-018-0639-3.
52. Eickhoff C, Müller U, Strunz AK, Seidling HM, Lampert A, Felberg M, Breiholz S, Klintworth D, Schulz M. Das Projekt PRIMA – Elektronische Erstellung und Aktualisierung von Medikationsplänen als gemeinsame Aufgabe von Ärzten und Apothekern [The PRIMA Project – Electronically-Supported Physician-Pharmacist Cooperation to Generate and Update Medication Plans in Germany]. Dtsch Med Wochenschr. 2019;144(18):e114–e120. German. DOI: 10.1055/a-0859-5862.
53. Amara S, Adamson RT, Lew I, Slonim A. Accountable care organizations: impact on pharmacy. Hosp Pharm. 2014 Mar;49(3):253–9. DOI: 10.1310/hpj4903-253.
54. Novik AA, Ionova TI. Rukovodstvo po issledovaniyu kachestva zhizni v medicine. 2nd ed. Moscow: ZAO «Olma Media Grupp. 2007:320p. Russian.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

AUTHORS

Irina A. Kirshchina – Candidate of Sciences (Pharmacy), Associate Professor of the Department of Management and Economics of Pharmacy of Perm State Pharmaceutical Academy. ORCID: 0000-0002-7952-9585. E-mail: irina.kirshina@mail.ru

Anna V. Soloninina – Doctor of Sciences (Pharmacy), the Head of the Department of Management and Economics of Perm State Pharmaceutical Academy. ORCID: 0000-0002-2745-7698. E-mail: soloninina@mail.ru

Valentina N. Mikhailova – Candidate of Sciences (Pharmacy), Associate Professor of the Department of Management and Economics of Pharmacy of Perm State Pharmaceutical Academy. ORCID: 0000-0002-1705-705X.

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2020-8-3-205-218

COMPARATIVE ANALYSIS OF LEBANON DEVELOPMENT. PROSPECTS FOR COOPERATION WITH THE RUSSIAN FEDERATION

M.A.E. El Moussawi[1,3], Zh.V. Mironenkova[1], S.Z. Umarov[1], O.I. Knysh[2], O.D. Nemyatykh[1]

[1] Saint-Petersburg State Chemical and Pharmaceutical University,
14, Bld. A, Prof. Popov St., St Petersburg, Russia 197376
[2] Tyumen State Medical University, 54, Odessa St., Tyumen, Russia 625023
[3] "Mohammed" Pharmacy, 12, Hadath, Beirut, Lebanon 90201

E-mail: shanna.mironenkova@pharminnotech.com

Received 10 February 2020 Review (1) 10 April 2020 Review (2) 20 April 2020 Accepted 28 April 2020

The objective of the research was to conduct a comparative analysis of the development of Lebanon based on a number of demographic, economic and social indicators characterizing the health care of Lebanon, and to determine the prospects for the cooperation with the Russian Federation (RF) in the pharmacy field.

Materials and methods. The studies were conducted from 2009 to 2016. The objects were the statistical data accumulated on the basis of the data from national institutions and international organizations. These data were published annually in the reports of the Department of Economic and Social Affairs, the United Nations Population Division for 11 countries in the **Middle East:** Bahrain, Jordan, Yemen, Kuwait, Lebanon, United Arab Emirates, Oman, Saudi Arabia (Asian countries); Egypt, Sudan, Tunisia (North African countries). The research methods were: a comparative analysis, analytical grouping of data, ranking.

Results and discussion. A comparative analysis of demographic, economic and social indicators revealed that low mortality rates and high life expectancy in Lebanon were achieved both due to a satisfactory level of health care financing (Rank 5) and due to the adoption of adequate decisions in organizing and managing the Lebanese health care system. The positive trends that were inherent in the Lebanese health care system in previous decades continued to operate within the framework of earlier inertia, while migration flows intensified. However, there has been a slowdown in the decline in infant mortality in the dynamics of growth rates, which is a signal of the emergence of negative processes in the social sphere of the country.

Conclusion. The current situation in the Lebanese health care system, associated with limited financial resources, poses new challenges in the search for managerial decisions in the field of organizational management. The import of drugs from the Russian Federation will provide a significant reduction in the financial costs of providing the population of Lebanon and migrants with medicines which will increase the monetary costs of providing medical care.

Keywords: demographic, economic and social indicators; health care system; medicines

Abbreviations: GDP by PPP – gross domestic product by purchasing power parity per capita; VEM – Vital and essential medicines; CJSC – closed joint stock company; PM-pharmaceutical medicines; MF – medicinal form; INN –International non-patented; OJSC – open joint stock company; UAE – United Arab Emirates; UN – United Nations; LLC – limited liability company; COR – certificate of registration; USA, Beirut-CIP – carriage and insurance paid to Beirut. Freight/transportation and insurance paid to Beirut.

Для цитирования: М.А.Э.Х. Эль Муссави, Ж.В. Мироненкова, С.З. Умаров, О.И. Кныш, О.Д. Немятых. Компаративный анализ развития Ливана. Перспективы сотрудничества с Российской Федерацией. *Фармация и фармакология.* 2020;8(3):205-218. **DOI:** 10.19163/2307-9266-2020-8-3-205-218

For citation: M.A.E. El Moussawi, Zh.V. Mironenkova, S.Z. Umarov, O.I. Knysh, O.D. Nemyatykh. Comparative analysis of Lebanon development. Prospects for cooperation with the Russian Federation. *Pharmacy & Pharmacology*. 2020;8(3):205-218. **DOI:** 10.19163/2307-9266-2020-8-3-205-218

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

КОМПАРАТИВНЫЙ АНАЛИЗ РАЗВИТИЯ ЛИВАНА. ПЕРСПЕКТИВЫ СОТРУДНИЧЕСТВА С РОССИЙСКОЙ ФЕДЕРАЦИЕЙ

М.А.Э.Х. Эль Муссави[1,3], Ж.В. Мироненкова[1], С.З. Умаров[1], О.И. Кныш[2], О.Д. Немятых[1]

[1] ФГБОУ ВО «Санкт-Петербургский государственный химико-фармацевтический университет» Министерства здравоохранения Российской Федерации
197376, Россия, г. Санкт-Петербург, ул. Профессора Попова, д. 14, лит. А
[2] ФГБОУ ВО «Тюменский государственный медицинский университет» Министерства здравоохранения Российской Федерации
625023, Россия, г. Тюмень, ул. Одесская, д. 54
[3] Аптека «Мухаммед» г. Бейрут, Хадас, 12, Ливан, 0201

E-mail: shanna.mironenkova@pharminnotech.com

Получено 10.02.2020 Рецензия (1) 10.04.2020 Рецензия (2) 20.04.2020 Принята к печати 28.04.2020

