Научно-практический журнал
Scientific and Practical Journal

ISSN 2307-9266
e-ISSN 2413-2241

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

PHARMACY & PHARMACOLOGY

2020 Том / Volume VIII

№ 5

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Scientific and Practical Journal

PHARMACY & PHARMACOLOGY

Scientific and practical journal
Volume VIII, Issue 5, 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 5, 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

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ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

MUMPS: ACHIEVEMENTS, PROBLEMS AND WAYS OF SOLUTION

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Received 01 Oct 2020 Accepted 25 Oct 2020

The aim. The article highlights the current state of the problem of mumps in the world and the Russian Federation.
Materials and methods. The materials of the study were electronic resources WHO infection control, Cohrane, Elsevier, ScienceDirect, CDC infection diseases database, PubMed, eLibrary, CyberLeninka. The research methods were the analysis and generalization of scientific literature. The assessment is presented by the immunological structure of the population in different age groups to mumps (n = 593) in the study area (2018) according to the data of the Center for Hygiene and Epidemiology in the Perm Territory.
Results. The spread of mumps is found to be widespread and uneven in different regions of the world in the form of sporadic cases and large epidemic outbreaks, despite the world practice of vaccine prevention of mumps. Analysis of the immunological structure to mumps in different age groups revealed a fairly high number of seronegative individuals (the largest number was found among adults aged 20–39 years) in the study area (2018). A decrease in the tension of post-vaccination immunity is the main cause for the emergence of an outbreak among the adult population, in addition to vaccination failures among vaccinated children. The immune defenses created by the vaccine strain do not have the same intensity and duration as with natural infection, and some genotypes of "wild" variants of the mumps virus can break through the immune barrier and cause disease. Antigenic differences between vaccine and circulating strains, low inoculation dose can weaken immunity and reduce the effectiveness of mass vaccine prevention.
Conclusion. Ways of solving the problem were proposed to forestall an unfavorable epidemic situation with mumps.
Keywords: mumps; morbidity; diagnostics; vaccine prevention; circulating genotypes
Abbreviations: WHO – World Health Organization; RT-PCR – reverse transcription polymerase chain reaction; TDC – tissue cytopathogenic dose

ЭПИДЕМИЧЕСКИЙ ПАРОТИТ: ДОСТИЖЕНИЯ, ПРОБЛЕМЫ И ПУТИ РЕШЕНИЯ

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Получено 01.10.2020 Принята к печати 25.10.2020

Цель. Анализ современного состояния проблемы эпидемического паротита в мире и Российской Федерации.
Материалы и методы. В качестве материалов исследования использованы электронные ресурсы WHO infection

For citation: V.V. Semerikov, N.V. Yuminova, N.O. Postanogova, L.V. Sofronova. Mumps: achievements, problems and ways of solution. *Pharmacy & Pharmacology.* 2020;8(4):222-232. **DOI:** 10.19163/2307-9266-2020-8-4-222-232

Для цитирования: В.В. Семериков, Н.В. Юминова, Н.О. Постаногова, Л.В. Софронова. Эпидемический паротит: достижения, проблемы и пути решения. *Фармация и фармакология.* 2020;8(4):222-232. **DOI:** 10.19163/2307-9266-2020-8-4-222-232

DOI: 10.19163/2307-9266-2020-8-5-296-303

control, Cohrane, Elsevier, ScienceDirect, CDC infection diseases database, PubMed, eLIBRARY, CyberLeninka. Методы исследования – анализ и обобщение научной литературы. Дана оценка иммунологической структуры населения в различных возрастных группах к эпидемическому паротиту (n=593) на изучаемой территории (2018 г.) по данным ФБУЗ «Центр гигиены и эпидемиологии в Пермском крае».

Результаты. Несмотря на мировую практику вакцинопрофилактики эпидемического паротита установлено повсеместное и неравномерное распространение паротитной инфекции в различных регионах мира, как в виде спорадических случаев, так и крупных эпидемических вспышек. Оценка иммунологической структуры в различных возрастных группах к эпидемическому паротиту на изучаемой территории (2018) выявила достаточно высокое число серонегативных лиц (наибольшее число установлено среди взрослых в возрасте 20–39 лет). Помимо вакцинальных неудач среди привитых детей, главной причиной возникновения вспышечной заболеваемости среди взрослого населения является снижение напряженности поствакцинального иммунитета. Иммунная защита, создаваемая вакцинным штаммом, не имеет такой напряженности и такой длительности, как при естественной инфекции, и некоторые генотипы «диких» вариантов вируса эпидемического паротита могут прорывать иммунный барьер, вызывая заболевание. Антигенные различия между вакцинными и циркулирующими штаммами, низкая прививочная доза могут способствовать ослаблению иммунитета и снижению эффективности массовой вакцинопрофилактики.

Заключение. Предложены пути решения для упреждения неблагополучной эпидемической ситуации по эпидемическому паротиту.

Ключевые слова. Эпидемический паротит, заболеваемость, диагностика, вакцинопрофилактика, циркулирующие генотипы

Список сокращений: ВОЗ – Всемирная организация здравоохранения; ОТ-ПЦР – обратно-транскриптазная полимеразная цепная реакция**;** ТДЦ – тканевая цитопатогенная доза; ЭП – эпидемический паротит.

INTRODUCTION

Mumps continues to attract the attention of scientists and practitioners around the world for its epidemiological, social and economic significance in the second decade of the 21st century. Mumps is widespread, but unevenly in different regions of the world: in Europe, the Eastern Mediterranean, Southeast Asia, Africa, America and the Western Pacific [1, 2]. Large outbreaks took place in the USA (2006, 2014, 2017 years) with 6585, 1521, 5629 victims, Australia (2015–2016) – 893, Belgium (2012-2013) – 4061, Israel (2014–2015) – 262, Jerusalem (2009–2011) – 3130, Poland (2013) – 2436, Czech Republic (2005–2006) – 5998, Austria (2006) – 214, Norway (2015–2016) – 232, Scotland (2014–2015) – 341, Canada (2016) with 1242 cases and others [3–11].

The glandular organs are affected in mumps (mumps, submandibulitis, sublinguitis, pancreatitis, orchitis, prostatitis, oophoritis – in 5% of cases in girls, mastitis – in 31% of cases in girls over 14 years old, thyroiditis, dacryoadenitis), and severe conditions may develop – serous meningitis and meningoencephalitis, myelitis and encephalomyelitis, damage to the cranial nerves due to prolonged circulation of the pathogen in the blood. Mumps can lead to residual consequences of damage to the central nervous system, can form infertility in men (in 50% of cases over 25) and secondary diabetes mellitus, not uncommon [12].

The World Health Organization (WHO) classifies mumps as an infection that can be eradicated by specific vaccinations. It was possible to achieve the WHO goal of reducing the incidence by 2010 or earlier to a level of 1 or less per 100,000 population in Russia (2009 – the registered incidence rate was 0.65 per 100,000 population). However, at present, the incidence is recorded in many countries of the world in the form of sporadic cases and in the form of large epidemic outbreaks.

THE AIM. The article highlights the current state of the problem of mumps in the world and the Russian Federation.

MATERIALS AND METHODS

Research materials are electronic resources WHO infection control, Cohrane, Elsevier, ScienceDirect, CDC infection diseases database, PubMed, eLIBRARY, CyberLeninka. Research methods are analysis and generalization of scientific literature. The immunological structure of the population was analyzed in different age groups to mumps (n = 593) in the study area (2018) according to the data of the Center for Hygiene and Epidemiology in the Perm Territory, the study was carried out by a serological method using the Vector Best test system «Vector Parotitis-IgM», «Vector Parotitis-IgG».

RESULTS AND DISCUSSION

Etiology of mumps

The viral nature of mumps was first established in 1934 by researchers E. Goodpascher and K. Johnson. The mumps virus belongs to the family *Paramyxoviridae*, genus *Rubulavirus* (Fig. 1). The mumps virus has biological properties: a spherical virion with a diameter of 100–300 nm; the genome is represented by a single-stranded, unsegmented infectious RNA comprising seven genes organized by 3'-NP-P-M-F-SH-HN-L-5'. The surface proteins hemagglutinin, neurominidase (HN), and fusion protein (F) are responsible for the adhesion and aggregation of the viral envelope with the cell membrane and have an important role in infection, and it is to them that virus neutralizing antibodies are formed [2].

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

Currently, 12 genotypes of the virus (A, B, C, D, E, F, G, H, I, K, L) circulate in the world, they are isolated on the basis of differences in the nucleotide sequence of the SH and HN transmembrane protein genes. The heterogeneity of the nucleotide sequence of wild virus genes ranges from 6 to 20% [2, 14, 15]. Exogenous (imported) strains of the mumps virus can appear along with the endogenous circulation of a particular genotype of the virus in a particular area. Thus, the prevalence of circulating mumps virus of genotype G in Australia in 2007–2008 was established with a wide endogenous circulation of the virus of genotype J in recent years (2015) [3].

The mumps virus genotype G has been circulating in the world for the last ten years, it is the most widespread and is most often detected during epidemiological investigations of large epidemic outbreaks (20 or more cases of infection) in the USA, Great Britain, the Netherlands, Australia, southern China, Canada, Norway. India, Scotland, Israel, Japan, Korea and France [3, 15–24]. The genotype of the mumps virus F circulates in the central part of China, the genotype of the virus K circulates in Vietnam [25–27].

Epidemiology of mumps

In Russia, as in the rest of the world, there has been a decrease in the incidence of EP (from 483.0 to 1.38 per 100 thousand population in 2018) since the introduction of mass routine immunization of children against mumps (since 1981) within the framework of the National Calendar preventive vaccinations (Fig. 2) [28, 29].

The analysis of the incidence of mumps identified the territory of risk in the Russian Federation – the North Caucasian District according to the federal statistical observation in the period 2016–2018 (Table 1) [30].

Comparative assessment of the age structure of patients with mumps revealed a shift in morbidity in adolescents and adults. At present, the proportion of schoolchildren and adults aged 17–19 and 20–25 is more than 60% [3, 16, 21]. In the study area (Perm Territory), out of 36 cases in 2018, 34 cases (94,5%) accounted for adults of working age from 18 to 49 years old [31].

Diagnosis of mumps

Etiological laboratory diagnostics in mumps is not provided for in the standard for the provision of specialized medical care for children. In the existing clinical guidelines for the provision of medical care to children, the use of the enzyme immunoassay method is recommended as a confirmatory laboratory test; in the case of verification of atypical forms of infection, the molecular biological method should be used [32–34].

Specific IgM antibodies to mumps are detected 1–4 days after the onset of the first clinical symptoms, their concentration rapidly increases and becomes maximum by 40–50 days of illness. It is believed that their diagnostic value increases from the fifth day of the disease. Specific IgM antibodies to mumps may be absent altogether or circulate for a short time in vaccinated individuals [35, 36]. The presence of specific IgG antibodies to mumps in the blood serum of patients does not allow establishing the age of the disease. A dynamic increase in the titer of specific IgG antibodies to mumps virus by 4 or more times after 2–3 weeks from the onset of the disease is considered diagnostically significant [24, 29].

During an outbreak of mumps in the study area from November 2017 to February 2018, with 12 cases of mumps aged 21 to 27 years in 100% of individuals, mumps was confirmed by a serological method using the Vector Best test system “Vector Parotitis-IgM”, “Vector Parotitis-IgG”. Of the 12 patients specific IgM antibodies to the mumps virus were detected in 4 people (33,3%), a dynamic four-fold increase in specific IgG antibodies was in 8 people (66.7%). The performed screening serological dynamic examination of contact persons with the source of the infectious agent revealed initially 26 (84%) seropositive persons and 2 (6%) with dubious results (the coefficient of positiveness antibodies-IgG was 0.8-1.0). Subsequently, there was an increase in the number of cases with dubious results to 3 (10%).

The use of molecular genetic methods (PCR) among patients previously vaccinated against mumps in the study of non-invasive biological material – the contents of a buccal smear and nasopharyngeal secretions is the most informative for the verification of mumps [2, 24, 37]. The mumps virus is detected within 9 days of the onset of clinical symptoms [2, 38, 39]. However, among the vaccinated, the isolation of the virus occurs short-term and is observed up to 2–3 days [24, 36]. The informative value of the molecular genetic and serological diagnostic methods used in everyday clinical and epidemiological practice directly depends on the timing of the disease. The greatest diagnostic value in confirming the diagnosis in the first days of the disease has the method of reverse transcriptase PCR (RT-PCR) with real-time detection, this method reveals the genetic material of the mumps virus in the contents of nasopharyngeal secretions and buccal smears from patients [24, 29, 37]. The PCR method in clinical practice, as a confirmatory test, provides an etiological decoding of patients with mumps, and timely prescribes adequate and systemic therapy for the sick. This method determines the circulating genotypes of the virus in a separate territory – endogenous strains, this allows differentiating the endogenous circulating strains of mumps from imported (exogenous) ones, isolating “wild” mumps viruses and comparing them with a “vaccine” strain, confirming or excluding the emerging post-vaccination complications, revealing changes in the mumps virus of adaptive and phylogenetic nature [14, 15, 40].

DOI: 10.19163/2307-9266-2020-8-5-296-303

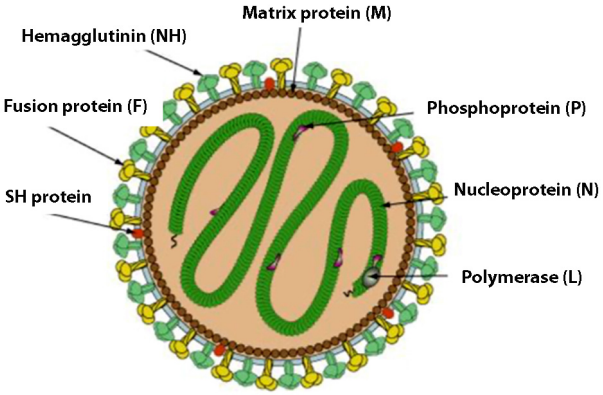

Figure 1 – The structure of the mumps virus according to N. Litusov, 2018 [13]

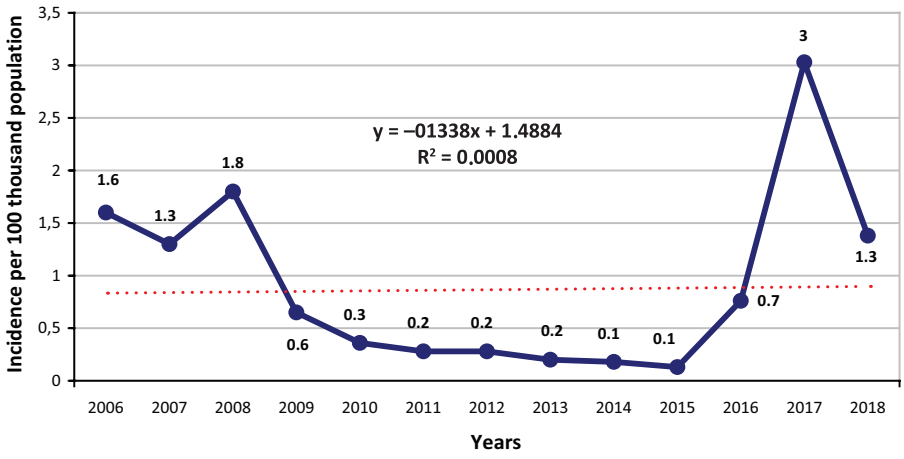

Figure 2 – Dynamics of the incidence of mumps in the Russian Federation (in terms of per 100 thousand population)

Table 1 – Territories of the Russian Federation with a high incidence of mumps in 2018

Territory	Absolute number	Indicator per 100 thousand population
Russian Federation	2027	1.38
Republic of Dagestan	1390	45.53
Chechen Republic	165	11.57

Table 2 – Characteristics of vaccine strains

Vaccine type	Vaccine name	Characteristics of the vaccine strain of mumps
Monovaccine (mumps)	Cultured live dry vaccine	Vaccine strain Leningrad-3 (one inoculation dose contains more than 20000 TCD50)
Divaccine (measles-mumps)	Cultured live dry divaccine	Vaccine strain Leningrad-3 (one inoculation dose contains more than 20000TCD50)
Trivaccine (measles-mumps-rubella)	MMR-II	Jeryl-Lynn vaccine strain (one vaccination dose contains at least 20000 TCD50)
	Priorix	Vaccine strain RIT 43/85 (one vaccination dose contains at least $10^{3,7}$ TCD50)
	Measles-mumps-rubella	Vaccine strain Leningrad-Zagreb (one vaccination dose contains at least 5000 TCD50)
Quadrivaccine (measles-mumps-rubella-varicella)	Priorix-Tetra	Vaccine strain RIT 43/85 (one vaccination dose contains at least 4,4 lg TCD50)

ISSN 2307-9266 e-ISSN 2413-2241

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The RT-PCR method is used quite widely; in the world clinical and epidemiological practice, along with enzyme immunoassay, it is used to establish, in the course of an epidemiological investigation, the causes and conditions of the spread of large epidemic outbreaks. The RT-PCR method was used for typing biological material in epidemic outbreaks in Germany (2008–2011), USA (2016), Canada (2007–2017), Australia (2007–2015), France (2013), Norway (2016), Israel (2017) [3, 16, 24, 37, 41, 42]. The molecular genetic method RT-PCR by isolating nucleic acids was used along with serological methods in the diagnosis of mumps in the course of a detailed epidemiological investigation of an epidemic outbreak of mumps with 176 cases in the Udmurt Republic (2008). An identical causative agent of the mumps virus with common biological properties has been established among the diseased along with common clinical manifestations [29].

The procedure for identifying, treating, isolating patients, official registration and statistical observation of mumps cases are determined in the Russian Federation in accordance with the current sanitary and epidemiological rules. About 300–600 thousand people suffered from mumps per year before the introduction of mass routine vaccine prophylaxis in 1970–1980, for comparison, in 2018, 2027 cases of mumps were registered in the Russian Federation [30].

Mumps prevention

Vaccine prophylaxis of mumps has been carried out in the Russian Federation since 1981; it has reduced the incidence and mortality rate, and has reduced the severity rate and the number of complications. Vaccine prophylaxis saved more than 2,500 lives, prevented about 2.5 million cases of serous meningitis, tens of thousands of cases of orchitis, oophoritis, pancreatitis, and subsequently diabetes mellitus, mastitis and premature abortions. To date, over 200 million people have been vaccinated. Coverage of preventive vaccinations should be at least 95% among decreed persons in order to achieve sufficient population immunity to mumps. In the Russian Federation, coverage with timely vaccination has exceeded 97,5% annually since 2002. However, the immune layer in mumps did not reach the normative level when assessing the immunological structure of the population in different age groups. For example, the proportion of seronegative individuals for mumps in different age groups ranged from 4,0% at the age of 40-49 to 21,4% at the age of 20–29 and 16,7% at the age of 30–39 among the total population of Moscow. and the Moscow region in 2007–2011 [29, 43]. At the same time, in 2017–2018, the increase in the incidence of mumps was noted to 3.03 per 100 thousand population in 2017 and 1,38 per 100 thousand population in 2018 [29, 44].

The vaccine strains used in the world practice of vaccine prevention of mumps: Jeryl Lynn and its derivative Rit 43/85 (USA), Leningrad-3 (Russia), Urabe, Hoshino, Torit, Miyahara (Japan), Leningrad-Zagreb (Croatia), Rubine (Switzerland), Sofia-6 (Bulgaria) [45].

In the Russian Federation, specific prophylaxis is carried out with a live mumps vaccine within the framework of the National Calendar of Preventive Vaccinations and the Calendar for Epidemic Indications (vaccination is carried out within 7 days from the moment the first case is detected in the epidemic focus). Mumps mono vaccine, mumps-measles divaccine, tri-vaccine (measles-mumps-rubella) and quadrivalent vaccine (measles-mumps-rubella-varicella) are licensed and registered in the prescribed manner for the implementation of vaccine prevention of mumps in the Russian Federation (Table 2) [44].

The Leningrad-3 strain is a part of mono- and divaccines and is cultivated in the primary culture of Japanese quail fibroblasts [44]. Jeryl-Lynn vaccine strain and RIT43/85 (derived from Jeryl-Lynn) are included in the MMR-II, Priorix (trivaccine) and Priorix-Tetra vaccines; it is cultured separately in a chicken embryo cell culture [46-48]. The Leningrad-Zagreb vaccine strain is a part of the trivaccine (measles-mumps-rubella), it is cultivated in fibroblasts of chicken embryos [11, 29, 44]. Currently, the domestic combined trivaccine (measles-mumps-rubella) “Vaktrivir” is registered in the Russian Federation [50].

Assessment of the immunological structure of the population revealed a fairly high number of seronegative individuals to mumps in different age groups (n = 593) in the study area (2018). Among children aged 3-4 years, their share was 9,5%, at the age of 16–17 years – 6%, at the age of 20–29 years – 13,3%, 30–39 years – 19,4% and 40–49 year olds – 8,4%. The largest number of seronegative individuals is found among adults aged 20–39 years. At the same time, the decreed age group for revaccination among adults is not defined in the National Calendar of Preventive Vaccinations.

In the second half of the 20th century, in many countries of the world, mass vaccine prophylaxis of mumps was introduced into national immunization programs and the incidence of the disease decreased significantly. However, the unfavorable epidemic situation in mumps continues to grow in some countries of the world, outbreak incidences are recorded in them in collectives with ideal vaccination coverage (up to 98%). According to M. Maillet (2013), P.A. Maple (2015), V.S. Fields (2019) among patients with mumps, previously received two doses of the vaccine to 62–92% of individuals [2, 16, 24]. The main reasons and conditions for the emergence of epidemic outbreaks are the lack of normative coverage of vaccination and revaccination in the past among the decreed groups, vaccination failures among vaccinated, decreased post-vaccination immunity, untimely and ineffective primary anti-epidemic (preventive) measures in the emerging epidemic foci of infection [2,

DOI: 10.19163/2307-9266-2020-8-5-296-303

19, 51]. G.E. Nelson (2013), C.V. Cardemil (2017), A.M. May (2017), M. Marin (2018) found that the incidence stopped after the use of the third dose of vaccine among contact persons in outbreaks against the background of high coverage with two doses of vaccine earlier in mainstream schools and universities as a preventive measure [43, 51–53].

The lack of production control of the produced vaccines for the prevention of mumps – the full compliance of the applied vaccine strain with circulating "wild" strains leads to insufficient protection of the population from circulating "wild" strains [21, 25, 54]. The immune defense created by the vaccine strain does not have the same intensity and duration as in natural infection, and some genotypes of "wild" variants of the mumps virus can break through the immune barrier and cause disease [11, 37]. Antigenic differences between vaccine and circulating strains, low inoculation dose can weaken immunity and reduce the effectiveness of the implemented mass vaccine prevention [44, 47, 50]. In connection with the possibility of adaptive and phylogenetic variability of the circulating "wild" strain of mumps, it is necessary to introduce regulated production control of the conformity of the vaccine strains used with the circulating "wild" strains of the virus [21, 25, 37].

CONCLUSION

The ongoing epidemic trouble with mumps in certain territories of the Russian Federation, the shift in the incidence in the age structure of patients towards adolescence and adults dictates the need to develop and introduce into medical practice a standard clinical definition of a mumps case in order to correctly verify the diagnosis with subsequent laboratory confirmation of the clinical diagnosis, taking into account the existing epidemiological data.

The main reason for the emergence of an outbreak is a decrease in the tension of post-vaccination immunity among the adult population, in addition to vaccination failures among vaccinated children.

Anticipating an unfavorable epidemic situation in mumps requires the introduction of regulated production control over the use of vaccine strains and the determination of the correspondence between the vaccine and circulating strains of the mumps virus with a justification for an adequate vaccination dose.

FUNDING

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

AUTHORS' CONTRIBUTION

Semerikov V.V. – concept and design of the study, obtaining data for analysis, writing the text of the manuscript;
Yuminova N.V. – concept and design of the study, partly collection of material;
Postanogova N.O. – review of publications on the topic of the article, writing the text of the manuscript;
Sofronova L.V. – review of publications on the topic of the article, writing the text of the manuscript

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

CORRECTION OF MORPHOFUNCTIONAL DISORDERS IN EXPERIMENTAL PREECLAMPSY BY COMBINED USE OF TRIMETAZIDINE AND PURIFIED MICRONIZED FLAVONOID FRACTION AS WELL AS THEIR COMBINATIONS WITH METHYLAMPSY

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Received 08 Oct 2020 Accepted 30 Oct 2020

The aim of the experiment was to determine the effectiveness of the combined use of trimetazidine and a purified micronized flavonoid fraction, as well as their combinations with methyldopa, in comparison with monotherapy with the same drugs in the correction of morphofunctional disorders arising in the conditions of experimental preeclampsia.

An integrated/multimethodology approach is the most effective way of treatment for preeclampsia. Therefore, an urgent task of modern pharmacology is to study the effectiveness of new drugs when used in combinations, as well as the drugs included in the standards for treatment.

Materials and methods. The study was carried out at the Research Institute of Pharmacology of Living Systems of Belgorod State National Research University. The experiment was performed on 200 female Wistar rats, weighing 250–300 g, in which an ADMA-like model of preeclampsia had been reproduced. To assess the degree of correction of emerging morphological and functional disorders, the following parameters were involved: blood pressure, a coefficient of endothelial dysfunction, microcirculation in the placenta, proteinuria, fluid contents in the greater omentum, morphometric indicators of placental tissues and fetal height and weight parameters.

Results. The combined use of trimetazidine (Preductal® MB) 6 mg/kg and a purified micronized flavonoid fraction (Detralex®) 260 mg/kg, as well as their combination with methyldopa (Dopegit®) 86 mg/kg, leads to a more pronounced decrease in the blood pressure, compared with a decrease in the coefficient of endothelial dysfunction by 2.22, 2.19 and 1.94 times, respectively, in relation to “untreated” animals. There was an increase in microcirculation indices in the placenta by 2.35, 2.21 and 2.03 times, respectively. In addition, there was an improvement in morphological parameters in the placenta and fetuses.

Conclusion. The results of the study showed a greater effectiveness of the combined use of the studied drugs in experimental preeclampsia compared to their monotherapy. This indicates the prospects for the use of trimetazidine and purified micronized flavonoid fraction in the complex therapy for preeclampsia and the need for further research in this direction.

Keywords: trimetazidine; purified micronized flavonoid fraction; preeclampsia; endothelial dysfunction; rats

Abbreviations: L-NAME – L-Nitro-Arginine Methyl Ester; VEGF – vascular endothelial growth factor; CED – coefficient of endothelial dysfunction; SBP – systolic blood pressure; DBP – diastolic blood pressure.

For citation: O.E. Antsiferova, M.P. Teleshchenko, Yu.M. Tsuverkalova, M.V. Pokrovsky, V.V. Gureev, M.A. Zatolokina, A.V. Gureeva. Correction of morphofunctional disorders in experimental preeclampsy by combined use of trimetazidine and purified micronized flavonoid fraction as well as their combinations with methylampsy. *Pharmacy & Pharmacology.* 2020;8(5):304-315. **DOI:** 10.19163/2307-9266-2020-8-5-304-315

Для цитирования: О.Е. Анциферова, М.П. Телещенко, Ю.М. Цуверкалова, М.В. Покровский, В.В. Гуреев, М.А. Затолокина, А.В. Гуреева. Коррекция морфофункциональных нарушений при экспериментальной преэклампсии сочетанным применением триметазидина и очищенной микронизированной флавоноидной фракцией, а также их комбинаций с метилдопой. *Фармация и фармакология.* 2020;8(5):304-315. **DOI:** 10.19163/2307-9266-2020-8-5-304-315

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2020-8-5-304-315

КОРРЕКЦИЯ МОРФОФУНКЦИОНАЛЬНЫХ НАРУШЕНИЙ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ПРЕЭКЛАМПСИИ СОЧЕТАННЫМ ПРИМЕНЕНИЕМ ТРИМЕТАЗИДИНА И ОЧИЩЕННОЙ МИКРОНИЗИРОВАННОЙ ФЛАВОНОИДНОЙ ФРАКЦИЕЙ, А ТАКЖЕ ИХ КОМБИНАЦИЙ С МЕТИЛДОПОЙ

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Получено 08.10.2020 Принята к печати 30.10.2020

Цель. Провести исследование эффективности сочетанного применения триметазидина и очищенной микронизированной флавоноидной фракцией, а также их комбинаций с метилдопой в сравнении с монотерапией этими же препаратами при коррекции морфофункциональных нарушений, возникающих в условии экспериментальной преэклампсии.

Комплексный подход является наиболее эффективным способом терапии преэклампсии. Поэтому актуальной задачей современной фармакологии остается исследование эффективности новых лекарственных препаратов при комбинированном использовании, в том числе, и с препаратами, входящими в стандарты лечения.

Материалы и методы. Исследование проводилось в НИИ фармакологии живых систем ФГАОУ ВО НИУ БелГУ. Эксперимент проводили на 200 самках крыс линии Wistar, массой 250–300 г, у которых воспроизводили ADMA-подобную модель преэклампсии. Для оценки степени коррекции возникающих морфофункциональных нарушений использовали следующие параметры: артериальное давление, коэффициент эндотелиальной дисфункции, микроциркуляцию в плаценте, протеинурию, содержание жидкости в большом сальнике, морфометрические показатели плацентарных тканей и ростовесовых показателей плодов.

Результаты. Комбинированное применение триметазидина (Предуктал® МВ) 6 мг/кг и очищенной микронизированной флавоноидной фракции (Детралекс®) 260 мг/кг, а также их сочетаное применение с метилдопой (Допегит®) 86 мг/кг, приводит к более выраженному снижению артериального давления, по сравнению со снижением коэффициента эндотелиальной дисфункции в 2,22, 2,19 и 1,94 раза соответственно по отношению к «нелеченным» животным. Происходило повышение показателей микроциркуляции в плаценте в 2,35, 2,21 и 2,03 раза соответственно. Кроме этого, наблюдалось улучшение морфологических показателей в плаценте и плодов.

Заключение. Результаты проведенного исследования показали большую эффективность комплексного применения исследуемых препаратов при экспериментальной преэклампсии по сравнению с их монотерапией. Это свидетельствует о перспективности применения триметазидина и очищенной микронизированной флавоноидной фракциии в комплексной терапии преэклампсии и необходимости проведения дальнейших исследований в этом направлении.

Ключевые слова: триметазидин; очищенная микронизированная флавоноидная фракция; преэклампсия; дисфункция эндотелия; крысы

Список сокращений: L-NAME – N-нитро-L-аргинин-метиловый эфир; ФРСЭ – фактор роста сосудистого эндотелия; КЭД – коэфициент эндотелиальной дисфункции; САД – систолическое артериальное давление; ДАД – диастолическое артериальное давление.

INTRODUCTION

For many decades, preeclampsia has remained an important medical and social problem in the countries all over the world. It occupies a leading place among the causes for maternal morbidity rate and mortality and, according to various authors, is from 9% to 25% [1, 2]. In Russia, this pathology stably occupies the 3–4th places [3]. The incidence ranges from 2% to 10% of all pregnancies and has no tendency to decrease [1, 4, 5]. In addition, preeclampsia leads to the development of pathological conditions not only in women, but also in the fetus, contributing to the disability of mothers and children [4, 6].

The problem of preventing and treating preeclampsia, as well as assessing the severity of its course and perinatal risks, is to a great extent due to the lack of con-

sensus among the medical community about its etiology and pathogenesis, although an extraordinary number of studies around the world are devoted to the investigation of this pregnancy complication [7, 8]. Herewith, preeclampsia is increasingly considered from the point of view of endothelial dysfunction [9–11], and "an oxidative stress" as a result of depletion of the antioxidant system under the conditions of placental ischemia, is one of its development mechanisms [12, 13]. The endothelial dysfunction developing against this background, leads to impaired microcirculation and tissue hypoxia and, as a result, to the development of multisystemic lesions that constitute the clinical manifestations of preeclampsia [14–16]. Therefore, the search for new drugs for the prevention and treatment of preeclampsia, is an urgent task of modern pharmacology.

In experimental studies on the model of L-NAME-induced preeclampsia in rats, the protective properties of resveratrol [17, 18], recombinant erythropoietin [19], and tadalafil [20, 21], which have endothelioprotective properties, have been demonstrated. Another promising area of prevention and correction of morphofunctional disorders that occur in preeclampsia, is the use of drugs with anti-ischemic and antioxidant properties. As a result of the previous studies, pronounced protective effects of trimetazidine and a purified micronized flavonoid fraction in the correction of morphofunctional disorders in experimental preeclampsia have been revealed, however, it should be notified that the target level has not been achieved [22–24].

Preeclampsia is a multifactorial disease. It is obvious that the increase in the effectiveness of therapy can be achieved by the complex use of drugs. Therefore, the urgent task of modern pharmacology is not only the search for new drugs for the treatment and prevention of preeclampsia, but also the study of their effectiveness in combined uses, as well as the drugs included in the standards for treatment.

THE AIM of the experiment was to determine the effectiveness of the combined use of trimetazidine and a purified micronized flavonoid fraction, as well as their combinations with methyldopa, in comparison with monotherapy with the same drugs in the correction of morphofunctional disorders arising under the conditions of experimental preeclampsia.

MATERIALS AND METHODS

Compliance with ethical principles

The study has been carried out at the Center for Preclinical and Clinical Research of the Belgorod State National Research University. The experiment was accomplished in accordance with legislative acts and guidelines regulating the conduct of experimental research in the Russian Federation: Order of the Ministry of Health of Russia dated 01.04.2016 No.199n "On the approval of the Rules of good laboratory practice" and "Guidelines for conducting preclinical studies of new drugs" ed. by A.N. Mironov. The ethical principles of handling laboratory animals were in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The study was approved of at the meeting of the Bioethical Commission of the Research Institute of Pharmacology of Living Systems of the Belgorod State National Research University, Protocol No.D2019/13. Keeping the animals was regulated by the norms of the Decree of the Chief State Sanitary Doctor of the Russian Federation dated 08.29.2014 No.51, GOST 33044-2014. The animals were kept in plastic cages, equipped with steel lattice lids, with a feeding recess, with a free access to food and water, on a balanced diet appropriate for the animal species. The ambient temperature was maintained at the level of 20–25°C with a relative humidity of 60–65%. The wood sawdust that had undergone preliminary UV sterilization, was used as a litter.

Modeling ADMA-like preeclampsia

The experiment was carried out on 200 white Wistar female rats weighing 250–300 g. An ADMA-like agent, a non-selective NO synthase blocker N-nitro-L-arginine methyl ether (L-NAME), was injected intraperitoneally at the dose of 25 mg/kg/per day for seven days (from the 14^{th} to the 20^{th} days of pregnancy) [25, 26]. To assess the effectiveness of the studied drug combinations, the animals had been divided into several groups (n=10).

1. Intact (Int.) (animals with oral administration of NaCl in equivalent doses from the 14^{th} to the 20^{th} days of pregnancy).
2. Modeling of ADMA-like preeclampsia (L-NAME) (N-nitro-L-arginine-methyl ester, Sigma-Aldrich), 25 mg/kg/per day.
3. L-NAME + methyldopa (Dopegit®, ZAO "Pharmaceutical Plant EGIS", Hungary), 86 (2×43) mg/kg/per day.
4. L-NAME + trimetazidine (Preductal® MB, ZAO "Servier", Russia), 6 mg/kg/per day.
5. L-NAME + purified micronized flavonoid fraction (Detralex®, ZAO "Servier", Russia), 260 mg/kg/per day.
6. L-NAME + trimetazidine 6 mg/kg/per day + purified micronized flavonoid fraction 260 mg/kg/per day.
7. L-NAME + trimetazidine 6 mg / kg + methyldopa 86 (2x43) mg/kg.
8. L-NAME + purified micronized flavonoid fraction 260 mg/kg/per day + methyldopa 86 (2×43) mg/kg/per day.

Assessment of the degree of endothelial dysfunction development in pathology modeling

The development of endothelial dysfunction in experimental animals, as well as the degree of its correction by the studied drugs and their combinations, were assessed by the calculated coefficient of endothelial dysfunction (CED) [27, 28].

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DOI: 10.19163/2307-9266-2020-8-5-304-315

$$CED = \frac{S_{NP}}{S_{ACH}}$$

The endothelial dysfunction coefficient is the ratio of the triangle area above the blood pressure recovery curve in response to 30 μg/kg nitroprusside (S_{NP}) to the triangle area above the blood pressure recovery curve in response to 40 μg/kg acetylcholine (S_{ACH}). The legs in the both triangles were indicators of the blood pressure recovery time (reaction duration) and the changes in the blood pressure in response to the intravenous administration of acetylcholine and nitroprusside, respectively.

Placental microcirculation assessment

To obtain the data on the state of microcirculation in the placenta, the equipment manufactured by Biopacsystems was used: polygraph MP100 with an LDF100C Laser Doppler Flowmetry (LDF) module and an invasive TSD144 needle probe. On the 21st day of pregnancy, under anesthesia, the level of microcirculation was measured in the projection of the placental disc at a distance of 1 mm at 4 points. The registration and processing of LDF results were carried out using the AcqKnowledge version 3.8.1. The microcirculation values were stated in perfusion units (PU) [29].

Proteinuria research

Urine collection was carried out for 12 hours using special metabolic cells. The determination of the protein amount in daily urine, was carried out by the pyrogall method. It is based on the determination of the optical density of a solution of a colored complex, formed by the interaction of protein molecules with the molecules of the complex of the pyrogallol red dye and sodium molybdate. The color intensity of the solution is proportional to the protein content. The measurements were carried out using a PE-5400 V spectrophotometer at the wavelength of 600 nm [30].

Study of the greater omentum сальник edema

On the 21st day of pregnancy, the greater omentum was removed under anesthesia, and weighed. Within 24 hours it was dried at 37°C, then weighed again. From the difference in the mass of the greater omentum before and after drying, the amount of the evaporated water was obtained in each specific piece. The water content was stated in %, relative to the total weight of the gland while the first weighing [31].

Morphological methods for assessing changes in placenta when modeling experimental gestosis

A histological study of the placenta was carried out in all the series of the experiment for a morphological confirmation of the development of modeled pathological processes and giving a comprehensive assessment of the drugs' effectiveness. The material was fixed in 10% formalin with subsequent embedding in paraffin. Histological sections of the placenta were made in a strictly vertical direction through the middle of the placental disc with the capture of all layers of the placenta and the wall of the uterine horn. The study of microslides, photorecording and morphometry, were carried out on a Leica DM4000B microscope with a video recording and an image processing system. All morphological studies were stained with hematoxylin and eosin [25].

Fetus study

The fetuses were removed from the uterine cavity, weighed, and the fetus size (craniocaudal size) was measured, followed by the calculation of the statural-weight coefficient [32].

Statistical processing of research results

Descriptive statistics was applied to all the data: the data were checked for a normal distribution. The distribution type was determined by the Shapiro-Wilk test. In case of a normal distribution, the mean (M) and a standard error of the mean (m) were calculated. The intergroup differences were analyzed by parametric (Student's *t*-test) or nonparametric (Mann-Whitney *U*-test) methods, depending on the type of the distribution. The calculations were performed using statistical programs Microsoft Excel 7.0. The groups were compared in pairs.

RESULTS

Effect of the combined trimetazidine and detralex use, as well as their combined use with methyldopa, for the correction of morphofunctional disorders in ADMA-like preeclampsia

The combined use of preductal and detralex, as well as their combined use with dopegit for the correction of morphofunctional disorders in ADMA-like preeclampsia, led to a more pronounced decrease in blood pressure. Thus, when the combined administration of preductal and detralex took place, the systolic and diastolic kinds of blood pressure decreased to 145.7±3.93 mm Hg and 100.1±3.59 mm Hg, respectively. When detralex was used individually, it decreased to 169.3±5.40 mm Hg and 125.7±4.91 mm Hg. When preductal was used individually, it decreased to and 152.5±1.99 mm Hg and 112.3±3.90 mm Hg. Herewith, the decrease of diastolic pressure was statistically significant in comparison with the reference groups (Table 1). The combined use of preductal and dopegit led to a statistically significant decrease in systolic blood pressure compared with monotherapy, and when detralex and dopegit were combined, both systolic and diastolic kinds of blood pressure decreased statistically significantly ($p<0.05$) compared with monotherapy (Table 1).