Цель: провести компаративный анализ развития Ливана на основе ряда демографических, экономических и социальных показателей, характеризующих здравоохранение Ливана и определить перспективы сотрудничества с Российской Федерацией (РФ) в фармацевтической сфере.
Материалы и методы. Исследования проводились за период с 2009 по 2016 гг. Объектами явились статистические данные, аккумулируемые на основе данных национальных институтов и международных организаций, ежегодно публикуемые в отчетах Департамента по экономическим и социальным вопросам, Отдела народонаселения Организации Объединенных Наций по 11 странам Ближнего Востока: Бахрейн, Иордания, Йемен, Кувейт, Ливан, Объединенные Арабские Эмираты, Оман, Саудовская Аравия (страны Азии); Египет, Судан, Тунис (страны Северной Африки).
Методы исследований: компаративный анализ, аналитическая группировка данных, ранжирование.
Результаты. Компаративный анализ демографических, экономических и социальных показателей выявил, что низкий уровень смертности населения и высокий уровень продолжительности жизни в Ливане достигнуты как вследствие удовлетворительного уровня финансирования здравоохранения (ранг 5), так и вследствие принятия адекватных решений в организации и управлении в системе здравоохранения Ливана. Положительные тенденции, которые были заложены в системе здравоохранения Ливана в предыдущие десятилетия, продолжали действовать в рамках полученной ранее инерции при усилении миграционных потоков. Однако произошло замедление снижения показателей младенческой смертности в динамике темпов роста, что является сигналом возникновения негативных процессов в социальной сфере страны.
Заключение. Сложившаяся ситуация в системе здравоохранения Ливана, связанная с ограниченностью финансовых ресурсов, ставит новые задачи поиска управленческих решений в сфере организационного управления. Импорт лекарственных препаратов (ЛП) из РФ обеспечит существенное снижение финансовых затрат на лекарственное обеспечение населения Ливана и мигрантов, что позволит увеличить денежные затраты на обеспечение медицинской помощи.
Ключевые слова: демографические, экономические и социальные показатели; система здравоохранения; лекарственные препараты
Список сокращений: ВВП ППС – валовой внутренний продукт по паритету покупательной способности на душу населения; ЖНВЛП – жизненно необходимые и важнейшие лекарственные препараты; ЗАО – закрытое акционерное общество; ЛП – лекарственные препараты; ЛФ – лекарственная форма; МНН – международное непатентованное название; ОАО – открытое акционерное общество; ОАЭ – Объединенные Арабские Эмираты; ООН – Организация Объединенных наций; ООО – общество с ограниченной ответственностью; РУ – регистрационное удостоверение; США – Соединенные Штаты Америки; CIP Бейрут (carriage and insurance paid to Beirut) – фрахт/перевозка и страхование оплачены до г. Бейрут.

INTRODUCTION

A significant growth in the population of Lebanon, due to the increased flow of refugees from the border country of Syria, since the outbreak of the civil war (March 2011), from 4,145.57 thousand people to 6,071.69 thousand people from 2009 to 2016, had revealed the need to eliminate the lack of knowledge regarding the processes currently occurring in the country's health care, and the trends of its further development.[1,2,3]

THE AIM of the work is to conduct a comparative analysis of the development of Lebanon on the basis of a number of demographic, economic and social indicators that characterize the health of Lebanon and to determine the prospects for cooperation with the Russian Federation in the pharmaceutical field.

MATERIALS AND METHODS

The research was conducted from 2009 to 2016. The objects were statistical data accumulated on the basis of the data from national institutions and international organizations, published annually in the reports of the Department of economic and social Affairs and the United Nations population Division[4] for 11 countries in the Mid-

[1] Naufal, Hala. Syrian Refugees in Lebanon: The humanitarian approach under political divisions / Hala Naufal // Migration Policy Centre Research Report. – 2012/13. – URL: http://www.migrationpolicycentre.eu (accessed: 2019 Apr 26).
[2] Rating of countries in the world by population // the United Nations Population Fund (UNFPA). – URL: http://www.un.org (date accessed: 2018 Feb 5).
[3] National Health Statistics. Report in Lebanon. 2011. – URL: http://habitat3.org/wp-content/uploads/National-Report_LEBANON.pdf (accessed: 2019 Apr 24)
[4] The Department of economic and social Affairs of the United Nations. – URL: https://esa.un.org (date accessed: 2019 Apr 26).

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-205-218

dle East: Bahrain, Jordan, Kuwait, Lebanon, Oman, Saudi Arabia (Asian countries), Egypt, Sudan, Tunisia (North African countries), Yemen. The research methods were: a comparative analysis, analytical grouping of data, ranking.

RESULTS AND DISCUSSION

One of the main demographic indicators is the population size in a certain period of time (Fig. 1).

We found out that for the period of 2009–2016, the average population growth rate (relative to the base year of 2009) in Lebanon was 123.51% (Tab. 1).

Previously, in 2004–2009 (relative to the base year of 2004), they were significantly lower: the average value was 104.73%. The analysis of the population growth rate in Lebanon relative to the previous period showed that the average value in 2009–2016 was 105.61% (Table 2). For comparison, in 2004–2009 it was 101.34%.

Based on the United Nations data of the population, it was found out that among the 11 countries analyzed, the largest population (Rank 1) was in Egypt – 92519.54 thousand people (Table 3). It should be notified that Jordan had a close value to the population in Lebanon in the descending order of the indicator value in 2016 – 7734.38 thousand people (Rank 7). For all the countries analyzed in the region, there was a steady increase in the population, which is typical for the countries of the Middle East.

The interaction between the processes of renewal of new generations and the replacement of one generation by another ensures a continuous reproduction of the population. To characterize the social and demographic well-being of Lebanon and the degree of the development of its public health services, not only the basic and chain growth rates of the population were analyzed, the mortality rates of men, women, and infants separately as the indicators that more objectively reflect the level of development of the country's health care, were also taken into account.

In the analysis of the mortality rates of men and women it was found out that in Lebanon they were the lowest among the analyzed countries in the region, both in absolute and relative values (Rank 1), in the ascending order of the indicator value. The absolute values of mortality rates for men and women in Lebanon by 2016 had decreased simultaneously by 14.78% and 14.23% compared to 2009. The similar dynamics of the negative growth was observed when analyzing the chain growth rates of mortality of men and women during the analyzed period: on average, minus 2.26% and minus 2.17%, respectively.

In Lebanon, the negative dynamics of the chain growth rate of male mortality from 2009 to 2012 was stable, and it was minus 2.84% per year at an average. However, in the period from 2013 to 2016, the opposite trend was observed: in 2016, relative to 2015, the chain growth rate of male mortality increased from minus 2.84% (2009–2012) to minus 1.00%.