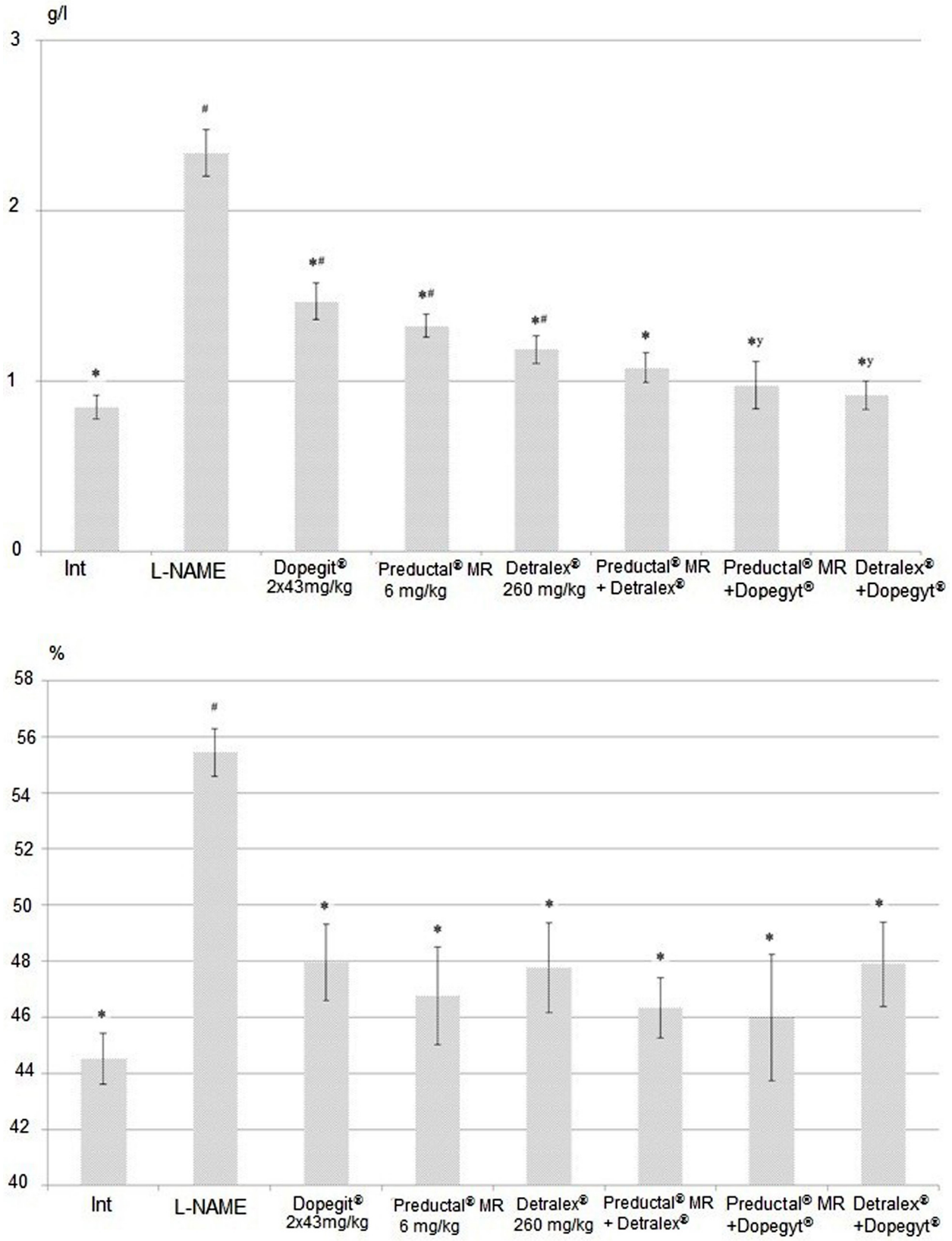

Figure 1 – The effect of the Preductal® MB and Detralex® combination, as well as their combination with Dopegit®, on proteinuria and fluid content in the tissue of the greater omentum in ADMA-like preeclampsia

Note: # – at p <0.05 in comparison with intact pregnant rats; * – at p <0.05 in comparison with the group of pregnant animals treated with L-NAME; y – at p <0.05 in comparison with both monotherapy options.

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DOI: 10.19163/2307-9266-2020-8-5-304-315

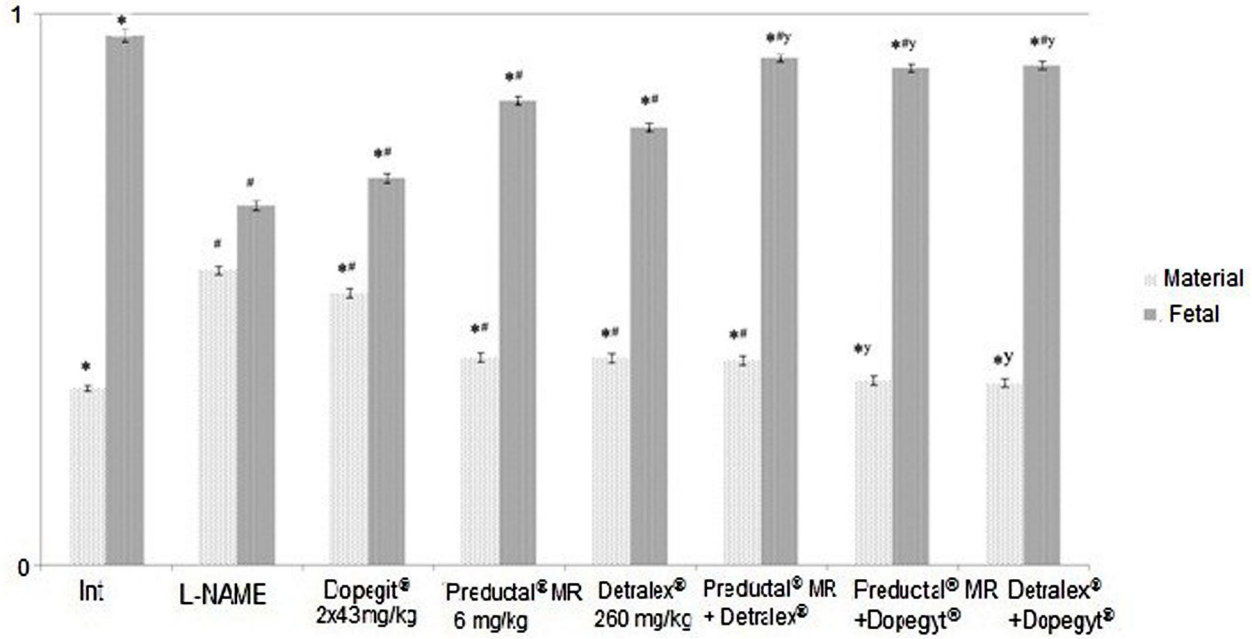

Figure 2 – The effect of the Preductal® MB and Detralex® combination, as well as their combination with Dopegit®, on the fetal and maternal size parts of the placenta, in ADMA-like preeclampsia

Note: # – at p <0.05 in comparison with intact pregnant rats; * – at p <0.05 in comparison with the group of pregnant animals treated with L-NAME; y – at p <0.05 in comparison with both monotherapy options

Table 1 – The effect of the Preductal® MB and Detralx® combination, as well as their combination with Dopegit®, on the functional parameters of the cardiovascular system in ADMA-like preeclampsia.

Indicator/Index / Group	SBP, mm Hg	DBP, mm Hg	CED, relative units.	Microcirculation, PU
Int	123.4±3.54*	83.8±5.47y*	1.21±0.13*	472.6±22.44*
L-NAME	193.6±6.28#	150.8±4.80#	2.89±0.25#	215.6±9.29#
L-NAME+ Dopegyt® 86 mg/kg	155.5±3.14*#	114.4±7.13*#	2.49±0.28#	297.8±13.41*#
L-NAME+ Preductal® MR 6 mg/kg	152.5±1.99*#	112.3±3.90*#	1.57±0.15*	402.3±15.81*#
L-NAME+Detralex® 260 mg/kg	169.3±5.4*#	125.7±4.91*#	1.79±0.11*#	394.0 ±9.87*#
L-NAME+ Preductal® MR + Detralex®	145.7±3.93*#	100.1±3.6*# y	1.30±0.05*	505.9±17.83*y
L-NAME+ Preductal® MR + Dopegyt®	138.6±3.16*#y	97.6±5.84*	1.32±0.08*	477.4±27.61*y
L-NAME+ Detralex®+ Dopegyt®	145.5±2.75*#y	97.8±2.2*#y	1.49±0.05*y	437.0±19.87*

Note: # – at p <0.05 in comparison with intact pregnant rats; * – at p <0.05 in comparison with the group of pregnant animals treated with L-NAME; y – at p <0.05 in comparison with both monotherapy options

Table 2 – The effect of the combined use of Preductal® MB and Detralex®, as well as their combined use with Dopegit®, on the density of the cell pool in the fetal and maternal parts of the placenta; the diameter of the chorionic villi and the growth-weight index of the fetuses in the correction of ADMA-like preeclampsia (M±m)

Indicator/Index / Group	Density of decidual cells, 0,008 mm²	Cell density in fetal part of placenta, 0,008 mm²	Diameter of villi, x10⁻³mkm	Growth-weight index, mm/g
Int.	118.3±2.14*	235.8±2.75*	32.40±0.41*	14.78±0.22*
L-NAME	23.1±0.33#	80.7±2.57#	17.19±0.26#	15.79±0.23#
L-NAME+ Dopegyt® 86 mg/kg	55.6±0.45*#	98.9±1.73*#	18.78±0.17*#	15.62±0.15#
L-NAME+ Preductal® MR 6 mg/kg	102.7±0.77*#	150.5±1.71*#	29.93±0.17*#	15.36±0.22*#
L-NAME+Detralex® 260 mg/kg	104.8±0.87*#	151.3±1.69*#	29.90±0.16*#	15.31±0.58
L-NAME+ Preductal® MR + Detralex®	132.7±1.92*#y	179.3±1.60*#y	34.89±0.16*#y	14.42±0.63*
L-NAME+ Preductal® MR + Dopegyt®	141.3±2.21*#y	177.6±1.59*#y	31.79±0.14*y	14.86±0.30*
L-NAME+ Detralex®+ Dopegyt®	138.8±2.29*#y	181.0±1.69*#y	26.85±0.15*#y	14.53±0.69*

Note: # – p <0.05 in comparison with the group of intact animals; * – p <0.05 in comparison with the L-NAME group; y – p <0.05 in comparison with the groups in monotherapy.

When the combinations of preductal + detralex, preductal + dopegit and detralex + dopegit were used, the coefficient of endothelial dysfunction (CED) decreased to 1.30±0.05, 1.32±0.08 and 1.49±0.05, respectively, and the microcirculation improvement was up to 505,9±17.83 PU, 477.4±27.61 PU and 437.0±19.87 PU, respectively. It should be notified that CED reached a statistically indistinguishable value from the group of intact animals when the combinations of preductal + detralex and preductal + dopegit were used. Herewith, when the combination of detralex + dopegit was used, microcirculation improved to the level of intact animals.

In the described groups, a decrease in proteinuria resulted in 1.08±0.09 g/L, 0.92±0.09 g/L, and 0.92±0.09 g/L, respectively (Fig. 1A), and the fluid content in the greater omentum decreased to 46.33±1.08%, 45.98±2.26% and 47.89±1.50%, respectively (Fig. 1B). It should be notified that in comparison with the group with monotherapy, a statistically significant ($p < 0.05$) decrease in proteinuria, was observed when using preductal + dopegit. The fluid content in the greater omentum in all the groups with a combined use of drugs, was at the level of intact animals.

A histological examination of the placenta revealed the following: a combined administration of the studied drugs to the animals with experimental preeclampsia, led to a pronounced positive dynamics of the morphological picture, which was approximate to the group of intact animals. There was a statistically significant ($p < 0.05$) increase in the density of the cellular component of the placental tissues of the maternal and fetal parts of the placenta, and the diameter of the chorionic villi, in comparison with the groups of the animals in which the studied drugs had been used in the monotherapy mode (Table 2). Only the diameter of the villi reached the level of the intact animals when the combination of Preductal® MB + Dopegit® had been used. In addition, there was a statistically significant ($p < 0.05$) increase in the fetal part of the placenta and a decrease in the maternal part of the placenta (Fig. 2). The exception was the group using Preductal® MB + Detralex®, in which there was no statistically significant change in the maternal part of the placenta compared to the groups with monotherapy with the same drugs. It should be notified that the level of the intact animals had not been reached.

The study of the height-weight ratio in fetuses in the groups with the combined administration of the investigated drugs, showed an improvement in this indicator up to the level of the intact animals (Table 2).

Thus, the combined use of Preductal® MB and Detralex®, as well as their combined use with Dopegit®, led to a significantly positive effect in the correction of ADMA-like preeclampsia compared with the use of the same drugs in monotherapy. This was reflected in a more pronounced decrease in blood pressure. The microcirculation level reached the target values. There was a pronounced positive effect on the size of the fetal and maternal parts of the placenta, the concentration of the cell pool in the maternal and fetal parts of the placenta increased, the diameter of the chorionic villi was restored. There was also a significant improvement in the morphometric parameters of the fetus.

DISCUSSION

The most pronounced positive effects in the correction of morphofunctional disorders arising under the conditions of experimental preeclampsia when using combinations of the studied drugs in comparison with the use of the same drugs in monotherapy, can be explained by the possibility of influencing various points of pathogenesis. This is ensured by the fact that each drug has its own, different from the others, mechanism for the implementation of effects.

The positive effects of trimetazidine, are explained by its capacity to improve the energy metabolism of tissues under ischemic conditions. During the oxygen starvation, under the influence of trimetazidine, the cells activate the oxidation of pyruvate for the synthesis of ATP. This leads to a decrease in oxygen deficiency by 10-12% compared to the oxidation of fatty acids, which makes it possible for the cells to use oxygen more efficiently under the conditions of the oxygen deficiency [33, 34].

In addition, trimetazidine prevents the accumulation of insufficiently oxidized fatty acid products in the mitochondria of cells, and increases the stability of cell membranes due to the inclusion of fatty acids in phospholipids [35, 36]. This leads to a decrease in the severity of the oxidative stress and, as a result of a decrease in the synthesis of reactive oxygen species by mitochondria, its negative effect [37, 38]. In addition, the endothelioprotective properties of trimetazidine due to an increase in the amount of eNOS and the synthesis of nitric oxide as one of the most important factors of vasorelaxation, also explain the effectiveness of its use [39, 40]. The capacity of trimetazidine to reduce the formation of pro-inflammatory cytokines [41, 42], can promote both a decrease in the systemic content of markers of the oxidative stress, and a decrease in the eNOS activity. In addition, the endothelioprotective properties of trimetazidine may lie in its capacity to protect the endothelium from the direct damaging action of free radicals [43] and to reduce the inactivation of nitric oxide by inactivating lipid peroxidation processes [44, 45]. The result of the realization of direct and indirect effects of trimetazidine on the endothelium, is an improvement in endothelium-dependent vascular relaxation not only in this experiment, but also in patients with chronic heart failure (CHF) [46, 47].

The effective use of Detralex is explained by the presence of its several protective properties. One of them is a pronounced anti-inflammatory and antioxidant effect [48- 51]. The anti-inflammatory effect is associated with the capacity of diosmin to reduce the production of pro-inflammatory cytokines: IL-6, IL-1β, TNF-α, etc. [52–54]. In addition, diosmin is can reduce the induced

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DOI: 10.19163/2307-9266-2020-8-5-304-315

production of NO by inhibiting eNOS [48, 53]. The antioxidant activity includes the capacity of the studied drug to increase the activity of glutathione peroxidase, superoxide dismutase, catalase [53, 55, 56], and to prevent lipid peroxidation with an increase in the activity of the antioxidant system [56, 57]. A decrease in the formation of proinflammatory cytokines and markers of the oxidative stress, leads not only to a decrease in the injury of the placental tissues, but also to the improvement in the endothelial function.

This can be confirmed by the results of the studies by other authors. Endothelioprotective effects are manifested both in the treatment for varicose veins [58, 59] and in the correction of arterial pathology [60–62]. Special attention should be paid to the data on the protective effects of diosmin in ischemia-reperfusion injuries, since this is comparable with the pathogenetic features of preeclampsia [63, 64], especially at the capillary level [65]. The molecular mechanisms by which endothelioprotective effects are realized, include: suppression of the synthesis of proinflammatory humoral factors, a decrease in the production of cell adhesion molecules, a modulating effect on the permeability of the vascular wall, a favorable effect on the ratio of prooxidant and antioxidant factors [66].

In the protective effects of flavonoids, an important role is played by their capacity to improve the drainage function of tissues [60, 67–69]. Since edema increases in ischemia or inflammatory phenomena, this disrupts tissue trophism, and an improvement in the drainage function causes the opposite effect.

Methyldopa is a prodrug by its nature, and belongs to the group of centrally acting antihypertensive drugs. Passing through the blood-brain barrier (BBB), methyldopa turns into alpha-methylnorepinephrine, which depletes the resources of norepinephrine, displacing it from the granules (which brings this drug closer to sympatholytics), excites the central α2-adrenergic receptors of the vasomotor center, causes a decrease in its rhenium, and inhibits the formation of angiotensin [70]. A decreased peripheral vascular tone is an important addition to the mechanisms of the studied drugs' action. In addition, there is evidence of the endothelioprotective properties of this drug. In an *in vitro* study, it was established that the incubation of endothelial cell culture with methyldopa, promoted the leveling of the inhibitory TNF-α effect on the endothelial NO-synthase, and also led to an increase in the content of the vascular growth factor VEGF [71]. Herewith, in the absence of TNF-α, this drug had no effect on the eNOS expression [72].

CONCLUSION

The combined use of drugs in the treatment of a lot of diseases, is the most urgent direction. The results of this study witness the fact that the use of trimetazidine and a purified micronized flavonoid fraction in the complex therapy of preeclampsia, is a promising direction necessary to continue the research in.

FUNDING

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

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

O.E. Antsiferova – administration of drugs to the animals, modeling of ADMA-like preeclampsia, functional tests and other studies;
M.P. Teleschenko – administration of drugs to the animals, modeling of ADMA-like preeclampsia, functional tests and other studies;
Yu.M. Tsuverkalova – administration of drugs to the animals, modeling of ADMA-like preeclampsia, carrying out functional tests and other studies;
M.V. Pokrovsky – idea, research planning, consultations on the implementation of individual stages of the experimental work;
V.V. Gureev – article writing, development of the research design;
M.A. Zatolokina – preparing samples for histological examination, morphological description of placenta sections;
A.V. Gureeva – article writing, preparing samples for histological examination, formalization of the bibliography, working with graphic materials.

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ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-5-304-315

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RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

УДК 615.12

COMPARATIVE ANALYSIS OF DRUG EFFICACY IN THE TREATMENT FOR COVID-19 SEVERE FORMS, BASED ON ATTRIBUTE-BASED STATISTIC METHODS AND ANALYSIS OF DRUG INTERACTIONS

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Received 31 Aug 2020 Accepted 20 Oct 2020

Severe and critical forms of COVID-19 are beset by the development of “a cytokine storm”, which is characterized by an increased secretion of proinflammatory cytokines. Therefore, one of the leading strategies for treating patients with severe forms of COVID-19 is the reduction of concentration of proinflammatory cytokines and leveling out their effect on the patient. Among the drugs aimed at reducing the concentration of proinflammatory cytokines, IL-6 inhibitors, IL-1 inhibitors, JAK inhibitors and systemic glucocorticosteroids have been found useful in COVID-19. All of these drugs are currently prescribed off-label.

The aim of the work is a comparative analysis of the data from the literature sources in the PubMed system, devoted to the clinical efficacy and safety of IL-6, IL-1, JAK inhibitors and systemic glucocorticosteroids in the treatment for severe forms of COVID-19.

Materials and methods. In the treatment for severe forms of COVID-19, materials for the comparative analysis were the data from the literature sources in the PubMed system, on the studies devoted to the use of the systemic glucocorticosteroid dexamethasone, IL-6 inhibitor tocilizumab, IL-1 inhibitor anakinra, and JAK inhibitor ruxolitinib. The analysis was performed by statistical evaluation of the drugs effect within the 28-day survival rate among the patients with severe COVID-19. Attributive statistics was used as a statistical tool. The safety of the drug use was assessed by analyzing potential drug interactions. The information about potential drug interactions, was obtained from a specialized website – Drugs.com. Knowmore. Besure (https://www.drugs.com/interaction/list/).

Results. As a result of the analysis, it has been established that tocilizumab has the highest efficacy rates. In this respect, it is followed by dexamethasone. The attributive efficacy rates and 95% confidence interval values for the both drugs were statistically significant. The indices of relative and population attributive kinds of efficacy, were also higher for tocilizumab, but a 95% confidence interval of these indices, get into the range of statistically insignificant values, requiring additional evidence of their efficacy. According to the data obtained, tocilizumab efficacy is higher than that of the other drugs compared: NNT (dexamethasone) – 32; NNT (tocilizumab) – 4, NNT (ruxolitinib) – 7; NNT (anakinra) – 35.

Conclusion. The choice of a drug should be based on the patient’s condition, comorbidities, and medications used in therapy to minimize the risk of undesirable drug interactions. Against the background of the lowest efficacy among the compared drugs, a high efficacy for the patients with concomitant hepatobiliary disorders and DIC syndrome, has been established for the inhibitor IL-1 anakinra, which makes it the drug of choice among the patients with these diseases and under these conditions in the development of “a cytokine storm”.

Keywords: severe forms of COVID-19; systemic glucocorticosteroid; IL-6 inhibitor; IL-1 inhibitor; JAK-inhibitor; “cytokine storm”; attributive statistics; drug interactions

For citation: O.V. Zhukova, I.N. Kagramanyan, A.L. Khokhlov. Comparative analysis of drug efficacy in the treatment for COVID-19 severe forms, based on attributive statistic methods and analysis of drug interactions. *Pharmacy & Pharmacology*. 2020;8(4):316-324. **DOI:** 10.19163/2307-9266-2020-8-5-316-324

Для цитирования: О.В. Жукова, И.Н. Каграманян, А.Л. Хохлов. Сравнительный анализ эффективности лекарственных препаратов в терапии тяжелых форм COVID-19 на основании методик атрибутивной статистики и анализа межлекарственных взаимодействий. *Фармация и фармакология.* 2020;8(5):316-324. **DOI:** 10.19163/2307-9266-2020-8-5-316-324

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2020-8-5-316-324

СРАВНИТЕЛЬНЫЙ АНАЛИЗ ЭФФЕКТИВНОСТИ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ В ТЕРАПИИ ТЯЖЕЛЫХ ФОРМ COVID-19 НА ОСНОВАНИИ МЕТОДИК АТРИБУТИВНОЙ СТАТИСТИКИ И АНАЛИЗА МЕЖЛЕКАРСТВЕННЫХ ВЗАИМОДЕЙСТВИЙ

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Получена 31.08.2020 Принята к печати 20.10.2020

Тяжелые и критические формы COVID-19 сопровождаются развитием «цитокинового шторма», который характеризуется повышенной секрецией провоспалительных цитокинов. Поэтому одной из ведущих стратегий лечения пациентов с тяжелыми формами COVID-19 является снижение концентрации провоспалительных цитокинов и нивелирование их действия на организм пациента. Среди лекарственных препаратов, направленных на снижение концентрации провоспалительных цитокинов, нашли применение при COVID-19 ингибиторы ИЛ-6, ИЛ-1, ингибиторы JAK и системные глюкокортикостероиды. Все эти лекарственные препараты в настоящее время назначаются off-label.

Цель – сравнительный анализ по данным литературных источников, представленных в PubMed, клинической эффективности и безопасности использования ингибиторов ИЛ-6, ИЛ-1, JAK и системных глюкокортикостероидов в терапии тяжелых форм COVID-19.

Материалы и методы. Материалами для проведения сравнительного анализа послужили данные литературных источников в системе PubMed, посвященные исследованиям использования системного глюкокортикостероида дексаметазона, ингибитора ИЛ-6 тоцилизумаба, ингибитора ИЛ-1 анакинры и ингибитора JAK – руксолинитиб в терапии тяжелых форм COVID-19. Анализ проводили путем статистической оценки влияния лекарственных препаратов на показатель выживаемости в течение 28 дней среди пациентов с тяжелым течением COVID-19. В качестве статистического инструмента были использованы методики атрибутивной статистики. Оценку безопасности использования лекарственных препаратов проводили путем анализа потенциальных лекарственных взаимодействий. Информацию о потенциальных взаимодействиях лекарственных препаратов получали на специализированном сайте – Drugs.com. Knowmore. Besure (https://www.drugs.com/interaction/list/).

Результаты. В ходе проведенного анализа установлено, что наибольшие показатели эффективности имеет тоцилизумаб, далее следует дексаметазон. Показатель атрибутивной эффективности и значения 95% доверительный интервал для обоих лекарственных препаратов оказался статистически значимым. Показатели относительной и популяционной атрибутивной эффективностей также выше для тоцилизумаба, однако, 95% доверительный интервал этих показателей попадают в область статистически незначимых значений, что требует дополнительных подтверждений их эффективности. Согласно полученным данным, эффективность использования тоцилизумаба выше эффективности других сравниваемых лекарственных препаратов. NNT (дексаметазон) – 32; NNT (тоцилизумаб) – 4, NNT (руксолитиниб) – 7; NNT (анакинра) – 35.

Заключение. Выбор лекарственного препарата должен осуществляться исходя из состояния пациента, сопутствующих заболеваний и используемых в терапии лекарственных препаратов с целью минимизации риска нежелательных межлекарственных взаимодействий. Для ингибитора ИЛ-1 анакинры на фоне самой низкой эффективности среди сравниваемых лекарственных препаратов установлена высокая эффективность для пациентов с сопутствующими гепатобилиарными расстройствами и ДВС-синдромом, что делает ее препаратом выбора среди пациентов с данными состояниями и заболеваниями при развитии «цитокинового шторма».

Ключевые слова: тяжелые формы COVID-19; системный глюкокортикостерод; ингибитор ИЛ-6; ингибитор ИЛ-1; ингибитора JAK; «цитокиновый шторм»; атрибутивная статистика; межлекарственные взаимодействия

INTRODUCTION

COVID-2019 is currently a global social problem that is particularly challenging for health systems [1].

Regarding a part of medicinal preparations (MPs), clinical studies are being conducted to obtain data on the effectiveness of their use in the treatment for COVID-19 [2]. A special feature of COVID-2019 is the possibility of rapid development of severe and critical conditions, which are characterized by high mortality rates, more specifically, from 49% [3] to 60.5% [4].

Severe and critical forms of COVID-19 are beset by the development of “a cytokine storm”, which is characterized by an increased secretion of proinflammatory cytokines. Therefore, one of the leading strategies for

treating patients with severe forms of covid-19 is the reduction of concentration of proinflammatory cytokines and leveling out their effect on the patient. [5].

Among the drugs aimed at reducing the concentration of proinflammatory cytokines, IL-6 inhibitors, IL-1 inhibitors, JAK inhibitors and systemic glucocorticosteroids have been found useful in COVID-19. All of these drugs are currently prescribed off-label. More research on the efficacy and safety of these drugs in COVID-19 therapy, is currently being conducted.

THE AIM of the work is a comparative analysis of the clinical efficacy and safety of IL-6, IL-1, JAK inhibitors and systemic glucocorticosteroids in the treatment for severe forms of COVID-19, according to the literature presented in PubMed.

MATERIALS AND METHODS

The materials for the comparative analysis were the data from the literature sources published in the PubMed system and devoted to 4 studies of the use of the systemic glucocorticosteroid dexamethasone [6], the IL-6 inhibitor tocilizumab [7], the IL-1 inhibitor anakinra [8] and the JAK inhibitor ruxolitinib [9] in the treatment of severe forms of COVID-19, including the analysis of the therapy data of 7406 patients. The selected sources contain comparable study endpoints (a drug effect on the 28-day survival).

The analysis was carried out by statistical evaluation of the drugs effect within the 28-day survival rate among the patients with severe COVID-19. The methods of attribute-based statistics were used as a statistical tool. The basis of the analysis with the use of attribute-based statistics is a contingency table (Table 1).

After compiling a contingency table, the following hypothesis has been formed: the use of the studied MPs makes it possible, to a greater extent, to achieve an increase in the survival rate within 28 days among the patients with a severe COVID-19 course compared to the controls.

The first stage is to determine the absolute efficacy (AE), which comes to calculating the frequency of the onset of positive clinical effects in the groups of patients who received and who did not receive MPs. Formula 1 was used to find the frequency of positive clinical outcomes in the exposed group (the patients receiving MPs) for each of the analyzed drugs.

$$AEe = \frac{a}{A} \quad (1)$$

Similarly, according to Formula 2, the frequency of occurrence of positive clinical effects in the unexposed group (the patients who did not receive MPs), was calculated.

$$PEn = \frac{c}{B} \quad (2)$$

As a result, the point estimates of the onset of positive clinical outcomes from the prescription of therapy regimens were obtained, including and not including the analyzed MPs (exposed and unexposed groups of patients). These frequencies were calculated on the basis of not the entire population, but only on its representative part, which approximately reflects the properties of the population. These point estimates were subjected to a statistical error. Therefore, the standard error of the obtained AEs was further calculated.

Since the obtained frequencies can change while calculating on another sampling, it was determined how significant these changes would be, and what minimum intervals of values would cover the actual exact values of the sought frequencies. In other words, what is the minimum interval that contains the real value of the sought frequency with a probability of 95% was to be determined. In statistics, this kind of interval is statistically 95% and is called “a confidence interval” (95% CI).

At the next stage, the attribute-based efficacy (AbE) was calculated. It characterizes the part of the efficacy (its share) that is associated with the studied MP and is explained by it. AbE was calculated according to Formula 3.

$$AbE = AEe - PEn = \frac{a}{c} - \frac{c}{B} \quad (3)$$

Based on the calculation of the relative efficacy (RE) according to Formula 4, the bonding force between the effect of MPs on the treatment and the outcome was shown, i. e., how many times the clinical efficacy of the therapy increases when the analyzed MPs are used.

$$RE = \frac{AEe}{PEn} = \frac{a/A}{c/B} \quad (4)$$

Population attribute-based efficacy (PAbE) is the absolute difference in indicators in the whole population and in the unexposed group. PAbE is similar to AbE but unlike the latter. It characterizes the population component of efficacy (Formula 5).

$$PAbE = \frac{C}{Q} = \frac{c}{B} \quad (5)$$

The safety assessment of the MPs products was carried out by analyzing potential drug- interactions. The information about potential drug interactions was obtained on a specialized website – Drugs.com. Know-more. Besure (https://www.drugs.com/

RESULTS

Statistically significant indicators are AbE, RE, and PAbE (Table 2).

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DOI: 10.19163/2307-9266-2020-8-5-316-324

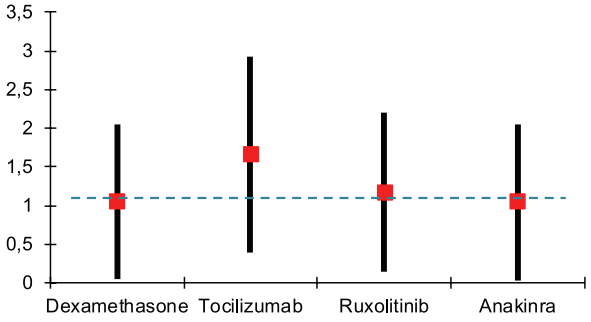

Figure 1 – Corridors of fluctuations in RE values with 95% CI of the MPP effect on the survival rates within 28 days

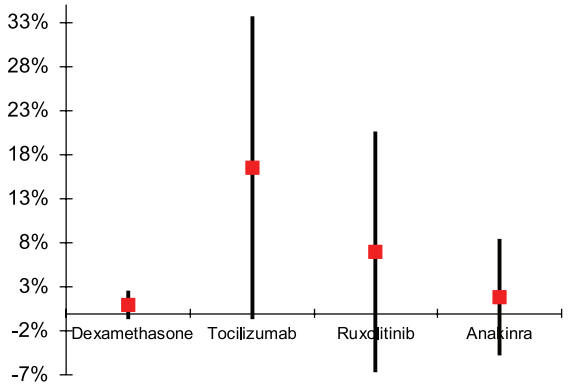

Figure 2 – Corridors of fluctuations in PAbE values with 95% CI of the effect of the studied drugs on the survival rate in the treatment of severe forms of COVID-19

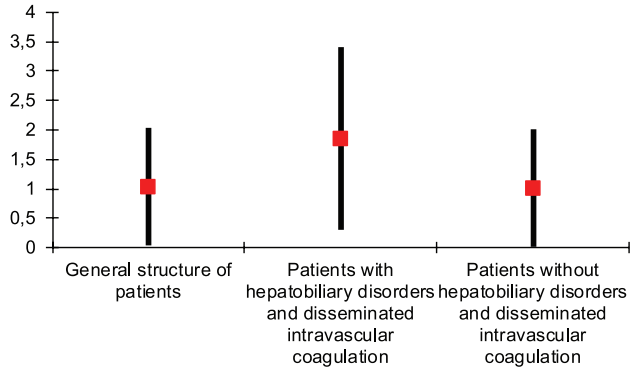

Figure 3 – Corridors of fluctuations in RE values with 95% CI of the effect of anakinra on survival rates within 28 days in different groups of patients

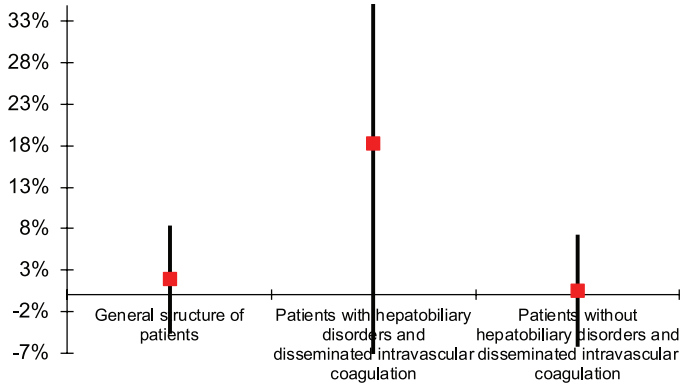

Figure 4 – Corridors of fluctuations in PAbE values with 95% CI of the anakinra effect on the survival rate in the treatment for severe forms of COVID-19 in different groups of patients

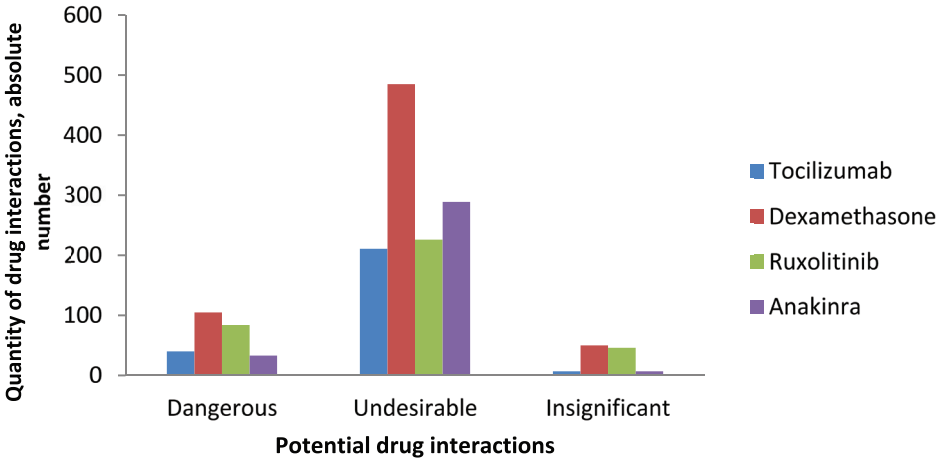

Figure 5 – Potential drug interactions of the drugs aimed at eliminating "the cytokine storm" in the treatment for severe COVID-19 conditions

Note: according to the specialized site – Drugs.com.Knowmore. Besure (URL: https: //www.drugs.com/interaction/list/)

Table 1 – Contingency table

Studied MP	Hypothetical effect of the studied drug		Total
	Yes	No	
Yes	**(a)** Group **in** a hypothetical state **with the effect** of the studied MP	**(b)** Group **out of** hypothetical state **with the effect** of the studied MP	**(A)** Sum a+b
No	**(c)** Group **in** a hypothetical state **without the effect** of the studied MP	**(d)** Group **out of** hypothetical state **without the effect** of the studied MP	**(B)** Sum c+d
Total	**(C)** Sum a+c	**(D)** Sum b+d	**(Q)** Sum A+B or C+D

Table 2 – The results of evaluating the clinical efficacy of various drugs in terms of survival within 28 days in the treatment for severe forms of COVID-19

Statistical value	MPs			
	Dexamethasone	Tocilizumab	Ruxolitinib	Anakinra
Attribute-based efficacy	3.1%	22.5%	14.3%	2.8%
Relative efficacy	1.04	1.66	1.17	1.04
Population attribute efficacy	1%	16.5%	7%	1.9%
NNT	**32**	**4**	**7**	**35**

Table 3 – The results of the clinical efficacy estimation of (anakinra's IL-1 inhibitor) in terms of the survival rate within 28 days in the treatment for severe forms of COVID-19 in patients with hepatobiliary dysfunction and disseminated intravascular coagulation

Statistical value	IL-2 inhibitor (anakinra)		
	General structure of patients	Patients with hepatobiliary disorders and disseminated intravascular coagulation	Patients without hepatobiliary disorders and disseminated intravascular coagulation
Attribute-based efficacy	2.8%	30.1%	0.8%
Relative efficacy	1.04	1.85	1.01
Population attribute efficacy	1.9%	18.2%	0.5%
NNT	**35**	**3**	**125**

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DOI: 10.19163/2307-9266-2020-8-5-316-324

Table 4 – Drug interactions aimed at eliminating "the cytokine storm" in the treatment for severe COVID-19 conditions

Drug interactions		Level (significance) of clinical interaction	Potential risk of clinical interaction
Dexamethasone	**Tocilizumab**	–	–
	Ruxolitinib	Undesirable	CYP450 3A4 inducers can reduce the concentration of ruxolitinib in the blood plasma; ruxolitinib is metabolized by isoenzyme.
	Anakinra	–	–
Tocilizumab	Dexamethasone	–	–
	Ruxolitinib	–	–
	Anakinra	Dangerous (life – threatening, should be avoided)	There is a risk of increased immunosuppression and an increased risk of developing an infectious process. Treatment with IL-6 inhibitors has been associated with serious, potentially life-threatening and fatal infections, including tuberculosis, invasive fungal infections such as candidiasis, aspergillosis and pneumocystosis, and other opportunistic infections. Cases occurred mainly in the patients administdated with concomitant immunosuppressive drugs or corticosteroids.
Ruxolitinib	Dexamethasone	Undesirable	CYP450 3A4 inducers can reduce the concentration of ruxolitinib in the blood plasma; ruxolitinib is metabolized by isoenzyme.
	Tocilizumab	–	–
	Anakinra	Undesirable	The use of interleukin-1 blockers with other immunosuppressive or myelosuppressive agents can increase the risk of infection. Interleukin-1 blockade can cause neutropenia and severe infections by itself, and the risk may be increased with another kind of immunosuppressive therapy.
Anakinra	Dexamethasone	–	–
	Tocilizumab	Dangerous (life – threatening, should be avoided)	There is a risk of increased immunosuppression and an increased risk of developing an infectious process.
	Ruxolitinib	Undesirable	The use of interleukin-1 blockers with other immunosuppressive or myelosuppressive agents can increase the risk of infection. Interleukin-1 blockade can cause neutropenia and severe infections by itself, and the risk may be increased with another immunosuppressive therapy.

Note: according to the specialized site – Drugs.com.Knowmore. Besure (URL:https://www.drugs.com/interaction/list/)

Table 5 – Potential drug interactions to be avoided in the treatment for severe COVID-19 conditions (drugs, the concomitant administration of which should be avoided: dangerous life-threatening clinically significant interaction)

Dexamethasone	Tocilizumab	Ruxolitinib	Anakinra
Fluroquinolone	Anakinra -	Clarithromycin	Tocilizumab
Amiodarone-	–	Fluconazole	–
–	–	Itraconazole	–
–	–	Ketoconazole	–
–	–	Voriconazole	–

For dexamethasone, AbE was 3.1% (95% CI 0.9% – 5.3%); for tocilizumab it was 22.5% (95% CI 4.6% – 40.4%); for ruxolitinibruxolitinib AbE was 14.3% (95% CI –1.7% – 30.2%); for anakinra it was 2.8% (95% CI -4.2% – 9.8%). This indicator is statistically significant for dexamethasone and tocilizumab.

As for the relative efficacy (RE), for dexamethasone it was 1.04 (95% CI 0.040 to 2.042); for tocilizumab – 1.66 (95% CI 0.400 to 2.917); for ruxolitinibruxolitinib – 1.17 (95% CI 0.139 to 2.194); for anakinra, RE was 1.04 (95% CI 0.038 to 2.046) (Fig. 1).

However, the lower limits of 95% of the confidence interval (CI) fall in the area of the negative values <1, which does not make it possible to consider this indicator statistically significant.

For the compared MPs, the lower limit of 95% of PAbE CI also falls into the area of the negative values, which does not make it possible to assert the statistical significance of the obtained indicator and requires additional confirmations (Fig. 2).

Comparing the 95% CI values for RE and PAbE, it is possible to speak of a greater advantage of the IL-6 inhibitor relative to the other analyzed MPs.

The Number Needed to Treat (NNT), the average indicator of the number of the patients who need to be treated with this drug, was also calculated to prevent one additional episode compared to the control group). For dexamethasone, the NNT is 32; for tocilizumab it is 4; for ruxolitinib – 7; for anakinra – 35. According to the data obtained, the effecacy of tocilizumab is higher than that of the other compared MPs. According to the results of the calculations, it is anakinra that has the lowest effecacy. However, the study carried out by Shakoory et al. [8], showed its high efficacy in terms of the survival rate within a 28-day period among the patients with disseminated intravascular coagulation (DIC) and hepatobiliary dysfunction (Table 3).

The results obtained, make it possible to speak about the choice of anakinra in the patients with severe forms of COVID-19, associated with disseminated intravascular coagulation, as well as with liver diseases.

RE of anakinra among the patients with concomitant is more than 1.5 times higher compared with the general structure of patients (Fig. 3).

The PAbE indicator is more than 9 times higher (Fig. 4).

According to the electronic resource Drugs.com, the data of the previous studies were the following: for dexamethasone, 640 potential interactions were identified, 105 of which were clinically dangerous, 485 were undesirable; for tocilizumab, 258 potential interactions were identified, 40 of which were clinically dangerous, 211 were undesirable; for ruxolitinib, 356 potential interactions were identified, 84 of which were clinically dangerous, 226 were undesirable; for anakinra, 329 potential interactions were identified, 33 of which were clinically dangerous, 289 were undesirable (Fig. 5).