In general, analyzing the basic growth rates of male mortality, it was found out that in all the 11 countries of the Middle East in the period of 2009–2016, the negative dynamics remained: at an average, the values of the basic growth rates of male mortality decreased by 5.28%.

In the Middle East, the mortality rates of women also tended to decrease during the analyzed period. According to this indicator, Lebanon had Rank 1 again. Rank 2 was assigned to the United Arab Emirates, where these indicators were higher than in Lebanon by 8% and 12% in 2009 and 2016, respectively.

An important result of the analysis of the chain growth rates of women's mortality relative to the previous period is the following: as in the chain growth rates of men's mortality, the opposite trend was observed in Lebanon, i.e. the negative vector showed its slowdown. Under these circumstances, in 2016, relative to 2015, the chain growth rate of mortality increased to minus 2.10% (Rank 2) from minus 2.50% in the period of 2009–2012.

It should be notified that in all the analyzed countries, there was a dynamic decrease in women's mortality. The baseline rate of increase in women's mortality was negative at an average (minus 5.82%), which indicates an improvement in women's living conditions and advances in hygiene and health in the Arab countries in general.

Reduction in the children's mortality, including the infant mortality, is one of the main goals of the Millennium Declaration adopted by the UN General Assembly on 08.09.2000. (Resolution No. A / RES/52 / 2)[5]. It was found out that there was a tendency to reduce the infant mortality in the analyzed countries by 12.86%. The rate of its changing was negative at an average. The analysis of the infant mortality showed that Lebanon had Rank 3 after Bahrain and the United Arab Emirates. For example, in Bahrain (Rank 1), the indicator was 7.60 and 5.10 in 2009 and 2016, respectively. In the UAE, the indicator of 7.60 in 2009 decreased to 5.70 in 2016. Lebanon (Rank 3) had the infant mortality rate of 9.20 in 2009 and 7.60 in 2016. Rank 3 of Lebanon in terms of the basic growth rate of the infant mortality reduction, corresponds to the average value for the analyzed period. In 2016, there was a slowdown in the decline in the negative values of the basic growth rate of the infant mortality, lowering the rating of Lebanon to Rank 8. According to the chain growth rate of the indicator, Lebanon also had Rank 8 out of the 11 countries analyzed.

[5] Millennium Declaration adopted by the UN General Assembly on 08.09.2000 (Resolution No. A / RES/52/2). – URL: http://www.un.org. (date accessed: 2019.Apr 26)

RESEARCH ARTICLE
ISSN 2307-9266 e-ISSN 2413-2241

Table 1 – Basic growth rates (relative to 2009) of demographic and socio-economic indicators in Lebanon

Year	Total-population	Male mortality	Female mortality	Total mortality per 1,000 adults	Infant mortality per 1,000 live births	Birthrate	Life expectancy	GDP (PPP) per capita	Volume of health care expenditures per capita, Intern. dollar	Volume of expenditures on Medicines per capita, Intern. dollar	Share of drugs expenses in the structure of health care expenses, %	Number of doctors per 10,000 population	Number of pharmacists per 10,000 population
2009	100.00	100.00	100.00	100.00	100.00	100.00	100,00	100.00	100.00	100.00	100.00	100.00	100.00
2010	102.75	97.24	97.46	97.33	94.57	102.54	100.26	106.37	101.01	109.70	108.62	110.98	99.23
2011	107.69	94.47	94.93	94.66	90.22	105.92	100.64	104.53	99.67	117.18	117.58	108.11	101.19
2012	114.77	91.71	92.39	91.99	85.87	109.46	100.86	102.71	100.35	124.66	124.24	108.67	102.86
2013	123.16	89.84	90.79	90.23	82.61	112.59	101.09	99.65	95.72	139.12	145.36	111.02	104.81
2014	131.45	87.96	89.21	88.48	79.35	114.98	101.32	96.93	91.15	145.67	159.83	110.71	107.04
2015	138.25	86.08	87.61	86.71	77.17	116.49	101.53	94.09	96.01	152.42	158.77	103.01	102.37
2016	146.47	85.22	85.77	85.44	82.61	125.00	101.66	90.95	99.68	186.60	187.21	98.11	100.77
Average:	**123.51**	**90.36**	**91.16**	**90.69**	**84.63**	**112.43**	**101.05**	**99.32**	**97.66**	**139.34**	143.09	107.23	102.61

Table 2 – The growth rate of the chain (relative to the previous period) of demographic, socio-economic indicators in Lebanon

Year	Total population	Male mortality	Female mortality	Total mortality per 1,000 adults	Infant mortality per 1,000 live births	Birth-rate	Life expectancy	GDP (PPP) per capita	Volume of health care expenditures per capita, Intern. dollar	Volume of expenditures on Medicines per capita, Intern. dollar	Share of health care expenses in the structure of health care expenses, %	Number of doctors per 10,000 population	Number of pharmacists per 10,000 population
2009	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2010	102.75	97.24	97.46	97.33	94.57	102.54	100.26	106.37	101.01	109.70	108.62	110.98	99.23
2011	104.81	97.15	97.40	97.25	95.40	103.30	100.38	98.27	98.67	106.82	108.25	97.42	101.97
2012	106.58	97.08	97.33	97.18	95.18	103.34	100.22	98.26	100.68	106.38	105.66	100.52	101.65
2013	107.31	97.96	98.27	98.09	96.20	102.86	100.23	97.02	95.39	111.60	117.00	102.16	101.90
2014	106.74	97.91	98.26	98.06	96.05	102.12	100.23	97.27	95.23	104.71	109.95	99.72	102.13
2015	105.17	97.85	98.21	98.00	97.26	101.32	100.21	97.07	105.33	104.63	99.34	93.05	95.64
2016	105.94	99.00	97.90	98.54	107.04	107.30	100.13	96.66	103.82	122.43	117.91	95.24	98.43
Average:	**105.61**	**97.74**	**97.83**	**97.78**	**97.39**	**103.25**	**100.24**	**98.70**	**100.02**	**109.47**	109.53	99.87	100.14