In the course of the study, drug interactions aimed at eliminating "the cytokine storm" in the treatment for severe COVID-19 conditions which could potentially occur in a hospital, were also analyzed (Table 4).

Potential drug interactions that should be avoided and that can often occur when treating the patients for severe COVID-19, have also been identified (Table 5).

For example, fluoroquinolone therapy could take place in the treatment for pneumonia in the patients with COVID-19. In this case, against the background of the fluoroquinols use, the prescription of dexamethasone is dangerous.

A certain danger is represented by the use of glucocorticosteroid dexamethasone in the infectious process. It contributes to the development of secondary infections, superinfections. However, the data presented in a systematic review on the use of corticosteroids in the treatment for sepsis, show no statistically significant difference in the incidence of superinfection with long-term low-dose courses of glucocorticosteroids (16.75% versus 16.11%) [10].

DISCUSSION

On 2 September, 2020, WHO published guidelines for the use of corticosteroids in patients with COVID-19. WHO recommends systemic corticosteroids for the treatment of patients with severe and critical (gravy) COVID-19. Herewith, it is not recommended to use corticosteroids in the treatment of patients with mild forms of COVID-19, as this is not beneficial and may aggravate a patient's condition [11].

Corticosteroid therapy should be used with an extreme caution in the patients with diabetes mellitus. The fact that among patients with a severe course of COVID-19 there are people with diabetes mellitus, should be taken into consideration. Then, when planning purchases as well as the budget, it is necessary to take into account the availability of tocilizumab to stabilize the condition of patients with a developed "cytokine storm". In such cases, the use dexamethasone is dangerous.

When tocilizumab was used, superinfection developed twice as often compared with controls in the patients with COVID-19 who were on artificial lung ventilation (ALV) (54% versus 26%) [7]. Herewith, no statistically significant change in mortality within 28 days was found in the group of patients with superinfection and without it.

A particular risk from COVID-19 is the transition of patients to grave and critical conditions. The hospitalized patients with a diagnosis of severe COVID-19, have increased levels of cytokines. This increase may be associated with a cytokine release syndrome ("cytokine storm"), which is triggered by a number of factors (sepsis, cancer, organ transplantation), and in particular, viral infection [12]. The pathogenesis is based on a violation of the mechanisms of cellular cytotoxicity, an excessive

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DOI: 10.19163/2307-9266-2020-8-5-316-324

activation of cytotoxic lymphocytes and macrophages with a massive release of pro-inflammatory cytokines (a tumor necrosis factor (TNF-α), interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), a granulocyte colony-stimulating factor, monocytic chemoattractive protein 1), and inflammatory markers (C-reactive protein, serum ferritin), infiltration of internal organs and tissues activated by T-lymphocytes and macrophages – all these factors lead to a high-intensity inflammatory response [13, 14].

There is evidence of the successful use of an IL-1 receptor antagonist in the development of "a cytokine storm" [15]. The analysis of the data from phase III randomized study of the use of an IL-1 receptor antagonist (anakinra), indicates a significant improvement in the survival and the absence of serious adverse reactions in the patients with the development of sepsis [8]. Therefore, the use of an IL-1 receptor antagonist in severe forms of COVID-19, may be a promising direction in therapy and requires additional research.

A special place in the development of "the cytokine storm" in patients with COVID-19, belongs to IL-6, therefore, the effect on IL-6 and/or the mechanisms associated with its production, are the point of application in the treatment for severe patients. Interleukin 6 (IL-6) blockers are used to treat the "cytokine storm" in COVID-19 [16]. Thus, tocilizumab, which is a recombinant humanized monoclonal antibody that antagonizes the IL-6 receptor and is used, as recommended, in the treatment for rheumatoid arthritis, may play a key role in the treatment for critically ill patients with COVID-19 [17]. When using tocilizumab, an improvement in the main indicators during COVID-19 and a decrease in mortality in severe and critical conditions, has been shown [18].

CONCLUSION

In the course of the analysis, it was found out that the IL-6 inhibitor tocilizumab has the highest efficacy indicators, followed by the systemic glucocorticosteroid dexamethasone. The AbE and 95% CI values for the both drugs were statistically significant. The RE and PAbE values, are also higher for tocilizumab, however, 95% of the CIs of these indicators, fall into the area of statistically insignificant values, which requires additional confirmation of their efficacy. The choice of MPs should be based on a patient's condition, comorbidities and the drugs used in therapy, in order to minimize the risk of undesirable drug interactions.

Against the background of the lowest efficacy among the compared MPs, for the IL-1 inhibitor anakinra, its high efficacy was established for the patients with concomitant hepatobiliary disorders and disseminated intravascular coagulation, which should be taken into account when treating such patients.

FUNDING

This work did not have funding from outside organizations.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

CONTRIBUTION OF AUTHORS

Zhukova O.V. – collection, processing of material, statistical processing, text writing;
Kagramanyan I.N. – text writing, editing;
Khokhlov A.L. – the concept and design of the study.

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Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2020-8-5-325-335

EFFECT OF THE GABA DERIVATIVE SUCCICARD ON THE LIPID AND CARBOHYDRATE METABOLISM IN THE OFFSPRING OF RATS WITH EXPERIMENTAL PREECLAMPSIA IN EARLY AND LATE ONTOGENY

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Received 02 Jan 2019 Accepted 08 Jul 2020

Maternal preeclampsia can bring about metabolic disorders in the offspring at different stages of ontogeny. Up to date, no ways of preventive pharmacological correction of lipid and carbohydrate metabolism disorders developing in different periods of ontogeny in the children born to mothers with this pregnancy complication, have been developed.

The aim of the experiment was to study the effect of the gamma-aminobutyric acid derivative succicard (22 mg/kg) and its reference drug pantogam (50 mg) administered per os in the course of treatment in puberty (from 40 to 70 days after birth), on the parameters of lipid and carbohydrate metabolism in the offspring of the rats with experimental preeclampsia, in different periods of ontogeny.

Materials and methods. To assess the activity of lipid and carbohydrate metabolism in the offspring, an oral glucose tolerance test was performed at 40 days, 3, 6, 12 and 18 months of age. The level of glycosylated hemoglobin was measured at the age of 6, 12, and 18 months, and the concentrations of total cholesterol, high-density lipoprotein cholesterol and triglycerides were tested at 40 days, 3, 6, 12, and 18 months of age.

Results. The offspring of the rats with experimental preeclampsia, were found out to have lipid and carbohydrate metabolism disturbances during early (40 days and 3 months of age) and late (6, 12, and 18 months of age) ontogeny. In comparison with the offspring of healthy females, these disturbances were manifested by significantly higher levels of glucose revealed during the oral glucose tolerance test, by high glycosylated hemoglobin in males, and with elevated concentration of total cholesterol and triglycerides and a low level of high-density lipoprotein cholesterol in the negative control rats. Both the gamma-aminobutyric acid derivative succicard and its reference drug pantogam, reduced the negative effect of experimental preeclampsia on lipid and carbohydrate metabolism in the offspring in late ontogeny (6, 12 and 18 months of age). The effectiveness of succicard was either higher or comparable with pantogam.

Conclusion. Thus, the negative impact manifestations of experimental preeclampsia on lipid and carbohydrate metabolism, are revealed in the offspring in early (40 days and 3 months) and late (6, 12 and 18 months of age) ontogeny. The gamma-aminobutyric acid derivative succicard reduces the negative effect of experimental preeclampsia. Based on this finding, the drug implies the possibility of the development of a safe and highly effective medicine for preventive correction of lipid and carbohydrate metabolism disorders in the children born to mothers with preeclampsia.

Keywords: experimental preeclampsia; offspring; GABA derivatives; lipid and carbohydrate metabolism

Abbreviations. AP – arterial pressure; ATP – adenosine triphosphate; GABA – gamma-aminobutyric acid; IUGR – intrauterine growth restriction; TC – total cholesterol; OGTT – oral glucose tolerance test; PE – preeclampsia; TG – triglycerides; HDL-C – high-density lipoprotein cholesterol; EP – experimental preeclampsia.

For citation: E.A. Muzyko, V.N. Perfilova, A.A. Nesterova, K.V. Suvorin, I.N. Tyurenkov. Effect of the gaba derivative succicard on the lipid and carbohydrate metabolism in the offspring of rats with experimental preeclampsia in early and late ontogeny. *Pharmacy & Pharmacology*. 2020;8(5):325-335. **DOI:** 10.19163/2307-9266-2020-8-5-325-335

Для цитирования: Е.А. Музыко, В.Н. Перфилова, А.А. Нестерова, К.В. Суворин, И.Н. Тюренков. Влияние производного ГАМК сукцикарда на углеводный и липидный обмены потомства крыс с экспериментальной преэклампсией в ближайшие и отдаленные периоды онтогенеза. *Фармация и фармакология.* 2020;8(5):325-335. **DOI:** 10.19163/2307-9266-2020-8-5-325-335

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

ВЛИЯНИЕ ПРОИЗВОДНОГО ГАМК СУКЦИКАРДА НА УГЛЕВОДНЫЙ И ЛИПИДНЫЙ ОБМЕНЫ ПОТОМСТВА КРЫС С ЭКСПЕРИМЕНТАЛЬНОЙ ПРЕЭКЛАМПСИЕЙ В БЛИЖАЙШИЕ И ОТДАЛЕННЫЕ ПЕРИОДЫ ОНТОГЕНЕЗА

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Получена 02.12.2019 Принята к печати 08.08.2020

Преэклампсия у матери может служить причиной развития метаболических расстройств у потомства на разных этапах онтогенеза. На сегодняшний день не разработаны способы превентивной фармакологической коррекции нарушений углеводного и липидного обменов, возникающих в разные периоды жизни детей, рожденных от матерей с данным осложнением беременности.

Цель. Изучение влияния курсового перорального введения в пубертатном периоде (с 40 по 70 день жизни) производного гамма-аминомасляной кислоты сукцикарда (22 мг/кг) и препарата сравнения пантогама (50 мг) на показатели углеводного и липидного обменов потомства крыс с экспериментальной преэклампсией в разные периоды онтогенеза.

Материалы и методы. Для определения состояния углеводного и липидного обменов у потомства проводили Пероральный глюкозотолерантный тест в возрасте 40 дней, 3, 6, 12 и 18 месяцев, измеряли уровень гликированного гемоглобина в возрасте 6, 12 и 18 месяцев и определяли концентрации общего холестерина, холестерина липопротеинов высокой плотности и триглицеридов в возрасте 40 дней, 3, 6, 12 и 18 месяцев.

Результаты. Было выявлено, что у потомства крыс с экспериментальной преэклампсией на ранних (40 дней и 3 месяца) и поздних (6, 12 и 18 месяцев) стадиях онтогенеза наблюдаются нарушения углеводного и липидного обменов. Это проявляется в значительно более высоких по сравнению с потомством здоровых самок приростах уровня глюкозы при проведении Перорального глюкозотолерантного теста, высоком уровне гликированного гемоглобина у самцов, а также повышенной концентрации общего холестерина, триглицеридов и низком уровне холестерина липопротеинов высокой плотности у крыс группы негативного контроля.

Производное гамма-аминомасляной кислоты сукцикард и препарат сравнения пантогам ограничивают негативное влияние экспериментальной преэклампсии на липидный и углеводный обмены потомства на поздних стадиях онтогенеза (6, 12 и 18 месяцев). По эффективности действия сукцикард превышал или был сопоставим с препаратом сравнения пантогамом.

Заключение. Таким образом, негативное влияние экспериментальной преэклампсии на показатели липидного и углеводного обменов потомства проявляется, как на ранних этапах (40 дней и 3 месяца), так и в более отдаленные периоды (6, 12 и 18 месяцев) онтогенеза. Производное гамма-аминомасляной кислоты сукцикард уменьшает отрицательное действие экспериментальной преэклампсии, что позволяет предположить возможность разработки на его основе безопасного и высокоэффективного препарата для превентивной коррекции нарушений углеводного и липидного обменов у детей, родившихся от матерей с преэклампсией.

Ключевые слова: экспериментальная преэклампсия; потомство; производные ГАМК; липидный обмен; углеводный обмен

Список сокращений: АД – артериальное давление; АТФ – аденозинтрифосфат; ГАМК – гамма-аминомасляная кислота; ЗВУР – задержка внутриутробного развития; ОХ – общий холестерин; ПГТТ – пероральный глюкозотолерантный тест; ПЭ – преэклампсия; ТГ – триглицериды; ХС ЛПВП – холестерин липопротеинов высокой плотности; ЭП – экспериментальная преэклампсия.

INTRODUCTION

Preeclampsia is (PE) is a severe pregnancy complication causing adverse sequelae for both mother and child at different stages of postnatal ontogeny. In children, early complications of PE include premature birth and intrauterine growth retardations (IUGR), whereas it's long-term effects are manifested by a higher risk of developing cardiovascular, neurological, endocrine and metabolic disorders [1].

The damaging action of this pregnancy complication is associated with impaired cytotrophoblast invasion and deficient spiral arterial conversion, endothelial dysfunction, changes in the correlation between pro- and anticoagulant factors, an enhanced production of vasoconstrictors, which finally lead to circulatory disturbances in the "mother-placenta-fetus" system and hypoxy [2, 3]. The latter may cause changes in the organs and tissues

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DOI: 10.19163/2307-9266-2020-8-5-325-335

in the critical periods of the fetus development, which result in their dysfunction in late ontogeny [4].

A prenatal exposure to preeclampsia increases the risk of metabolic disorders in children at different stages of their individual development [5, 6]. It has been demonstrated that the children with a past history of cerebral ischemia, have a higher level of blood glucose [7], those with IUGR show elevated concentrations of total cholesterol (TC), triglycerides (TGs) and low-density lipoprotein cholesterol with a simultaneous decrease in high-density lipoprotein cholesterol (HDL) as compared to healthy children [8]. Furthermore, prematurely-born infants and those with IUGR born to mothers with preeclampsia, tend to show insulin resistance and obesity, respectively [9], which may contribute to the development of hypertension and type II diabetes mellitus at a more mature age.

To date, no ways of preventive pharmacological correction of lipid and carbohydrate metabolism disorders occurring at different stages of postnatal development in the children born to mothers with preeclampsia, have been discovered. Gamma-aminobutyric acid (GABA) derivatives are of special interest, as earlier studies have demonstrated their endothelium-protective, antihypoxic, antioxidant, vasodilating and antithrombotic action [10, 11]. Moreover, they promote the activation of both tissue respiration and oxidative phosphorylation, and enhance glucose utilization by cells [12], which implies their potential use for correcting lipid and carbohydrate metabolism in the offspring exposed to PE.

THE AIM of the present research was to study the effect of the course administration of the GABA derivative succicard and its reference drug pantogam in the puberty period (from the 40th to the 70th days of life) on the parameters of lipid and carbohydrate metabolism in the offspring of the rats with experimental preeclampsia (EP) at different stages of ontogeny.

MATERIALS AND METHODS

Experimental animals

The study was conducted on the offspring of white outbred rats – females weighing 230-250 g – with a physiological pregnancy and PE: males and females at the age of 40 days, 3, 6, 12 and 18 months (n=121). The animals were delivered from "Rappolovo breeding ground for laboratory animals" (Leningrad region). The females and their offspring were kept and cared for in the Volgograd State Medical University vivarium settings according to the recommendations of the national standard of the Russian Federation GOST R-33044-2014 Principles of Good Laboratory Practice. The study was conducted in compliance with the requirements of the Decree of MH RF No. 199n dated 01.04.2016 "On the Approval of the Guidelines for Laboratory Practice and the Directives of the European Parliament dated 2010/63/EU and the European Union Council dated 22.09. 2010 on the protection of animals used for scientific purposes". The experimental study protocol was approved by the Regional Independent Review Board (SU "Volgograd Medical Research Centre"): No.2044-2017 dated 25 December, 2017.

Modeling experimental preeclampsia

To be mated, the rats were placed in separate cages for 12 hours at the ratio of 2 females and 1 male. The pregnancy was detected when vaginal smears showed the presence of sperm. After that, each pregnant female was put in a separate cage. To model PE, the rats received a 1.8% sodium chloride solution instead of drinking water from the 1st to 21st day of pregnancy [13]. Increased arterial pressure (AP) and elevated urine protein on the 20th day of pregnancy as compared to the 1st day, were the signs indicating the development of PE. AP was measured in the females on the 1st and 20th days of gestation using a non-invasive blood pressure monitoring system CODA'TM Non-Invasive Blood Pressure System (Kent Scientific Corporation, USA). For a 24-hour urine collection, the female rats were placed in a metabolic cage (Nalgene, Italy). To assess total urine protein, a CliniTest-BM PGK panel (ECO-SERVIS, Russia) was used.

On the 1st day of pregnancy, AP readings in rats with physiological pregnancy and simulated EP were 121.95±6.62 and 119.54±8.31 mmHg, respectively. On the 20th day they were 109.74±5.16 and 133.61±9.64 mmHg, with an increase of 17.9% ($p<0.05$).

The level of 24-hour urine protein showed no significant differences in both groups at the beginning of pregnancy. However, on the 20th day of gestation, it was 4.91±0.40 mg per 24 hours in the females with PE, whereas in the healthy females it amounted to 2.38±0.26 mg per 24 hours.

The obtained findings were considered as the evidence that the females receiving saline solution, developed EP.

Study design

On the 39th day after birth, the offspring were moved away from the females. The experiment was conducted in two stages. The first stage involved the division into groups: 1, 2 – positive control – offspring (males n=10, and females n=10) born to the females without PE; 3, 4 – negative control – offspring (males n=11 and females n=10) born to the females with EP.

At the second stage, the groups were arranged in such a way that each group was made up of 10 animals: 1, 2 – positive control – offspring (males and females) born to the females without EP and receiving distilled water; 3,4 – negative control – offspring (males and females) born to the females with PE and receiving distilled water; 5, 6, 7, 8 – offspring (males and females) born to the females with EP and receiving the GABA derivative succicard (composition of 4-phenylpiracetam

and succinic acid in the ratio of 2:1) (Fig. 1) at the dose of 22 mg/kg and the reference drug pantogam (hopantenic acid, PIK-PHARMA PRO Ltd, Russia; syrup 100 mg/ml), at the dose of 50 mg, respectively. The offspring received a half of the effective dose of succicard for adult animals, which had been detected while studying neuro- and cardioprotective, antihypoxic and antioxidant activities [15]. Pantogam was chosen as a reference drug since it is used in clinical practice to treat children with posthypoxic disorders caused by various conditions including preeclampsia. Hopantenic acid was applied in effective doses on the basis of literature findings [16]. Succicard and the reference drug, were administered *per os* at the same time once every 24 hours, from the 40th to 70th days of postnatal ontogeny. The positive and negative control animals received a similar regimen of distilled water.

Identifiable parameters of lipid and carbohydrate metabolism

The oral glucose tolerance test (OGTT) was performed at the age of 40 days, 3, 6, 12, and 18 months. The level of glycosylated hemoglobin was measured at the age of 6, 12, and 18 months, and the concentrations of TC, HDL and TG were tested at the age of 40 days, 3, 6, 12, and 18 months.

When OGTT was performed, the blood was collected from the caudal vein after a 12-hour food deprivation. Glucose solution was intraorally administered to the rats at the rate of 4g of the substance per 1 kg of the animal weight, then, its concentration in the blood was measured 30, 60, 90, and 120 min after loading to assess an endogenous insulin activity [17]. Oxochrom Glucosa S panel for enzymatic detection of glucose by GOD-POD method (Erba Lachema, Czech Republic), was used. The optical density of the specimens was measured using a PE-5400V spectrophotometer (Ekros, Russia) (the wavelength 498 nm).

Glycosylated hemoglobin indicates the total level of glucose interacting with hemoglobin within the period of 3-4 months. Its amount in the blood collected from the sublingual vein, was quantified using a Glycohemoglobin reagent panel (High Technology, Inc., USA). A hemolyzed sample was mixed up with weak cation exchange resin. After a 5-minute incubation, filters were used to separate the supernatant containing glycosylated hemoglobin from the resin. To determine the content of glycosylated hemoglobin, the optical density of glycosylated hemoglobin fraction and that of total hemoglobin, were evaluated. The amount of glycosylated hemoglobin in the sample, was calculated as a ratio of these two optical density types. The measurements were made with a PE-5400V spectrophotometer (Ekros, Russia) with a wavelength of 415 nm.

The content of TC, HDL and TG in blood serum was determined using the following reagent panels – Total Cholesterol (Olvex Diagnosticum, Russia), High-density lipoprotein cholesterol (Olvex Diagnosticum, Russia) and Triglycerides (Olvex Diagnosticum, Russia). The blood was collected from the sublingual vein. The optical density was assessed by a PE-5400V spectrophotometer (Ekros, Russia).

Methods of statistical analysis of data

The study findings were statistically processed with STATISTICA v.12.5 software (StatSoft Inc., USA), which involved the Mann-Whitney U test, the Student's t-test to compare paired samples, the Newman-Keuls test for multiple comparisons with a prior assessment of the distribution normality based on the Shapiro-Wilk test. The differences were statistically significant when $p<0.05$.

RESULTS

OGTT findings revealed that the offspring born to the females with PE, showed a higher increase in the glucose level as compared to the positive control rates, which may be suggestive of carbohydrate metabolism disturbances.

In 40-day-old males born to the females with EP compared with the males born to the healthy females, the increase in the glucose level with reference to the original values (before loading) 60 min after its administration, was 3 times higher ($p<0.05$), after 90 min it was 4 times higher ($p<0.05$) and after 120 min this indicator was negative in the positive control group. However, 40-day-old females demonstrated the opposite tendency (Fig. 2A). In 3-month-old negative control males, significant differences were observed 30 minutes after the glucose administration – the increase was 1.4 times as high ($p<0.05$) as in the animals born to the rats with physiological pregnancy, whereas 90 and 120 min after the introduction, in the females, the increase was 1.8 and 2.9 times higher ($p<0.05$), respectively (Fig. 2B). In 6- month-old males born to the rats with a complicated pregnancy, the gain in the glucose level was significantly higher – 1.6, 2.1, 1.8, and 2 times higher ($p<0.05$) 30, 60, 90 and 120 min after the administration, respectively, as compared to the offspring of healthy females; in the female offspring it was 1.8, 2.8, 2.7 and 2 times higher ($p<0.05$) (Fig. 2C). At the age of 12 months, the negative control males showed an increase, which was significantly higher 30 and 60 min after the introduction (1.9 and 1.6 times ($p<0.05$)), the females demonstrated a high increase throughout the test (1.6 times ($p<0.05$) 30 min after, 1.4 times ($p<0.05$) in 60, 90, and 120 min (fig. 2D). The same trend retained in 18-month-old offspring. 60, 90 and 120 minutes after the glucose introduction, the negative control males showed a higher increase (1.2, 1.3 and 1.5 times ($p<0.05$)) as compared to the positive control group, in the females it was 1.4, 1.2 and 1.2 times higher ($p<0.05$) (Fig. 2E).

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DOI: 10.19163/2307-9266-2020-8-5-325-335

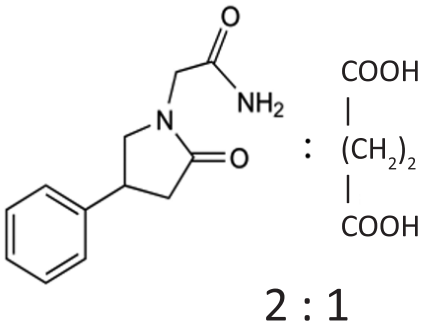

Figure 1 – Structural formula of succicard

Table 1 – Effect of GABA derivatives on the level of glycosylated hemoglobin in the offspring of the females with EP at the age of 6, 12 and 18 months (M±m)

Age	Animal groups	Rat gender	Level of glycosylated hemoglobin
6 months	Offspring of the females with physiological pregnancy receiving distilled water – positive control	Males	8.43±0.65
		Females	8.87±0.31
	Offspring of the females with EP receiving distilled water – negative control	Males	12.56±0.84$
		Females	9.01±0.61
	Offspring of the females with EP receiving succicard 22 mg/kg	Males	7.45±0.74#
		Females	8.94±0.74
	Offspring of the rats with EP receiving pantogam 50 mg	Males	10.69±0.73
		Females	10.71±0.78
12 months	Offspring of the females with physiological pregnancy receiving distilled water – positive control	Males	8.27±0.58
		Females	7.56±0.81
	Offspring of the females with EP receiving distilled water – negative control	Males	10.44±0.61*
		Females	7.49±0.83
	Offspring of the females with EP receiving succicard 22 mg/kg	Males	11.34±0.90
		Females	6.53±0.33
	Offspring of the rats with EP receiving pantogam 50 mg	Males	10.39±1.29
		Females	7.63±0.83
18 months	Offspring of the females with physiological pregnancy receiving distilled water – positive control	Males	9.6±1.00
		Females	10.55±0.94
	Offspring of the females with EP receiving distilled water – negative control	Males	12.26±0.87*
		Females	10.27±0.75
	Offspring of the females with EP receiving succicard 22 mg/kg	Males	9.85±0.76#
		Females	9.24±0.93
	Offspring of the rats with EP receiving pantogam 50 mg	Males	10.97±1.06
		Females	10.52±1.33

Note: $ – based on the Mann-Whitney test as compared to the positive control group; * – based on the Student's t-test as compared to the positive control group; # – based on the Newman-Keuls test as compared to the negative control group ($p<0.05$)

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

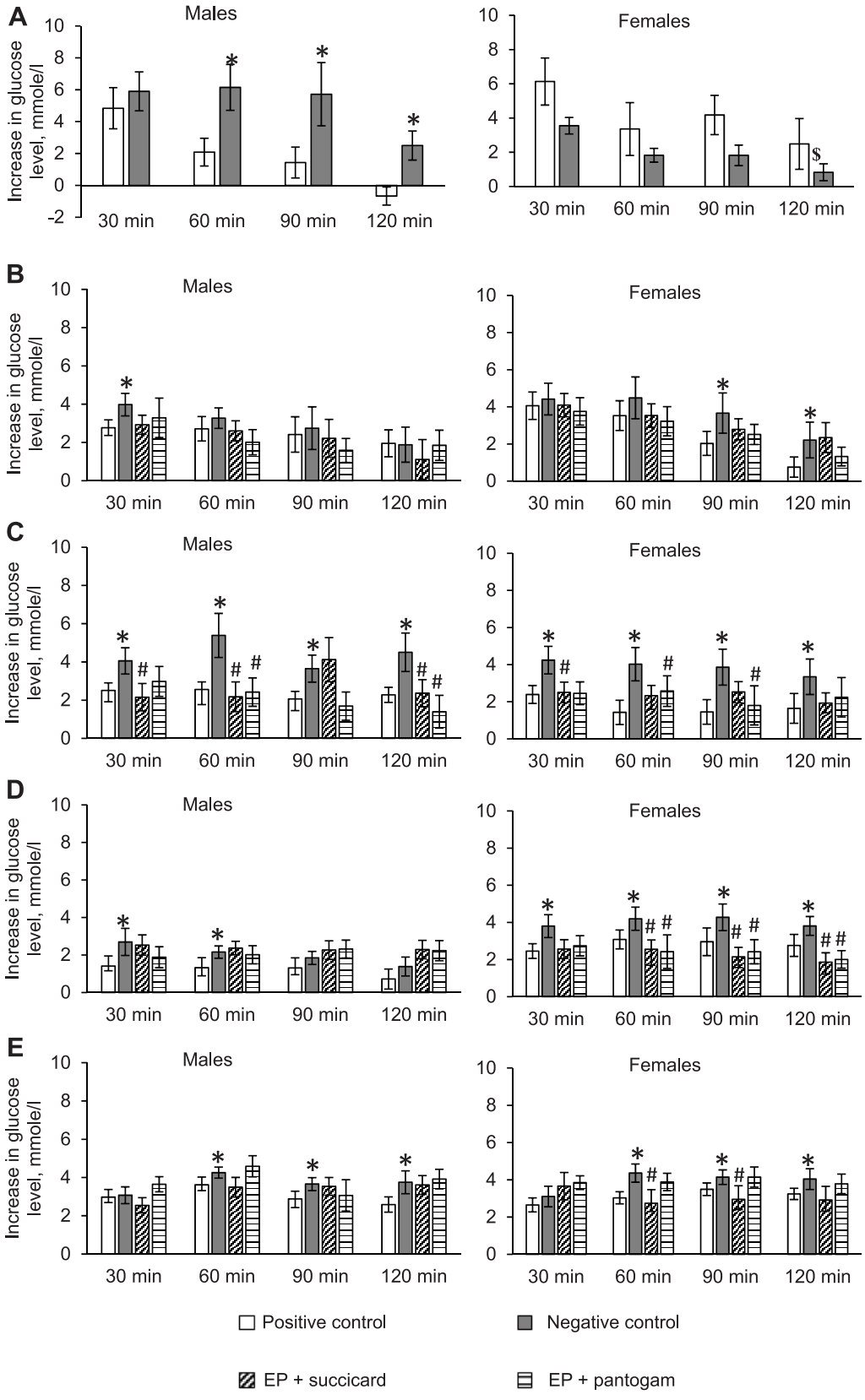

Figure 2 – Effect of GABA derivatives on the increase in glucose level in the offspring of the females with EP at the age of 40 days (A), 3 months (B), 6 months (C), 12 months (D), and 18 months (E) (M±m) on the basis of OGTT findings

Note: $ – based on the Mann-Whitney test as compared to the positive control group; * – based on the Student's t-test as compared to the positive control group; # – based on the Newman-Keuls test as compared to the negative control group ($p<0.05$)

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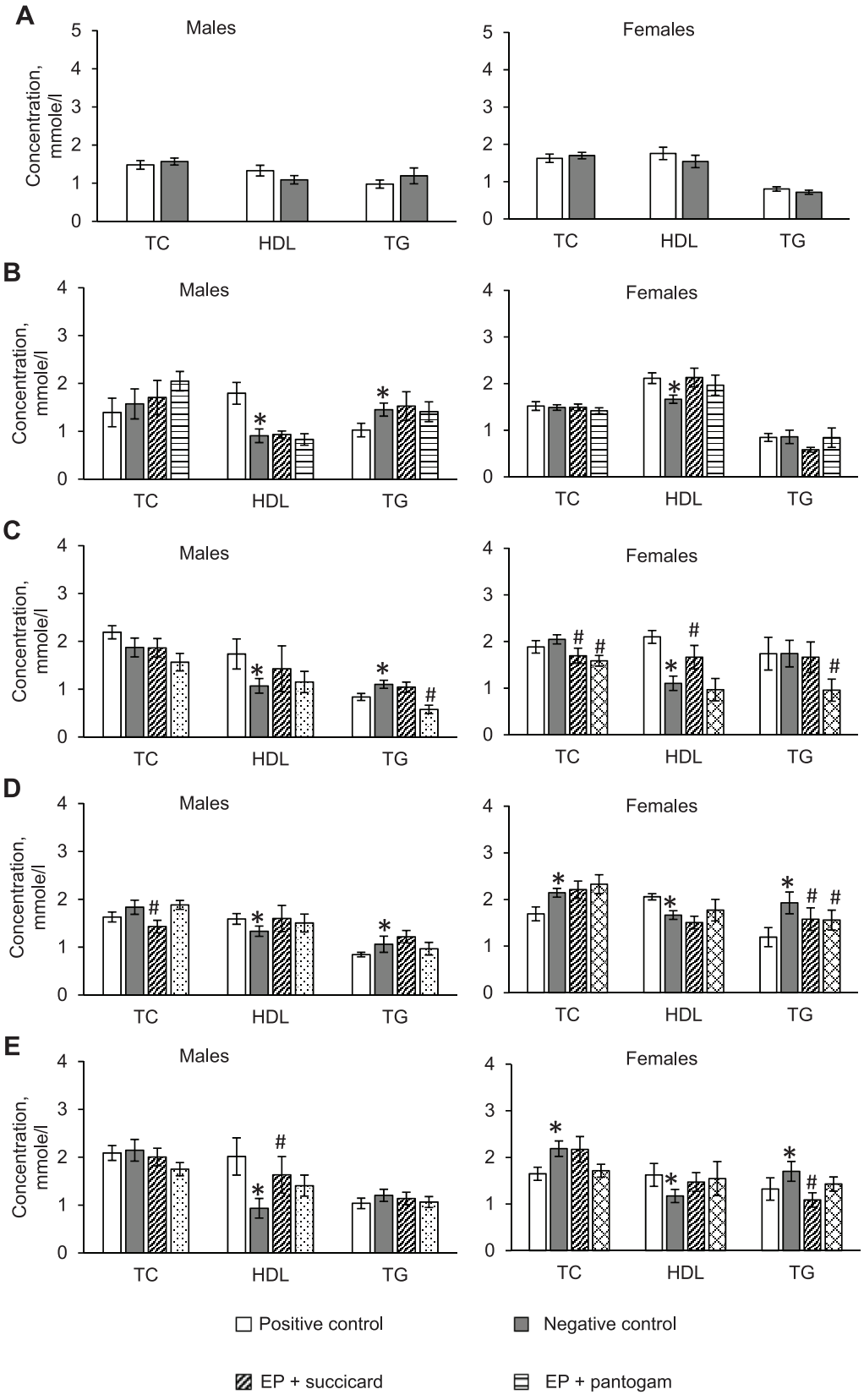

Figure 3 – Effect of the GABA derivatives on the lipid metabolism indices in the offspring of the females with EP aged 40 days (A), 3 months (B), 6 months (C), 12 months (D) and 18 months (E) (M±m)

Note: $ – based on the Mann-Whitney test as compared to the positive control group; * – based on the Student's t-test as compared to the positive control group; # – based on the Newman-Keuls test as compared to the negative control group ($p<0.05$)

In 3-month-old males and females receiving succicard and its reference drug, the increase was lower than in the negative control group, however, no significant differences were revealed (Fig. 2B). 6-month-old males receiving succicard, demonstrated a glucose increase which was 1.9, 2.5 and 1.9 times lower ($p<0.05$) 30, 60 and 120 minutes after the administration; in the rats receiving hopantenic acid, it was 2.2, 3.2 lower ($p<0.05$) after 60 and 120 minutes. In the females to which succicard had been administered, the increase was 1.7 times lower ($p<0.05$) 30 minutes after the introduction, in those receiving hopantenic acid it was 1.6 and 2.1 times ($p<0.05$) lower after 60 and 90 min (Fig. 2C). At the age of 12 months, significant differences were revealed only in the females to which GABA derivatives and the reference drug had been administered. Those receiving succicard, showed an increase which was 1.6 times ($p<0.05$) lower 60 min after the glucose introduction and 2 times lower after 90 and 120 min; in the rats receiving hopantenic acid it was 1.7, 1.8 and 1.9 times lower ($p<0.05$) after 60, 90 and 120 min (Fig. 2D). 18-month-old females, to which succicard had been administered, demonstrated significantly lower values of the increase 60 and 90 min after the glucose introduction (1.6 and 1.4 times ($p<0.05$)). No significant differences were revealed in the females receiving hopantenic acid as well as in the males receiving succicard and the reference drug as compared to the negative control group (Fig. 2E).

When estimating the level of glycosylated hemoglobin it was found out that in the males born to the rats with EP, this indicator was 1.5 times higher ($p<0.05$) at the age of 6 months, and 1.3 times higher ($p<0.05$) at the ages of 12 and 18 months as compared to the positive control group. Among the males, to which the studied GABA derivative and its reference drug had been administered, significant differences from the animals born to the females with the studied pregnancy complication were revealed only in the rats receiving succicard: at the age of 6 months their level of glycosylated hemoglobin was 1.7 times lower ($p<0.05$), and at the age of 18 months – 1.2 times lower ($p<0.05$). No significant differences between the females from the positive and negative control groups as well as those receiving succicard and hopantenic acid, were found out (Table 1).

No significant changes in the lipid metabolism indices were registered in the negative control animals aged 40 days as compared to the offspring born to the rats with physiological pregnancy (Fig. 3A).

The concentration of TC in blood in 3-month-old males and females born to the rats with EP did not change, their level of HDL was 2 and 1.3 times as low ($p<0.05$) as in the positive control group. The changes in TG concentration were observed only in the males of the negative control group – it was 1.4 higher ($p<0.05$) compared to the offspring of the healthy females (Fig. 3B). This tendency was retained at the age of 6 months: the males born to the rats with complicated pregnancy, showed a 1.6 times lower level of HDL ($p<0.05$), their TG level was 1.3 times higher ($p<0.05$); in the females the HDL, the concentration was 1.9 times lower ($p<0.05$) (Fig. 3C). 12-month-old males and females of the negative control group tended to demonstrate a lower level of HDL (1.2 times, $p<0.05$) and an increased concentration of TG in blood serum (1.3 and 1.6 times, $p<0.05$); the females also showed a higher level of EC (1.3 times, $p<0.05$) compared to the positive control group (Fig. 3D). The level of HDL in the males and females born to the rats with EP aged 18 months, was 2.2 and 1.4 times as low ($p<0.05$) as in the offspring of the healthy animals, respectively. At the same age, the TC and TG levels were 1.3 times higher ($p<0.05$) (Fig. 3E).

No significant differences were revealed in 3-month-old males and females receiving succicard and the reference drug compared to the negative control group (Fig. 3B). However, by the age of 6 months, the females which had been receiving succicard and hopantenic acid, showed a lower level of EC (1.2 and 1.3 times, $p<0.05$), respectively, in contrast to the offspring of the rats with EP. The concentration of HDL in the females, to which succicard had been administered, was 1.5 times higher ($p<0.05$). The offspring receiving hopantenic acid, showed lower levels of TG (1.9 and 1.8 times, $p<0.05$ in the males and females, respectively) compared to the negative control group (Fig. 3C). In 12-month-old males, to which succicard had been administered, a 1.3 times lower concentration of TC ($p<0.05$) was observed. The females, which had been receiving succicard and hopantenic acid, demonstrated a lower level of TG that was 1.3 times as low as in the offspring born to the rats with the complicated pregnancy (Fig. 3B). At the age of 18 months, statistically significant differences from the negative control group were registered only in the offspring receiving succicard: in the males, the HDL concentration was 1.8 times as high ($p<0.05$) and in the females the TG level was 1.6 times as low ($p<0.05$) (Fig. 3E).

DISCUSSION

To date, a number of studies have demonstrated that the impact of PE during the intrauterine period is associated with a higher risk of endocrine and metabolic disturbances both at early stages of postnatal development and in the late periods of life [5, 6, 18, 19]. According to the Weibull parametric survival model, hypertensive disorders of pregnancy including chronic hypertension, gestation hypertension and PE, significantly increase the risks of developing endocrine and metabolic disturbances in children up to the age of 18 and may be manifested as obesity, hyperlipidemia, and diabetes mellitus [9, 18]. In neonates, who suffered perinatal hypoxia, the level of EC and TG in blood serum, exceeds the age-specific normal values [20]. The study of lipid and carbohydrate metabolism in 3- and 6-month-old offspring born to the mice with EP, demonstrated an elevated concentration of blood glucose and its higher increase compared to the

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DOI: 10.19163/2307-9266-2020-8-5-325-335

control group of the animals registered by OGTT, with a simultaneous trend towards an insulin level decrease [21]. The male rats, which were on a high-fat diet and were subjected to perinatal hypoxia, showed a considerably elevated level of fasting glucose and impaired glucose tolerance [22].

The carried out experiments have revealed lipid and carbohydrate metabolism disturbances observed in the offspring of rats with EP both at early (40 days and 3 months) and late (6, 12, 18 months) stages of ontogeny. Compared to the offspring of healthy females, they were manifested by significantly higher increases in glucose levels, revealed during the oral glucose tolerance test; by the elevated glycosylated hemoglobin values in males, a high concentration of TC and TG, and low HDL in the negative control rats.

Hypoxic damage typical of this type of pregnancy complication, is likely to account for this effect of PE. The exposure to hypoxia during the critical periods of the fetal development, has an adverse impact on organs and tissues, and induces their functional impairments in the postnatal ontogeny [4].

B. Akhaphong et al. [2] demonstrated that the increased ß-cell death, a decrease in their area in the pancreas and changes in the mTOR protein level (mammalian target of rapamycin) regulating the growth and survival of cells, are observed in the offspring of rats born to the females with experimental gestation hypertension.

Another mechanism of PE adverse effect on children's metabolism is hypomethylation of imprinted genes. There is some evidence that children born to the mothers with PE, show alkylation-induced aberrations of differentially methylated gene regions IGF2, DLKI and MEST, which influence postnatal ontogeny. This process contributes to the development of obesity, diabetes, hypertension and other metabolic disorders in adulthood [24–26].

Impaired lipid and carbohydrate metabolism in the offspring caused by PE suffered by mothers, has a negative effect on their healths, decreases the quality of their lives, and reduces their lifespans. Therefore, a search for agents for correcting such PE complications, is of crucial importance.

The study carried out by the authors, has demonstrated that the cycle administration of the GABA derivative succicard and its reference drug in the puberty period, yielded a lesser increase in the glucose level registered by OGTT in the 6-month-old offspring. The administration of these agents at the age of 12 months, caused a significant decrease in this indicator only in the females, whereas at the age of 18 months the increase was significantly lower in the females, which had been receiving succicard, than in the negative control group. The assessment of glycosylated hemoglobin level has revealed that it was significantly lower only in 6- and 8-month-old males, to which succicard had been administered, as compared to the negative control group.