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-205-218

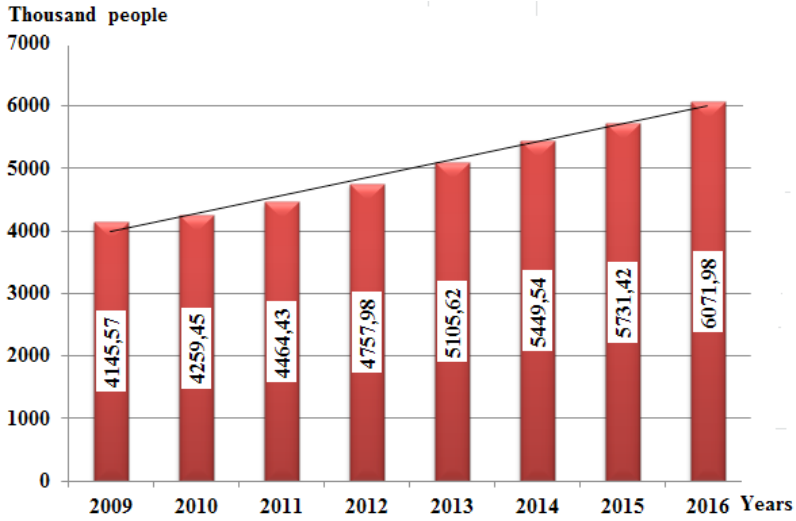

Figure 1 – The total population of Lebanon in 2009-2016, thousand people

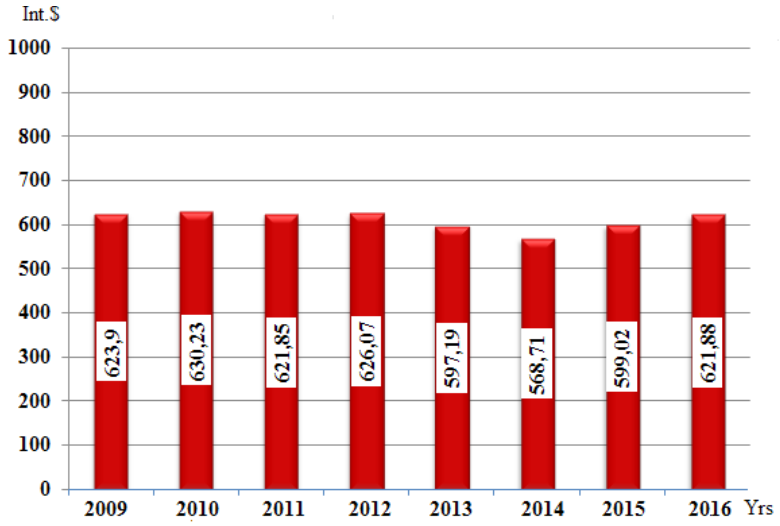

Figure 2 – Financial expenditures on health per capita in Lebanon in 2009–2016

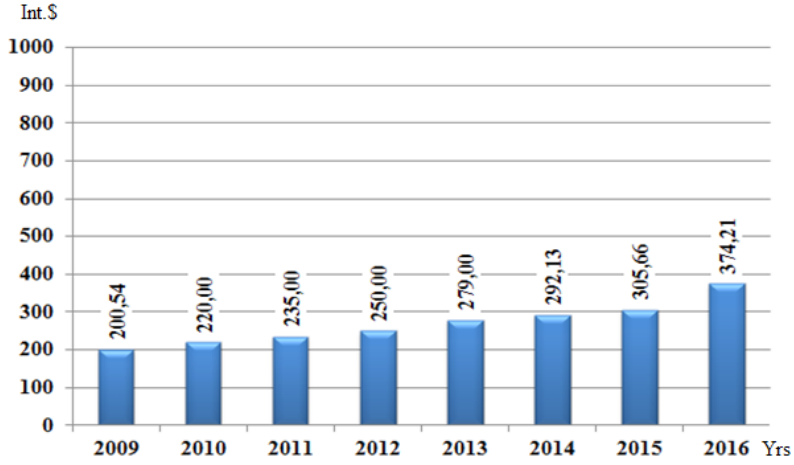

Figure 3 – Per capita expenditures on medicines in Lebanon in 2009–2016, in international dollars

Table 4 – Ranks of socio-economic indicators for the Middle East countries in 2009–2016

Country	Ranks of values						Ranks of base growth rates						Ranks of chain growth rates					
	GDP (PPP), per capita	Health care expenditure, per capita	Expenditure on Medicines, per capita	Share of drugs expenses in the structure of health care expenses,	Number of doctors per 10,000 population	Number of pharmacists per 10,000 population	GDP (PPP), per capita	Health care expenditure, per capita	Expenditure on Medicines, per capita	Share of drugs expenses in the structure of health care expens-es,per capita	Number of doctors per 10,000 population	Number of pharmacists per 10,000 population	GDP (PPP), per capita	Health care expenditure, per capita	Expenditure on Medicines, per capita	Share of drugs expenses in the structure of health care expens-es,per capita	Number of doctors per 10,000 population	Number of pharmacists per 10,000 population
Lebanon	6	5	5	3	5	3	8	10	1	1	6	11	9	11	1	1	10	11
UAE	2	1	1	1	7	5	5	6	5	4	7	6	4	8	5	4	6	6
Bahrain	5	3	6	4	9	9	4	2	7	10	10	2	3	2	7	10	9	3
Kuwait	1	2	3	10	4	8	9	11	3	2	2	10	8	7	3	2	3	10
Saudi Arabia	3	4	4	6	2	6	1	1	6	11	4	8	1	1	6	11	4	8
Oman	4	6	2	11	6	4	10	4	2	8	9	9	10	4	2	9	7	4
Tunisia	8	8	7	8	8	7	3	5	11	9	3	1	5	9	11	7	2	2
Jordan	9	7	9	7	1	2	6	9	4	3	5	7	7	10	4	3	5	9
Egypt	7	9	8	2	3	1	2	3	10	7	11	4	2	3	10	5	11	7
Yemen	11	11	11	5	10	10	11	7	8	5	8	3	11	6	8	6	8	5
Sudan	10	10	10	9	11	11	7	8	9	6	1	5	6	5	9	8	1	1

Table 3 – Ranks of demographic indicators for the Middle East countries in 2009–2016

Country	The ranks of values						Ranks of base growth rates						Ranks of chain growth rates					
	Total population in 2016	Male mortality	Female mortality	Total mortality per 1,000 adults	Birthrate	Average life expectancy	Total population in 2016	Male mortality	Female mortality	Total mortality per 1,000 adults	Birthrate	Average life expectancy	Total population in 2016	Male mortality	Female mortality	Total mortality per 1,000 adults	Birthrate	Average life expectancy
Lebanon	8	1	1	3	10	1	2	1	1	3	1	5	2	1	1	8	1	6
UAE	6	3	2	2	11	2	4	5	4	5	11	8	4	2	2	4	11	9
Bahrain	11	2	4	1	9	4	6	6	6	11	8	10	6	6	4	1	7	10
Kuwait	10	4	3	4	8	6	3	11	8	6	10	9	3	11	7	3	10	8
Saudi Arabia	3	5	5	7	5	7	8	4	5	9	9	4	8	5	6	10	8	2
Oman	9	8	7.5	5	6	3	1	10	10	11	7	3	1	9.5	9.5	11	9	3
Tunisia	5	7	6	6	7	5	11	2	11	4	3	11	11	3	11	6	3	11
Jordan	7	6	7.5	8	3	8	5	9	10	8	4	7	5	9.5	9.5	7	4	5
Egypt	1	9	9	9	4	9	10	7	2	7	2	6	10	7	3	5	2	7
Yemen	4	10	10	10	2	10	7	8	7	2	6	2	7	8	8	2	6	4
Sudan	2	11	11	11	1	11	9	3	3	10	5	1	9	4	5	9	5	1

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-205-218

The negative dynamics of the chain growth rates of men's and women's mortality since 2013 has slowed down, which is a signal of the presence of negative processes in the social sphere of Lebanon. This signal is also seen in infant mortality rates. The process of a slow decline in infant mortality rates reflected not only on the dynamics of the chain growth relative to the previous period, but also on the dynamics of the basic growth relative to 2009.