When the lipid profile was estimated in the males, which had been receiving the GABA derivative and the reference drug, a decrease in the TG level was observed at the age of 6 months in the rats receiving hopantenic acid; a reduced concentration of TC was registered at the age of 12 months and increased HDL was reported in 18-month-olds receiving succicard. 6-month-old females, which had been administered with succiard and the reference drug, showed a lower level of TC and TG, but higher HDL, whereas in 12-month-olds the concentration of TG in the blood serum was decreased. At the age of 18 months, significant differences from the negative control group were revealed only in the females receiving succicard: their TG level was lower.

These facts suggest that the GABA derivative succicard and hopantenic acid, promote improvement in lipid and carbohydrate metabolism indices at late stages of ontogeny (6, 12, 18 months) in the offspring of rats with EP. The efficacy of succicard either exceeded or was comparable with that of its reference drug.

The pharmacological properties of the succicard compounds (a composition of 4-phenylpiracetam and succinic acid), are likely to account for the obtained findings. S and R enantiomers of phenylpiracetam are selective inhibitors of dopamine reuptake and show affinity for the dopamine transporter, DAT [27]. Moreover, it is well-known that catecholamines and therapeutic agents, which stimulate the release or block the uptake of endogenous catecholamines, suppress appetite. In the experimental study, the oral administration of S-phenylpiracetam in the mice kept on a "western diet", and Zucker line obese rats for 8 and 12 weeks, resulted, respectively, in a considerable weight and body fat mass losses. Furthermore, they showed a significant decrease in the glucose level when OGTT was conducted [28].

Another assumptive mechanism of the favourable effect of succicard on the indices of lipid and carbohydrate metabolism in the offspring, can be associated with the fact that phenylpiracetam and succinic acid, which it is composed of, are capable of eliminating the hypoenergetic condition of pancreatic cells. The former has a pronounced antihypoxic action, stimulates oxidation-reduction processes, enhances glucose utilization by cells. Succinic acid is a Krebs cycle metabolite and can promote an increased production of NADH+H+, FADH+, which results in ATP production in mitochondria. The earlier studies have demonstrated that introduction of succicard after a chronic alcohol intoxication, leads to a decrease in the amount of primary and secondary products of lipid peroxidation, an increased activity of superoxide dismutase and glutathione peroxidase in cardiac and cerebral mitochondrial cells, and inhibits mitochondrial dysfunction [14].

CONCLUSION

Consequently, EP has a negative impact on the indices of lipid and carbohydrate metabolism in the offspring both at early (40 days and 3 months) and later (6, 12, 18 months) stages of individual development. The GABA derivative succicard decreases the negative effect of EP, which can suggest the likelihood of developing a safe and highly effective agent for a preventive correction of lipid and carbohydrate metabolism disturbances in children, born to mothers with EP on its basis.

FUNDING

The present study was not funded by any third-party organizations.

AUTHOR CONTRIBUTION

E.A. Muzyko – implementation of the main stages of the experiment, analysis and interpretation of the findings, article writing.
V.N. Perfilova – analysis and interpretation of the data, verification of the crucial intellectual content, approval of the manuscript for publication.
A.A. Nesterova – analysis and interpretation of the data.
K.V. Suvorin – implementation of the main stages of the experiment.
I.N. Tyurenkov – development of the concept and design, verification of the crucial intellectual content, a final approval of the manuscript for publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

COMPARATIVE REVIEW OF METHODOLOGIES FOR ESTIMATING THE COST OF ADVERSE DRUG REACTIONS IN THE RUSSIAN FEDERATION AND BRAZIL

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Received 28 May 2020 Accepted 25 Oct 2020

The aim of the review article was to highlight the methodologies for assessing the financial costs of adverse drug reactions exemplified by the Russian Federation and Brazil.
Materials and methods: for a comparative analysis, materials from open sources were used. The study of the experience of methods used for assessing the burden of adverse drug reactions, was carried out using the system for calculating payments for medical care by clinical-statistical and clinical-profile groups, the methodology for assessing the severity of adverse events of the US National Cancer Institute, drug-associated problems, and "the decision tree" model.
Results. When comparing the costs of ADR management in the Russian Federation and Brazil, the following results have been obtained: in the Russian Federation, the "cost" of reaction can be estimated only for a limited number of nosological groups that are regulated by the classification of diseases by clinical and statistical groups; in Brazil, when predicting the costs of adverse reactions management, the combination of "the decision tree" method and the Delphi method is used. In the Russian Federation, the cost of the 3rd and above severity adverse event (according to CTCAE v. 4.03), varies from 26,849.22 up to 26,196.37 RUB in the North-West region (St. Petersburg). In Brazil, the cost of ADR ranges from 13 USD (the best scenario for the patient) to 574 USD (the worst scenario for the patient), which is about 975 and 43,000 RUB, respectively. The introduction of methods that make it possible to predict the development and potential outcomes of adverse drug reactions, as well as taking into account the experiences of foreign colleagues in their modeling, will reduce economic costs in the Russian Federation at the federal level.
Conclusion: for the economic value analysis and further forecasting, an improvement of existing methodologies is required. The models used in the Russian Federation ("the decision tree", classification of diseases by clinical groups, Markov model) do not take into account the time factor, therefore, when planning the analysis of potential costs for adverse reactions, it is necessary to reinforce the methods with such tools as QALY, YLL, and YLD.
Keywords: adverse drug reactions; pharmacovigilance; pharmacoeconomics; modeling; disease burden
Abbreviations: ADRs – Adverse drug reactions; AIS – Automatic information system; CICU – Critical and intensive care unit; ALV – artificial lung ventilation; ARCADe – Adverse Reactions in Crimea, Autonomic Database; AWF – average weight factor; CPG – Clinical profile group; CSG – Clinical statistic group; DALY – Disability Adjusted Life Year; DRM – Drug-Related Morbidities; DRP – Drug Related Problems; GBD – Global Burden of Disease; GTA – General Tariff Agreement; HLE – Healthy Life Expectancy; ICU – intensive care unit; MedDRA – Medical Dictionary for Drug Regulatory Affairs; MMR – Maternal Mortality Ratio; MS – Medical substance; NMP – New Medical Problem; NPS – National Pharmacovigilance System; PCNE – Pharmaceutical Care Network Europe; PE – Pharmacoeconomics; PSG – The program of state guarantees of free provision of medical care to citizens; QALY – Quality-adjusted life year; SADR – Serious Adverse Drug Reaction; SAR – Serious Adverse Reaction; SGBP – Program of state guarantees for free provision of medical care to citizens; SOFA – Sequential Organ Failure Assessment; SUS – Sistema Unico de Sau; SUSAR – Suspected Unexpected Serious Adverse Reaction; TF – Treatment Failure; UADR – Unexpected Adverse Drug Reaction; UAE – Unexpected adverse events; USD – United States dollar; WACIF – Weighted average cost intensity factor; YLL – Years of Life Lost; YLD – Years Lived with Disability.

For citation: G.I. Syraeva, A.S. Kolbin, A.V. Matveev, V.S Panezhina. Comparative review of methodologies for estimating the cost of adverse drug reactions in the Russian Federation and Brazil. *Pharmacy & Pharmacology*. 2020;8(5):336-344. **DOI:** 10.19163/2307-9266-2020-8-5-336-344

Для цитирования: Г.И. Сыраева, А.С. Колбин, А.В. Матвеев, В.С. Панежина. Сравнительный обзор методологий оценки стоимости нежелательных лекарственных реакций в Российской Федерации и Бразилии. *Фармация и фармакология.* 2020;8(5):336-344. **DOI:** 10.19163/2307-9266-2020-8-5-336-344

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2020-8-5-336-344

СРАВНИТЕЛЬНЫЙ ОБЗОР МЕТОДОЛОГИЙ ОЦЕНКИ СТОИМОСТИ НЕЖЕЛАТЕЛЬНЫХ ЛЕКАРСТВЕННЫХ РЕАКЦИЙ В РОССИЙСКОЙ ФЕДЕРАЦИИ И БРАЗИЛИИ

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Получено 28.05.2020 Принята к печати 25.10.2020

Цель работы: рассмотреть методологии оценок финансовых затрат на сопровождение нежелательных лекарственных реакций на примере Российской Федерации и Бразилии.
Материалы и методы: для сравнительного анализа использовались материалы, находящиеся в источниках с открытым доступом. Изучение опыта применения методик по оценке бремени нежелательных лекарственных реакций проводился с использованием системы расчёта оплаты медицинской помощи по клинико-статистическим и клинико-профильным группам, методики оценки тяжести осложнений Национального института рака США, лекарство-ассоциированных проблем и модели «дерева решений».
Результаты: при сравнении затрат на ведение нежелательных реакций в РФ и Бразилии были получены следующие результаты: в РФ оценить «стоимость» реакций можно только для ограниченного числа нозологических групп, которые регламентированы классификацией заболеваний по клинико-статистическим группам; в Бразилии при прогнозировании затрат на ведение нежелательных реакций используется сочетание метода «дерева решений» и метода Дельфи. В РФ стоимость сопровождения реакций третьей степени тяжести по CTCAE v. 4.03 и выше составляет от 26849,22 руб. до 26196,37 руб. для Северо-Западного региона (г. Санкт-Петербург). В Бразилии стоимость сопровождения реакций составляет от 13 долларов США (затраты, при развитии лучшего для пациента сценария) до 574 долларов США (затраты, при развитии худшего для пациента сценария), что составляет около 975 и 43000 рублей, соответственно. Внедрение методик, позволяющих прогнозировать развитие и потенциальные исходы нежелательных реакций, а также учёт опыта зарубежных коллег при их моделировании, позволит снизить экономические затраты в РФ на федеральном уровне.
Заключение: для анализа экономической стоимости и дальнейшего прогнозирования требуется совершенствование существующих методологий. Применяемые в РФ модели («дерево решений», классификации заболеваний по группам, модель Маркова) не учитывают временной фактор, соответственно, при планировании анализа потенциальных затрат на НЛР требуется дополнение методов такими инструментами как QALY, YLL, YLD
Ключевые слова: нежелательные лекарственные реакции; фармаконадзор; фармакоэкономика; моделирование; бремя болезни
Список сокращений: АИС – Автоматизированная информационная система; ГТС – Генеральное тарифное соглашение; КПГ – Клинико-профильная группа; КСГ – Клинико-статистическим группа; ЛП – Лекарственный препарат; ЛС – Лекарственное средство; НЛР – Нежелательная лекарственная реакция; ННР – Непредвиденные нежелательные (лекарственные) реакции; ОРИТ – Отделение реанимации и интенсивной терапии; ПГГ – Программа государственных гарантий бесплатного оказания гражданам медицинской помощи; СКЗ – Средневзвешенный весовой коэффициент затратоемкости; СННР – Серьезные непредвиденные нежелательные (лекарственные) реакции; ФЭК – Фармакоэкономика; DALY – Disability Adjusted Life Year/эквивалент потери 1 года здоровой жизни; DRM – Drug-Related Morbidities/заболевания связанные с приемом лекарственных средств; DRP – Drug Related Problems/проблемы связанные с приемом лекарственных средств; GBD – Global Burden of Disease/глобальное бремя заболевания; HLE – Healthy Life Expectancy/ожидаемая продолжительность здоровой жизни; MedDRA – Medical Dictionary for Drug Regulatory Affairs/Медицинский словарь для регуляторной деятельности; MMR – Maternal Mortality Ratio/коэффициент материнской смертности; NMP – New Medical Problem/новая медицинская проблема; NPS – National Pharmacovigilance System/национальная система фармаконадзора; PCNE – Pharmaceutical Care Network Europe/Европейская сеть фармацевтической опеки; SAR – Serious Adverse Reaction/серьезные нежелательные (лекарственные) реакции; SOFA – Sequential Organ Failure Assessment/шкала оценки органной недостаточности у пациентов, находящихся на интенсивной терапии; SUS – Sistema Unico de Sau/система здравоохранения Бразилии; SUSAR – Serious Unexpected Serious Adverse Reaction/серьёзные непредвиденные нежелательные (лекарственные) реакции; TF – Treatment Failure/неудача лечения; QALY – Quality-adjusted life year/качество жизни с поправкой на год; UADR – Unexpected Adverse Drug Reaction/непредвиденные нежелательные (лекарственные) реакции; USD – United States dollar/доллар США; YLL – Years of Life Lost/потерянные годы жизни; YLD – Years Lived with Disability/годы жизни связанные с инвалидностью.

INTRODUCTION

The costs of drug provision come to the fore when planning the budget of the healthcare system [1]. It is necessary to take into account not only the benefits of prescribed medicinal products but also the potential risks and predictability of adverse drug reactions (ADRs) development. A comprehensive assessment of the risk/benefit ratio during the prescribing of medicinal products (MPs) should include an analysis of clinical efficacy, a safety profile, and potential economic consequences [2]. Methodological approaches to assessing pharmacoeconomic parameters are varying and include the analysis of the databases on known ADRs, and the subsequent draw of conclusions and formulation of recommendations to minimize ADRs costs; high-quality clinical trial data; the results of clinical and economic research as well as a pharmacoeconomic analysis [3].

Adverse drug reactions are harmful and unexpected reactions in response to the use or withdrawal of a medicinal product prescribed in therapeutic doses to a person for prevention, diagnosis, therapy, or changing physiological functions [4]. The aim of continuous ADRs monitoring is not only to identify previously unknown medical effects and potential drug interactions, but also to control the increase in the frequency of occurrence of known ADRs and/or their severity, to identify risk factors and possible mechanisms that cause them, and to spread information required to improve the prescription of drugs [5].

For a comprehensive assessment of an adverse event, in both global and Russian kinds of practice, the presence and/or the absence of seriousness criterion is also taken into account as well as the expectedness of the event. Therefore, reactions can be classified as unexpected adverse reactions (UADRs), Serious Adverse Drug Reactions (SADR), and Suspected Unexpected Serious Adverse Reactions (SUSAR) (Official website of Roszdravnadzor, 2020). The collection of ADRs data in different countries is carried out in a similar manner – notification forms that contain the data that make possible the identification of the patient, a suspected drug, the ADR description, and information about these sources [6].

According to the information from Roszdravnadzor, about 30% of the notification forms it receives, do not contain the minimum required data for a complete analysis [8].

The deviations in the ADR manifestations, are possible due to various characteristics of the population (demographic, genetic, etc.). In addition, they can be explained by the conditions of drug manufacturing and transportation. It does not make it possible to fully extrapolate the available foreign data to the Russian practice, and requires the study of ADRs on the territory of the Russian Federation.

The pharmacovigilance system, which was introduced into routine medical practice at the post-authorization stage of MPs use around the world, makes it possible to accumulate and evaluate the data on ADRs [9]. Working with these resources, is aimed at collecting and analyzing information on adverse events not specified in the instructions for medical use, UADR, SADR and SUSAR, and drug interactions. The collected information is evaluated by experts of regulatory authorities, often with the involvement of other expert organizations. ADRs databases maintained by WHO and national regulatory authorities, accumulate information on the events related to the safety of MPs, for example, Vigibase, Eudravigilance, FAERS, etc. An Automatic Information System (AIS) for the input of ADR information called "FARMAKONADZOR", has been introduced by the Russian Federation [10]. The ARCADe regional database (Adverse Reactions in Crimea, Autonomic Database) has been used since 2009 on the territory of the Republic of Crimea and cumulates the data of spontaneous reports in the region [11]. Brazil has a National Pharmacovigilance System (NPS) of their own, and it operates as a part of the Brazilian National Health Surveillance Agency (ANVISA) [12].

Regardless of the particular type of a local pharmacovigilance system and pharmacovigilance assessment methods, the obtained data make it possible to draw a preliminary conclusion regarding the safety profile and the effectiveness of drugs. However, spontaneous reporting does not take into account the time factor in the ADRs development, and does not make it possible to assess the pharmacoeconomic costs of a separate ADR, including indirect costs, and additionally, it cannot predict further ADRs development.

THE AIM of this review article is to highlight the methodologies for assessing the financial costs of adverse drug reactions exemplified by the Russian Federation and Brazil.

MATERIALS AND METHODS

The costs of ADRs must be taken into consideration in an integrated manner, i.e., with keeping account of a range of other problems associated with ADRs. A number of pharmacoeconomic (PE) methodological approaches can be used. In this case, the main stages of the analysis, can be divided into two large blocks (or directions): pharmacoeconomic and pharmacoarithmetic ones. In the first block, after assessing the economic and clinical components and their synthesis, the sustainability of the results should be assessed, a sensitivity analysis should be carried out, and after these stages, the conclusions are to be drawn and recommendations oriented towards decision-making in the healthcare sector are to be made. In the second block, the economic component (costs of pharmacotherapy) is assessed, and after assessing the sustainability of the results and the sensitivity analysis, the conclusions are drawn. Unlike the first approach, the conclusions are not prioritized in decision-making for the entire system of public healthcare.

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DOI: 10.19163/2307-9266-2020-8-5-336-344

Estimating the costs of ADRs obviously requires a more systematic approach, with an assessment of possible outcomes and a detailed patient routing. The route of a patient in PE models can be outlined using either the decision tree model or the Markov model (or a combination of these two methods). All the three approaches have certain advantages and limitations. Thus, the "decision tree" model considers the state of the system (in this case, the patient's condition) at the input and output, and its final state, i.e., the exit from the model is determined by the sum of initially specified events developing sequentially and having a certain degree of occurrence probability. In the final assessment, a number of factors, e.g. temporal, are excluded. Unlike models of the "decision tree" type, which consider the state of the system at the input and output, Markov models (Fig. 1) take into account the probability of the system transition from one state to another during the so-called Markov cycle, i.e. in a given time interval [13, 14].

Fig. 1 shows the graph of the Markov model, possible in our case. Several states of the patient are presented: "Health", "ADR", "Death" and the "Disease" is known, as well as the probabilities of transition from one state to another (Px) during a given time interval. This Markov cycle can be extended by adding additional states. The duration and frequency of cycles depend on a specific clinical situation. Additional factors taken into account in the process of the model building, make possible evaluating predictions more accurately [14].

The models described above were originally developed to predict the outcomes of infectious diseases. Subsequently, these methodologies were successfully applied in PE modeling of various therapeutic and surgical outcomes, as well as in the assessment of the effectiveness of health technologies. Such modeling algorithms, which include findings from existing databases indicating the frequency and quality of outcomes, can be extrapolated to the prediction and course of ADRs.

When assessing the ADR cost, it seems reasonable to use the European DRP PCNE (Drug Related Problems of Pharmaceutical Care Network Europe) system, which makes it possible to systematize the events related to pharmacological safety issues in validated reports. The versions of the PCNE system, are regularly updated through periodic revisions by a working group of experts. The PCNE approach is based on the coding of problems into several categories: problems, causes, interventions, and outcomes, which in turn are divided into subcategories [11, 15, 16]. It is necessary to take into consideration the fact that modeling, aimed specifically at assessing economic costs during the evaluation of the "global burden of disease" (GBD), also requires keeping account of such indicators as the cost/utility ratio, the analysis of the impact on the budget as well as discounting with a planning time horizon of more than one year. When using the time factor, the following indicators can be used: mortality, maternal mortality ratio (MMR), years lived with disability (YLD), years lost for life (YLL), healthy life expectancy (HLE), disability-adjusted life year (DALY), etc. [17].