In terms of the birth rate, Lebanon experienced an 18.03% growth trend from 2009 to 2016. The base and chain growth rates of this indicator were significant: 25.00% and 3.25%, respectively. Our ranking, calculated in the descending order by birth rate, showed that Lebanon had Rank 10. Bahrain and the United Arab Emirates had similar ranks. It should be notified that, in contrast to Lebanon, the birth rate in these two countries tended to decrease.

When analyzing the basic rate of birth rate growth, it was found out that Lebanon (Rank 1) was the only country among the 11 analyzed countries that had a stable increase in the birth rate of the population: in 2016, compared to 2009, by +25.00%. For example, in Kuwait (Rank 10), it was minus 10.59%; in the United Arab Emirates (Rank 11), it was minus 12.40%. It should be emphasized that the average chain growth rate of fertility was positive only in two countries: Lebanon and Egypt (the average values of 103.25% and 100.34%, respectively).

All the other countries of the analyzed statistics population had negative chain growth rates in the birth rate of the population.

The study found out the following: having low absolute birth rates (Rank 10), Lebanon had Rank 1 in terms of basic and chain growth rates, which indicates the absence of a birth control policy. On the whole, the analyzed countries showed a general decline in the rate of birth rate growth. In terms of life expectancy, Lebanon had Rank 1, with an average life expectancy of 79.02 years in the analyzed period. For example, in the UAE it was 77.07 years (Rank 2). Life expectancy in Lebanon increased by 1.4 years from 78.30 years in 2009 up to 79.60 years in 2016, while in the UAE, this figure increased by less than 1 year.

Therefore, an analysis of the economic indicators has also been carried out. In particular, the gross domestic product by purchasing power parity per capita (GDP PPP) was considered6. It should be notified that this indicator has a multidirectional character in different countries. In most countries, the dynamics were positive. From 2009 to 2016, the negative dynamics were observed annually in only four countries: Lebanon, Kuwait, Oman and Yemen. The average decline was 17.36%. At the same time, in Oman, Kuwait and Yemen in 2016 compared to 2014 and 2015, there was stabilization in the decline of this indicator, while in Lebanon the slowdown in the growth rate of this indicator increased (Table 1). Thus, while in 2013 and 2014 the indicator of basic growth rates decreased by 0.35% and 3.07%, in 2015 and 2016 it decreased by 5.91% and 9.05%, respectively. A more complex situation in the dynamics of chain growth rates (Rank 9, Table 4) should be highlighted. While in 2013–2015, the GDP PPP indicator was relatively stable at minus 2.93% in Lebanon, in 2016 there was a decrease of 0.41% compared to 2015 and it amounted to minus 3.34%. The analysis of GDP PPP dynamics in 2009–2016, revealed the existence of problems in the economy of Lebanon, and their impact on the development of the health care system was considered on this basis. An important condition that determines a positive demographic situation is the development of the health sector in the country and training of specialists in the fields of medicine and pharmacy. An analysis of financial expenditure on health showed that in Lebanon, it had increased by 45.99% between 2009 and 2016.

While in 2013–2015, the GDP PPP indicator in Lebanon was relatively stable at minus 2.93%, in 2016 there was its decrease by 0.41% compared to 2015 and it amounted to minus 3.34%. The analysis of the dynamics of GDP PPP during 2009–2016 revealed the presence of problems in the economy of Lebanon, on the basis of which their impact on the development of the health care system was considered. An important condition that determines a positive demographic situation is the development of the health sector in the country and training of specialists in the fields of medicine and pharmacy. An analysis of financial expenditure on health showed that in Lebanon, it had increased by 45.99% between 2009 and 2016

It should be notified that the volume of health care expenditures per capita did not undergo any significant changes in 2016 compared to 2009 due to an increase in the population by 46.47% (Fig. 2).

A comparative analysis of per capita health expenditures showed that Lebanon had Rank 5 (Tab.4). The volume of expenditures, while decreasing in 2013–2015, stabilized in 2016, slightly decreasing by 0.32% compared to 2009. The basic growth rate of health financing was stagnant (Rank 10). It is important to increase the volume of the expenditures on medicines per capita during the analyzed period (Fig. 3).

When analyzing the structure of the expenditures in the health sector in Lebanon, significant changes were found out: the share of expenditures on health care increased from 32.14% in 2009 to 60.17% in 2016, thereby reducing the expenditures on general health items.

[6] Global ranking of countries and territories of the world in terms of gross domestic product // Information and analytical portal "Humanitarian technologies and human development. URL: http://gtmarket.ru (date accessed:2019 Apr 24).

The conducted correlation analysis between this indicator and male, female and infant mortalities revealed a correlation, which, at first glance, is a contradictory situation: the decrease in health care funding had a positive impact on the reduction of male, female and infant mortalities: the correlation coefficient was 0.930.

However, at the stage of analyzing the chain growth rates of male, female and infant mortalities, there was no correlation: the correlation coefficient was minus 0.078. Accordingly, the inconsistency of the situation is explained by the difference in the rate of decline of the correlated parameters. The mechanism for reducing male, female and infant mortalities is more conservative, and the positive trends that had been laid down in the Lebanese health system in previous decades, continued to operate within the framework of the previously received inertia with reduced funding. The situation in which the chain growth rates in terms of mortality rates no longer correlated with funding indicators clearly showed a slowdown in the decline in mortality rates among the analyzed groups of the Lebanese population.

Further on, the hypothesis on the possibility of finding additional financial resources in the health care system by optimizing current expenses through the purchase of inexpensive medicines in the new for Lebanon dynamically developing pharmaceutical market of the Russian Federation was tested by the authors [7]. The imports of goods from the Pharmaceutical products group to Lebanon from the Russian Federation during the period of December 2017 – October 2018 amounted to 1.1 million US dollars, with a total weight of 14.6 tons.

The mainly imported products were the following: “human blood”; “animal blood”; “immune serums” (91%) and “Pharmaceutical products mentioned in Note 4 to this group” (9%), which include chemical contraceptives based on hormones, other compounds of the heading “Hormones, prostaglandins, thromboxane and leukotrienes, natural or synthesized; their derivatives and structural analogues, including chain modified polypeptides used mainly as hormones” or spermicides; contrast agents for x-ray examinations; diagnostic reagents intended for introduction to patients, and others.

In the structure of the exports of goods from the group “Pharmaceutical products” from the Russian Federation, Ukraine and Kazakhstan occupy the 1st (16%) and the 2nd (16%) places. Lebanon is Russia’s partner number 42 with a 0.2% share of all MP supplies.

The cooperation between the Russian Federation and Lebanon has a significant potential [8–10]. The implementation of joint projects in the pharmaceutical industry and trade will expand the cooperation between the countries [11–19]. The interest of the Russian Federation in the export of the MP according to Strategy of the Pharmaceutical Provision of the Population of the Russian Federation for the Period Until 2025 is the key to this mutually beneficial cooperation and expanding economic ties between Russia and Lebanon.

To study the economic feasibility of introducing a mechanism for purchasing medicines from the Russian Federation, wholesale prices according to the reference and analytical publication of “Farm index” (for medical and pharmaceutical specialists) in the segment of vital and essential drugs were considered. The analysis was performed for 31 international non-proprietary names produced in the Russian Federation from Russian substances and imported by Lebanon from other countries. The comparative analysis of wholesale prices was based on the calculation of the cost of 1 gram of MPs under the terms of delivery of CIP Beirut (carriage and insurance paid to Beirut). The terms of delivery included: cargo packing, customs clearance, delivery to the port of loading, loading on the ship, sea transportation, unloading from the ship in the port of Beirut, delivery to the destination, insurance.

For a better visual representation, the VEDs nomenclature was presented in the form of 4 quartiles, depending on the price ratio (Lebanon/Russia): quartile I – 7.6–10.0 and higher ranked quartiles; quartile II-5.1-7.5; quartile III – 2.6–5.0; quartile IV-2.5 and lower ranked quartiles. The carried out ranking showed that at the time of the study, the current prices of Lebanon for all positions exceeded the book prices of the Russian Federation. The following was established: the highest rank, No. 1, when the ratio of prices amounted to 11, 21, was appointed to Olanzapine, 10 mg film-coated tablets (according to the Russian Commodity Nomenclature it is Olanzapine-TL, in Lebanon it is Zyprexa).

The lowest rank was 31, wherein the minimum ratio of the price index was 1.13. It was represented by Gemcitabine, 200 mg, in the form of lyophilisate for preparation of infusion solutions (according to the Russian Commodity Nomenclature it is Gemzar®, in Lebanon it is Gemcitabine™).

A comparative analysis of the prices revealed that the MPs purchased in Lebanon in this segment, have an average of 4.54 times the price of MPs with the corresponding international non-proprietory name in the Russian Federation, taking into account the delivery to the port of Beirut.

At the same time, the calculations showed a high economic efficiency of importing 35.48% (11 nomenclature items) of medicinal products that had been included in quartiles I and II, from the Russian Federation, which showed the possibility of improving the drug supply in Lebanon by reducing the financial costs of purchasing medicinal products at the country level (Tab. 5).

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-205-218

Table 5 – Ranking of the ratio of wholesale prices for VEDs in the Russian Federation and Lebanon based on the cost of 1 gram of Medicines on the terms of CIP delivery to Beirut

RANK	INN	Trade name in Lebanon	Trade name in the Russian Federation	Manufacturer in Russia	Type of Dosage form	Dosage	Price ratio (Lebanon/ Russia)
Quartile I							
1.	Olanzapine	Zyprexa™	Olanzapine-TL	"Drugs technology", Rabochaya St., Himki, Moscow region	Film-coated tablets	10 mg	11.21
2.	Ceftriaxone	Ceftriaxone Panpharma®	Ceftriaxon – AKOS	"Sintez Pharmaceuticals", Kurgan	Powder for solution preparation for I.M. and I.V. administration	1g	9.88
3.	Olanzapine	Zyprexa™	Olanzapine-TL	"Drugs technology", Himki, Moscow region	Film-coated tablets	5 mg	9.22
4.	Ceftriaxone	Ceftriaxon Labatec®	Ceftriaxon – AKOS	"Sintez Pharmaceuticals", Kurgan	Powder for solution preparation for I.M. and I.V. administration	2 g	8.98
5.	Levofloxacin	Levofloxacin Hameln	Leflobact	"Sintez Pharmaceuticals", Kurgan	Infusion solutions	5 mg/ml	8.63
			Levofloxabol	"Abolmed" Company, Novosibirsk			
			Levofloxacin	JSC "Kraspharma", Krasnoyarsk			
Quartile II							
6.	Bortezomib	Velcade®	Boramilan	"Nativa", vil. Petrovo-Dal'neye, Moscow region 143422	Lyophilized for lyophilisate for preparation of I.V. and subcutaneous infusion solutions	3.5 mg	7.34
7.	Simvastatin	Simvastatin-Remedica®	Simvastatin	Valenta Pharm, Shchelkovo	Film-coated tablets	40 mg	7.23
			Simvastatin- SZ	"Severnaya Zvezda" CJSC, Vsevolozhsky district, the town of Kuzmolovsky, Leningrad region,			
			Simvastatin	"Ozon", Zhigulevsk (holder of reg. / UD. LLC «Atoll»)			
8.	Cefotaxime	Cefotaxime Panpharma®	Cefosin	"Sintez Pharmaceuticals", Kurgan	Powder for solution preparation for I.M. and I.V. administration	1 g	7.05
			CEFOTAXIME	DEKO Company LLC, Tver region, p. Zelenogorsky			
			Cefabol	Company «Abolmed», Novosibirsk			
			Cefotaxime	CJSC «Pharmaceutical company «LECCO», The Vladimir region, p. Volginsky			

RANK	INN	Trade name in Lebanon	Trade name in the Russian Federation	Manufacturer in Russia	Type of Dosage form	Dosage	Price ratio (Lebanon/ Russia)
9.	Gentamicin	Gentamicine Panpharma®	Gentamycine	JSC "MosChimPharmPreparaty" n.a. N.A. Semashko», Moscow	Solutions for for I.M. and I.V. administration	40 mg/ml	6.11
				"Atompharm", Vashutinsk highway, Himki, Moscow region Russia			
10.	Metronidazole	Metronidazole®	Metronidazole – AKOS	"Sintez Pharmaceuticals", Kurgan	Solution for infusions	5 mg/ml	5.49
			Metronidal	«Abolmed» Company, Novosibirsk			
			Metronidazole	"Dalkhimpharm", 22, Tashkent St, Khabarovsk,			
11.	Cefazolin	Cefazolin Inj.	Cefazoline -AKOS	Sintez Pharmaceuticals, Kurgan	Powder for preparation of solution for intravenous and intramuscular administration	1 g	5.20
			Cefazoline	"Redkinsky Experimental Plant", Zavodskaya St., vil. Redkino, Tver region.,			
				"DEKO Company" LLC, Vil. Zelenogorsky, Tver region.			
				"Biochimic", Saransk			
Quartile III							
12.	Vancomycin	Vancomycin Hikma®	Vancorus	"Sintez Pharmaceuticals", Kurgan	Lyophilisate for preparation of solutions for infusions and oral administration	500 mg	4.98
13.	Vancomycin	Vancomycin Hikma®	Vancorus	"Sintez Pharmaceuticals", Kurgan	Lyophilisate for preparation of solutions for infusions and oral administration	1000 mg	4.50
14.	Tamoxifen	Tamoxifen Ebewe	Tamoxifen	"Ozon pharmaceutical", Zhigulevsk	Tablets	10 mg	4.09
			Tamoxifen citrate	JSC" Obolenskoe – the pharmaceutical enterprise"			
15.	Cefepime	Cefepime Panpharma®	Maxicef	"Prebend Production and Pharmaceutical company", Novosibirsk	Powder for preparation of solution for I.M. and I.V. administration	1 g	4.02
16,5.	Tamoxifen	Tamoxifen Ebewe	Tamoxifen	"Ozon pharmaceuticals", Zhigulevsk	Tablets	20 mg	3.25

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-205-218

RANK	INN	Trade name in Lebanon	Trade name in the Russian Federation	Manufacturer in Russia	Type of Dosage form	Dosage	Price ratio (Lebanon/ Russia)
16,5.	Ketolac	Ketolac®	Ketorolac	FSUE SPC "Parmzashchita" FMBA of Russia, Khimki	Powder for preparation of solutions for I.M. and I.V. administration	30 mg/ml	3.25
				"Ozon Pharmaceuticals"- Himki, Russia			
				"Ellara", Pokrov			
				JSC «Biosynthesis», Penza			
				JSC "MosChimPharmPreparaty" n.a. N.A. Semashko», Moscow			
				JSC "Kurgan Joint-Stock company of medical preparations and products "Sintez", Kurgan			
			Dolomine	CJSC «FarmFirma "Sotex", Moscow region, vil. Belikovo			
			KETALGIN	JSC "Pharmstandard-Ufa VITA", Ufa			
18.	Paroxetin	Apo-Paroxetine®	Adepress	OJSC "Veropharm", Belgorod	Film-coated tablets	20 mg	2.90
19.	Clarithromycin	Klacid RM®	Arvicin	JSC "Obolenskoye", Obolensk, Moscow region,	Film-coated tablets	500 mg	2.84
			Clarithromycin	"Ozon pharmaceuticals", Zhigulevsk			
			Ecozitrin	JSC, Avva Rus, Kirov			
20.	Acetylsalicylic acid	Aspirin Protect	Aspinat	JSC "Valenta Pharm", Shchelkovo	Tablets	100 mg	2.80
			Aspicor	JSC "VERTEX",St Petersburg	Tablets coated with an intestinal-soluble film coating		
			Sanovasc	JSC "Irbit Chemical and Pharmaceutical Plant", Irbit			
			Quartile IV				
21.	Topiramate	Topamax®	Topiramate TL	LLC "Technology of Medicines", Himki	Film-coated tablets	100 mg	2.52
22.	Ketorolac	Ketolac®	Ketolorac	JSC "TatChemPharmPreparaty" Kazan	Film-coated tablets	10 mg	2.42
				JSC "Vertex", St Petersburg			
				"Sintez Pharmaceuticals", Kurgan			
				JSC "PFC "Update", Novosibirsk			
			Ketalgin	OJSC "Pharmstandard-Leksredstva", Kursk			
			Ketorolac-OBL	"Obolenskoye", vil. Obolensk, Moscow region			

RANK	INN	Trade name in Lebanon	Trade name in the Russian Federation	Manufacturer in Russia	Type of Dosage form	Dosage	Price ratio (Lebanon/ Russia)
23.	Irinotecan	Irinotecan Ebewe	Irinotecan	JSC "BIOCAD", vil. Petrovo-Dal'neye	Concentrate for preparation of infusion solutions	20 mg/ml	2.03
24.	Leflunomide	Arava®	Ralef	OOO "Evofarm", vil. Obolensk	Film-coated tablets	20 mg	1.96
			Leflaid	LLC "Technology of medicines", Himki			
25.	Cefuroxime	Cefuroxime – Panpharma®	Cefurus	"Sintez Pharmaceuticals", Kurgan	Powder for preparation of solution for I.M. and I.V. administration	1500 mg	1.68
26.						750 mg	1.63
27.	Topiramate	Topamax 25®	Topimate TL	LLC "Technology of medicines", Himki	Film-coated tablets	25 mg	1.58
28.	Acetylsalicylic acid	Aspirine	Aspinat 300	JSC "Valenta Pharmaceuticals", Shchelkovo	Tablets coated with an intestinal-soluble coating	300 mg	1.53
29.	Simvastatin	Simvastatin-Remedica®	Ovenkor	"Ozon pharmaceuticals", Zhigulevsk	Film-coated tablets	20 mg	1.36
			Simvastatin SZ	"Severnaya Zvezda" CJSC, vil. Kuzmolovsky, Vsevolozhsky district, Leningrad region			
			Simvastatin	JSC "Valenta Pharmaceuticals", Shchelkovo			
				CJSC «ALSI Pharma», Kirov			
				JSC "Vertex", St Petersburg			
				JSC "AVVA RUS", Kirov			
30.	Allopurinol	Apo-Allopurinol®	Allopurinol	JSC "Organica", Novokuznetsk	Tablets	100 mg	1.27
31.	Gemcitabine	Gemcitabine®	Gemcitare	JSC "BIOCAD", Vil. Petrovo-Dal'neye	Lyophilisate for preparation of infusion solutions	200 mg	1.13
				Average 4,54			

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-3-205-218

CONCLUSION

A correlation analysis of demographic and economic indicators in Lebanon, revealed a satisfactory level of health financing (Rank 5) and making adequate decisions in the organization and management of the Lebanese health system which resulted in the low mortality rate and a high life expectancy. The positive trends that had been laid down in the Lebanese health system in previous decades continued to operate within the framework of the inertia received before, with reduced funding. It should be emphasized that the number of doctors and pharmaceutical specialists (on average 21.50 and 14.67 per 10,000 population, respectively) is fairly stable in Lebanon, which ensures the effectiveness and sustainability of the health system, especially in times of crises and wars. However, due to the limited financial resources of the country, the current situation in the health care system poses new challenges in finding management solutions in the field of organizational management. One of them is to optimize current expenses by purchasing low-cost medicines in new and dynamically developing pharmaceutical markets in Lebanon. Import of medicinal products from the Russian Federation will significantly reduce the cost of medical CARE for the population of Lebanon and migrants from the neighboring countries, which will increase the costs of providing medical care to the population.

FINANCIAL SUPPORT

This study did not have any financial support from third-party organizations.

AUTHOR'S CONTRIBUTION

El Moussawi MAEH – collection and processing of the materials, statistical data processing, text writing; **Mironenkova Zh.V.** – statistical data processing, text writing, editing; **Umarov S.Z.** – a research concept and design, editing; **Knysh O.I.** – a research concept and design, editing; **Nemyatykh O.D.** – text writing, editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Ledov, DG, Bogdanov Yu. Migration crisis in Lebanon. Humanitarian scientific research. 2016; 9(61):188–190.
2. Ammar W, Kdouh O, Hammoud R, Hamadeh R, Harb H, Ammar Z, Atun R, Christiani D, Zalloua PA. Health system resilience: Lebanon and the Syrian refugee crisis. J Glob Health. 2016;6(2):020704. DOI: 10.7189/jogh.06.020704.
3. Fotoeva AV. The Main article of individual manufacturers as a component of drug safety pages. Bulletin Of Roszdravnadzor. 2017;5:55–58.
4. Trofimova, EO, Denisova MN. Russian pharmaceutical market: position of domestic companies. Pharmacy. 2018; 67(1):3–7. DOI: 10.29296/25419218-2018-01-01.
5. Mauskopf JA, Sullivan SD, Annemans L, Caro J, Mullins CD, Nuijten M, Orlewska E, Watkins J, Trueman P. Principles of good practice for budget impact analysis: report of the ISPOR Task Force on good research practices –budget impact analysis. Value Health. 2007;10(5):336–47. DOI: 10.1111/j.1524-4733.2007.00187.x.
6. Marchenko YuO. Improving the competitiveness of Russian companies in the global pharmaceutical market based on the use of import substitution tools. Fundamental and applied research: problems and results. 2015; 21:173–187.
7. Sazava NN. The current state, conditions and prospects of development of world pharmaceutical market. Russian foreign economic Bulletin.2015;12:118–129.
8. Trofimova, EO. Macroeconomic factors and financing of the healthcare system as prerequisites for the development of the pharmaceutical market. Remedium. Magazine about the Russian market of medicines and medical equipment. 2017;S13:22–34.
9. Lin, AA, Sokolova SV, Golant ZM. Pharmaceutical market: a model for supporting the export of medicines. Problems of the modern economy. 2016;1 (57):162–166.
10. Esangina, IA, Yushkov ES. analysis of the current state of the pharmaceutical market of Russia. Economic research and development: scientific research electronic journal. Nizhny Novgorod: NOO «Professional science». 2018;6:91–97.
11. Bahlol, MM, Lagutkina TP. Analysis of scientific research on the promotion of pharmaceutical products in foreign countries: practical recommendations for pharmaceutical companies. Bulletin of Siberian medicine. 2016;15(1):60–68. DOI: 10.20538/1682-0363-2016-1-60-68.
12. Sapir EV, Karachev IA Features of the world pharmaceutical market and problems of its development by Russian companies. Russian foreign economic Bulletin. 2016;8:97–111.
13. Andreeva EL, Sapir EV, Karkh DA, Karachev IA. Comparative analysis of foreign economic development of the pharmaceutical sector in the Russian Federation and the USA. Regional Economy. 2019;15(2):576–589. DOI 10.17059 / 2019-2-20.
14. Demidenok DA, Petrova TA, Narkevich IA, Markova VA. Trends in the global pharmaceutical market: unrealized development opportunities. Development and registration of medicines. 2017;4(21):282–287.
15. Filatova, YuM, Romanova LV, Larikova II. Current state of the world pharmaceutical market. Proceedings of the Tula state University. The Economic Series.2016;2:167–174.
16. Sapir EV, Karachev IA, Zhang M. Export potential of Russian pharmaceutical enterprises in emerging regional clusters. Regional economy.2016;12(4):1194–1204.
17. Mironenkova ZhV, Moussawi ME, Davletyanova AF. Main characteristics of ART in HIV-infected citizens of the Lebanese Republic. Journal of Infectology.2017; 9(3):109–116. DOI: 10.22625 / 2072-6732-2017-9-3-109-116.

18. Karachev IA. Development of the Russian pharmaceutical market at the present stage. Bulletin of the Samara state University of Economics. 2016; 8(142):71–77.
19. Ryzhova, OA, Moroz TL. Results of the analysis of import substitution of antitumor drugs in the Russian Federation for 2013-2018. Pharmacy and pharmacology.2019;7(2):105–111. DOI: 10.19163/2307-9266-2019-7-2-105-111.

AUTHORS

El Moussawi Mohamad Abd El Hussein – candidate of the Department of medical and pharmaceutical commodity science of St Petersburg State University of Chemical and Pharmaceutical Sciences; the head of the Pharmacy "Mohammed", 12, Hadath, Beirut, Lebanon 90201.ORCID ID: 0000-0001-5432-7680. E-mail: drmohamadmoussawi@hotmail.com

Zhanna V. Mironenkova – Doctor of Sciences (Pharmacy), Associate Professor, Professor of the Department of medical and pharmaceutical commodity science of St Petersburg State Chemical and Pharmaceutical University. ORCID ID: 0000-0003-1029-093X. E-mail: shanna.mironenkova@ pharminnotech.com

Sergey Z. Umarov – Doctor of Sciences (Pharmacy), Professor, the Head of the Department of Medical and Pharmaceutical Commodity Science of St Petersburg State Chemical and Pharmaceutical University. ORCID ID: 0000-0003-0771-6143.E-mail: sergei.umarov@pharminnotech.com

Olga I. Knysh – Doctor of Sciences (Pharmacy), Professor, the Head of the Department of Pharmaceutical Disciplines of Tyumen State Medical University. ORCID ID: 0000-0001-6150-1683. E-mail: knysho@mail.ru

Oksana D. Nemyatykh – Doctor of Sciences (Pharmacy), Professor, Professor of the Department of Pharmacy Management and Economics of St Petersburg State Chemical and Pharmaceutical University. ORCID ID: 0000-0001-5933-2120. E-mail: oksana.nemyatyh@ pharminnotech.com