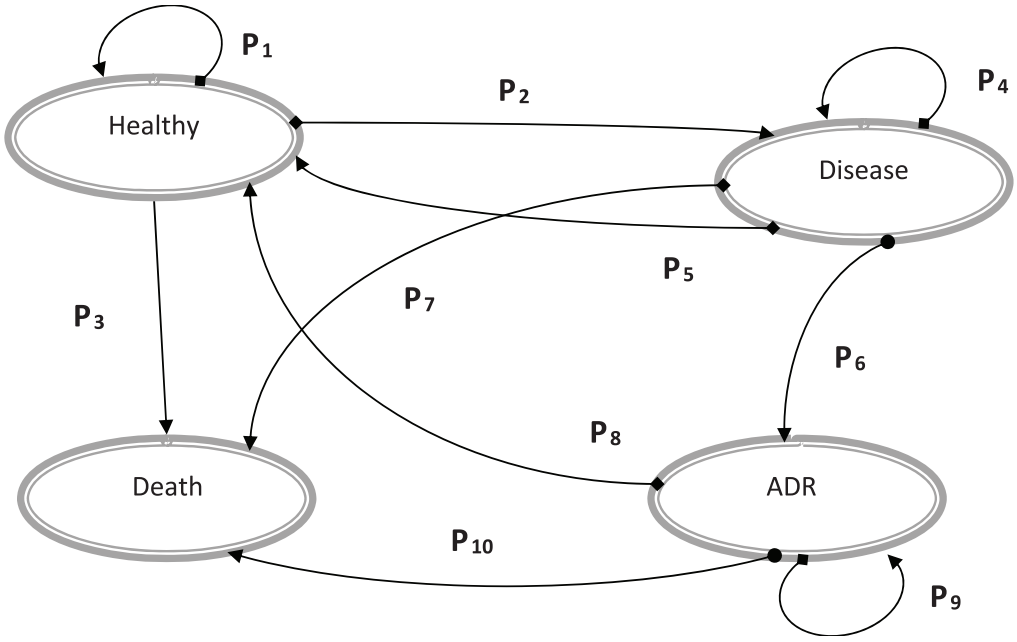

Figure 1 – An example of a possible Markov cycle, where P_x are the probabilities of transition from one condition to another

Table 1 – Cost of adverse drug reactions for 2019, calculated on the basis of the CSG

Adverse events	The cost of drug A therapy, ADR ≥ Stage 3 (roubles)	The cost of drug B therapy, ADR ≥ Stage 3 (roubles)
GTA of Saint-Petersburg	26 849,22	26 196,37
Basic tariff of SGBP	36 790,16	35 593,73
Federal CSG	70 613,19	76 218,45

Note: GTA – General Tariff Agreement; SGBP – Program of state guarantees for free provision of medical care to citizens; CSG – clinical and statistical group; ADR – Adverse Drug Reaction

Table 2 – Results of an expert assessment of ADRs costs in various scenarios according to the data de Feritas [19]

Outcome	Base analysis		Best scenario		Worst scenario	
	Costs (USD)		Costs (USD)		Costs (USD)	
Without additional treatment	Visiting a doctor and primary prescription of drugs	28	Primary prescription of drugs	13	Visiting a doctor and primary prescription of drugs	28
Additional therapy prescribed	Visiting a doctor and primary prescription of drugs + additional therapy prescribed	42	Primary prescription of drugs + additional therapy prescribed	27	Visiting a doctor and primary prescription of drugs + additional medical consultation + additional therapy prescribed	57
Consultation of specialist needed	Visiting a doctor and primary prescription of drugs + additional medical consultation + additional therapy prescribed	71	Visiting a doctor and primary prescription of drugs	28	Special medical consultation and primary prescription of drugs + additional special consultation + additional therapy prescribed	86
Urgent care required	Visiting a doctor and primary prescription of drugs + 2 days of in-hospital treatment	86	Visiting a doctor and primary prescription of drugs + 1 days of ICU treatment	43	Visiting a doctor and primary prescription of drugs + 5 days of in-hospital treatment	173
Hospitalization or prolongation of hospitalization	Visiting a doctor and primary prescription of drugs + 6 days of in-hospital treatment	374	Visiting a doctor and primary prescription of drugs + 3 days of in-hospital treatment	186	Visiting a doctor and primary prescription of drugs + 9 days of in-hospital treatment	574
Long-term follow-up in a medical facility	Visiting a doctor and primary prescription of drugs + 30 days of in-hospital treatment	2715	Visiting a doctor and primary prescription of drugs + 10 days of ICU treatment	1418	Visiting a doctor and primary prescription of drugs + 30 days of ICU treatment	4191
Death	Visiting a doctor and primary prescription of drugs	374	Visiting a doctor and primary prescription of drugs	101	Visiting a doctor and primary prescription of drugs	480

Note: USD – USA dollars; ICU – intensive care unit

As seen from the description of the approaches for collecting information about ADRs, these methodologies do not imply an assessment of the time factor, and therefore, forecasting the ADRs "burden" in the Russian Federation has a number of limitations. Expenditures on diagnostics, treatment, and prolongation of hospitalization, all in relation to a specific nosology, should be taken into account in the assessment of the economic costs of ADRs. In addition, the indirect costs associated with missing working days, disability, and a decrease in the quality of life, should be also taken into consideration.

RESULTS AND DISCUSSION

At the federal level, the Russian Federation maintains a unified safety database. The information is recorded on the basis of Federal Law No. 61-FZ dated 12 April 2010 (as amended on 03.04.2020) "On Circulation of Medicinal Products" and Article 64 "Pharmacovigilance". Since April 2019, coding of ADRs in the Auto-

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DOI: 10.19163/2307-9266-2020-8-5-336-344

matic information system of Roszdravnadzor is carrying out using the MedDRA (Medical Dictionary for Drug Regulatory Affairs) classifier with obligatory indicating of the system-organ class of the reaction that has arisen. In addition, the contact person who provided the information, the international non-proprietary name that caused the ADR, its dosage, start and end dates, the causal relationship type for the drug-event pair, the concomitant therapy, de-challenge and re-challenge results for the suspected drug (if applicable), are indicated. According to WHO recommendations, the minimum information to be reported also includes the severity and seriousness of ADR.

Since 2013, the Russian Federation has introduced a system for calculating the reimbursement of medical care from the state foundation of compulsory medical insurance based on diseases classification according to the so-called "Clinical and statistical groups" (CSG). They represent the groups of diseases belonging to the same profile of medical care, and similar in the methods used for diagnosing and treating patients, as well as in average resource intensity (cost, cost structure and set of resources used).

A concept of "clinical profile group" (CPG) which is a group of CSGs and/or individual diseases, united by one profile of medical care", can also be used for such calculations [19]. The group of CPGs includes conditions that require hospitalization. Tariffs to assess the complications of pharmacotherapy, were developed for CPG.

The formation of such groups is carried out on the basis of the parameters that determine the relative capacity of treatment costs: diagnosis, type of health technology or intervention used, patient's age, gender, concomitant pathology or complications of the disease, duration and treatment regimen, duration of staying on artificial lung ventilation (ALV) if necessary. The results of assessing the patient's condition according to clinical scales (for example, the scale for assessing the organ failure in the patients in the intensive care department (Sequential Organ Failure Assessment (SOFA), rehabilitation routing scale, etc.), are also taken into consideration. The average weight factor (AWF) which is calculated according to the given formula and the number of cases in the previous year, are used to calculate the amount of expected similar cases in the next year. The quantity of financial support for a medical organization by each CSG or CPG, is calculated as the sum of the costs of all hospitalizations in a hospital.

According to the data for 2019, Table 1 represents the data on the costs of severity grade 3 ADRs (CTCAE v. 4.03 classification) in medical institutions in St. Petersburg.

It should be notified that in the Russian Federation a unified methodology for assessing the costs of ADRs, has not been developed yet. As a rule, the results of the observational studies and/or database data are analyzed by an expert group, which then interprets the results based on their assessment.

Thus, a standardized approach to assessing pharmacoeconomic costs in connection with the ADRs development in the Russian Federation, has not been developed, either. The existing methodologies are applicable only to a limited number of nosological forms, and only to those events that require hospital observation. Therefore, it is impossible to take into account the time factor and indirect health care costs provoked by ADRs. In addition, extrapolation of results even during hypothetical planning can be difficult due to the fact that different systems for coding clinical manifestations and diseases, are used in pharmacovigilance and pharmacoeconomics (MedDRA and ICD 10).

In the healthcare system of some countries in Latin America, for example, in Brazil (the approach to burden assess in this country is well represented in the literature). A different approach using the DRPs concept (a system that evaluates all medical adverse events caused by ineffective pharmacotherapy and/or non-adherence to recommended treatments) is applied. For example, to assess DRPs, the Brazilian healthcare system SUS (Sistema Unico de Sau) uses a methodology originally developed by Jonson and Butman. It is based on a "decision tree" model representing potential clinical outcomes and direct economic costs. Herewith, direct economic costs also take into account the development of "new diseases" associated with the use of drug therapy (Drug-Related Morbidities; DRM). Costs can be direct, i.e. costs for the ADRs correction, and indirect ones such as lost working days or a period of reduced work ability [19, 20]. The model of ADRs assessment is based on 8 basic characteristics: untreated clinical conditions, inappropriate drug choices, subtherapeutic doses, drug refusals, overdoses, ADRs, drug interactions, and a drug use without appropriate indications. Further modeling involves analyzing the probabilities and costs, associated with the following therapeutic outcomes: 1) no need in additional treatment; 2) additional treatment; 3) visiting a doctor (visiting a medical specialist); 4) emergency department visit with a hospital stay less than 24 hours; 5) hospitalization or hospitalization with a stay in hospital for more than 24 hours; 6) long-term treatment or preliminary hospitalization with a minimum stay of 30 days in hospital or hospitalization to the intensive care unit; 7) death.

Further on, when analyzing an event, the "decision tree" can be represented by several branches. In the first branch of the "optimal outcome," DRMs are divided into three axes of mutually exclusive, sequential negative events: 1) Treatment Failure (TF); 2) New Medical Problem (NMP); 3) a combination of new medical problems and treatment failure (NMP/TF). A New Medical Problem (NMP) represents effects that are superior to those expected after pharmacotherapy (or undesirable effects of pharmacotherapy), including ADRs, dependence, and

overdose. Treatment failure (TF) includes inadequate therapeutic effects arising from inappropriate treatment or dose selection, drug and food interactions, inappropriate drug prescription, medication errors, unnecessary drug use, and inadequate adherence to the drug regimen as well. The second part of the "decision tree" estimates the supposed proportion of DRMs, and the third one consists of clinical negative results from previous DRMs (NMPs, TFs, and NMPs/TFs) [19, 20].

The branches of the tree are mutually exclusive. Therefore, the score should represent the worst scenario for the patient. For example, a patient who is hospitalized due to DRMs has probably already been consulted for treatment adjustments during previous consultations by a healthcare professional. However, this patient should be allocated to those who had been "hospitalized" and should not be taken into account in other branches ("additional treatment" or "visiting–a specialist").

The method has been refined through the involvement of clinical experts using a double-stage Delphi approach. At the first stage, the model can be presented to clinical experts, each of whom assesses the likelihood of the results development described above, in accordance with their own practical and clinical kinds of experience. At the second stage, clinical experts review the predictions of all other participants. If the prognosis of a particular medical expert differs, it explains such a position in order to reach a consensus [21].

An example is the cost estimate of the ADRs, was carried out in one of the hospitals in accordance with the methodology described above by de Freitas et al. [20].

48 medical specialists were recruited as experts. 44 of them were clinical pharmacologists. The results of assessing the ADR costs in the various scenarios, calculated by the authors, are presented in Table 2.

It should be notified that this study is representative of 36% of the Brazilian population [21]. By the group of clinical experts, it was determined that more than half (59%) of patients had DRMs when using at least one drug after a visit to a medical organization/certified physician. The research also shows that the result of an outpatient visit is a prescription for at least one drug. This estimate is consistent with other Brazilian and global data, which indicate that from 50% to 86% of consultations lead to drug prescriptions [22]. Based on the "decision tree" model and the expert review, the average prediction costs of the various ADRs were as follows: NMP, TF, and NMP/TF ratios were 216, 240, and 282 USD, respectively. The results show that among the patients, who received outpatient care and at least one drug, were as follows: 19.5%, 26.8%, and 13% had NMP, TF, and NMP/TF, respectively. These data are comparable to other studies that used the same method (NMP: 10.3%, 28.7%; TF: 16.0%, 23.4%; and NMP/TF: 6.5%, 14. 0%). The cost of medical care for a patient with ADRs was 155 USD. Brazil's average annual costs for ADRs services, range from 9.1 billion USD to 27.2 billion USD (the best and worst-case scenarios, respectively). Of these costs, 3 billion USD will be spent on hospitalization, 10.8 billion USD – on prolongation of hospitalization, which will be the main outcome of ADR. Additional consultations by specialists, as well as the stay of patients in the ICU, will cost 2 billion USD and 900 million USD, respectively [19–21].

The reasons for the aforementioned problems can be explained by failures in treatment monitoring, difficulties with therapeutic adherence, and issues related to the choice or prescription of MPs. According to the experts' estimates, from 53% to 60% of new medical problems (NMPs) and treatment failures (TFs), could have been avoided, in case patients received pharmaceutical care service out of hospital.

The current literature data confirm that ADRs are an urgent problem of modern pharmacoeconomics. Unfortunately, the current pharmacovigilance systems cannot solve a number of problems, such as a low activity of researchers in relation to the ADRs detection, recording, and transmission of information about ADRs, low awareness of the population about the potential risk of ADRs, a low quality of the sent spontaneous reports, etc.

One of the priority-oriented areas of the Russian healthcare system over the past few years has been monitoring the effectiveness, safety, and quality of drugs used at various stages of the clinical and diagnostic process. The experience of the Brazilian drug safety system demonstrates that the introduction of a pharmacologist/clinical pharmacologist consultation prior to prescribing drugs in a specific clinical situation, is cost-effective. Inappropriate prescriptions and lack of patients' follow-up, are the most common causes of ADRs. New diseases and deterioration of existing conditions requiring hospitalization associated with MPs could often be avoided.

Most of ADRs are explained by communication impairments (between patients, caregivers, and healthcare providers), poor adherence to treatment, and lack of knowledge about MPs, their dosages, dosing regimens, and potential drug interactions. The leading task for medical professionals is comprehensive counseling of patients in relation to the prescribed drugs. Sufficient knowledge of health professionals in the field of drug safety will allow them to reduce the frequency of SUSARs, as well as to prevent negative drug-related conditions.

Comprehensive analysis of the information related to drug safety issues, can be an effective economic tool for optimizing costs in the healthcare system. When assessing economic costs, various modeling methodologies or their combinations can be used. However, when predicting, it is important to take into account not only direct and indirect costs but also the ADRs outcomes, which, in turn, can seriously affect a further quality of

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DOI: 10.19163/2307-9266-2020-8-5-336-344

life. The methods of the economic assessment of ADRs, are almost not used in Russian practice, with the exception of the cases of the CSG/CPG classification of the diseases, used for the calculation of the reimbursement for medical care from the state foundation of compulsory medical insurance. In general, the number of studies on this issue is limited, and requires active development.

CONCLUSION

The models used in the Russian Federation for the analysis of economic value and further prediction of costs, in contrast to the Brazilian approach, do not take into account the time factor. Respectively, during the planning of the analysis of ADRs potential costs, it is necessary to strengthen the methods with such PE tools as QALY, YLL, YLD, etc.

The introduction of methods for assessing the ADRs economic burden into the Russian routine medical practice, will reduce the direct and indirect costs associated with complications of pharmacotherapy. As shown by the review of Brazilian practice, the analysis of the economic feasibility of prescribing a particular drug should be also subjected to a critical analysis, and the final decision should be made by the teams involving a clinical pharmacologist. This approach will make it possible not only to predict the costs of medical interventions, but also to take into account the potential costs of modifying pharmacotherapy due to the development of ADRs.

FUNDING

This work did not have funding from outside organizations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTION OF AUTHORS

G.I. Syraeva – carrying out a literary search, writing the original text of the article and the resume;
A.S. Kolbin – the formulation of the aim and objectives of the study, text editing;
A.V. Matveev – article editing, preparing the English language text, preparing illustrations;
V.S. Panezhina – carrying out a literary search, preparing the “Materials and Methods” section.

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Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2020-8-5-345-353

HEMORHEOLOGICAL PROPERTIES OF THE 5-HT2A-ANTAGONIST OF THE 2-METHOXYPHENYL-IMIDAZOBENZIMIDAZOLE DERIVATIVE OF THE RU-31 COMPOUND AND CYPROHEPTADINE, IN COMPARISON WITH PENTHOXYPHYLLINE

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Received 31 Jul 2020 Accepted 15 Oct 2020

Migraine and its comorbid conditions are pathogenetically associated with many factors, including hemorheological disorders. A class of drugs with a 5-HT2A antagonistic mechanism of action, is promising for the prevention and treatment of migraine attacks and concomitant pathologies.
The aim of the research is to study and compare a hemorheological activity of anti-migraine drugs, antagonists of 5-HT2A receptors of cyproheptadine, and a new drug that completed preclinical studies of the 1-(2-diethylaminoethyl)-2-(4-methoxyphenyl)-imidazo[1,2-a]benzimidazole derivative of the RU- 31 compound.
Materials and methods. The study of the hemorheological activity of the RU-31 compound and cyproheptadine, was carried out using an experimental model of rabbit blood hyperthermia *in vitro*. Pentoxifylline was used as a reference drug. In the course of the work, the parameters of blood viscosity, aggregation and deformability of erythrocytes were recorded.
Results. It has been established that in the concentration of 1 μM, the RU-31 compounds reduce blood viscosity by 17% at high shear rates, which is comparable with pentoxifylline in the concentration of 100 μM on the activity level. In the concentration of 1 μM, cyproheptadine also causes a general tendency to reduce blood viscosity at high shear rates, being inferior in activity to the RU-31 compound and pentoxifylline.
In the concentration of 1 μM, the RU-31 compound has a pronounced effect on the aggregation ability of erythrocytes in autologous plasma, reducing the aggregation rate by 70%, while the level of activity is not inferior to the drug compared to pentoxifylline in the concentration of 100 μM, and surpasses the drug cyproheptadine. For the RU-31 compound and cyproheptadine, no significant effect on the deformability of erythrocytes has been shown.
Conclusion. The capacity of cyproheptadine and the RU-31 compound to influence the rheological properties of blood by reducing blood viscosity and aggregation of erythrocytes has been revealed.
Keywords: migraine; 5-HT2A-antagonists; hemorheology; microcirculation; deformability; aggregation

ГЕМОРЕОЛОГИЧЕСКИЕ СВОЙСТВА 5-НТ$_{2A}$-АНТАГОНИСТА ПРОИЗВОДНОГО 2-МЕТОКСИФЕНИЛ-ИМИДАЗОБЕНЗИМИДАЗОЛА СОЕДИНЕНИЯ РУ-31 И ЦИПРОГЕПТАДИНА В СРАВНЕНИИ С ПЕНТОКСИФИЛЛИНОМ

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Получено 31.08.2020 Принята к печати 15.10.2020

For citation: D.S. Yakovlev, L.V. Naumenko, K.T. Sultanova, A.A. Spasov. Hemorheological properties of the 5-HT$_{2A}$-antagonist of the 2-methoxyphenyl-imidazobenzimidazole derivative of the RU-31 compound and cyproheptadine, in comparison with penthoxyphylline. *Pharmacy & Pharmacology*. 2020;8(5):345-353. **DOI:** 10.19163/2307-9266-2020-8-5-345-353

Для цитирования: Д.С. Яковлев, Л.В. Науменко, К.Т. Султанова, А.А. Спасов. Гемореологические свойства 5-НТ$_{2A}$-антагониста производного 2-метоксифенил-имидазобензимидазола соединения РУ-31 и ципрогептадина в сравнении с пентоксифиллином. *Фармация и фармакология.* 2020;8(5):345-353. **DOI:** 10.19163/2307-9266-2020-8-5-345-353

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Мигрень и ее коморбидные состояния патогенетически ассоциированы со многими факторами, в том числе и с гемореологическими нарушениями. Перспективным в отношении профилактики и лечения мигренозных атак и сопутствующих патологий является класс препаратов с 5-HT_{2A}-антагонистическим механизмом действия.

Цель. Изучение и сравнение гемореологической активности противомигренозных средств антагонистов 5-HT_{2A}-рецепторов ципрогептадина и нового средства, завершившего доклинические исследования производного 1-(2-диэтиламиноэтил)-2-(4-метоксифенил)-имидазо[1,2-а]бензимидазола соединения РУ-31.

Материалы и методы. Изучение гемореологической активности соединения РУ-31 и ципрогептадина проводили с использованием экспериментальной модели гипертермии крови кроликов *in vitro*. В качестве препарата сравнения использовался пентоксифиллин. В ходе работы регистрировались параметры вязкости крови, агрегации и деформируемости эритроцитов.

Результаты. Установлено, что соединение РУ-31 в концентрации 1 мкМ снижает вязкость крови на 17% при высоких скоростях сдвига, что по уровню активности сопоставимо с пентоксифиллином в концентрации 100 мкМ. Ципрогептадин в концентрации 1 мкМ также вызывает общую тенденцию снижения вязкости крови при высоких скоростях сдвига, уступая по активности соединению РУ-31 и пентоксифиллину. Соединение РУ-31 в концентрации 1 мкМ оказывает выраженное действие на агрегационную способность эритроцитов в аутологичной плазме, снижая показатель агрегации на 70%, при этом, по уровню активности, не уступая препарату сравнения пентоксифиллину в концентрации 100 мкМ и превосходя препарат ципрогептадин. Для соединения РУ-31 и ципрогептадина не показано значимого влияния на деформируемость эритроцитов.

Заключение. Выявлена способность ципрогептадина и соединения РУ-31 оказывать влияние на реологические свойства крови путём снижения вязкости крови и агрегации эритроцитов.

Ключевые слова: мигрень; 5-HT_{2A}-антагонисты; гемореология; микроциркуляция; деформируемость; агрегация

INTRODUCTION

Migraine is a common disease that is associated with an impaired blood supply to the brain. Migraine often accompanies the development of disabling complications. For this disease, the presence of concomitant disorders, such as arterial hypertension, ischemic heart disease, myocardial infarction, stroke, is possible [1, 2]. The associative links between these pathological conditions are confirmed by the data obtained in a number of epidemiological, clinical, as well as experimental studies of their pathological physiology. Risk factors (smoking, the female sex, hormonal contraceptives, a high frequency of migraine attacks and symptoms of aura) significantly increase the risk of these comorbid conditions, including ischemic stroke [3]. It is known that the risk of ischemic stroke increases significantly the presence of migraine pathology in patients, especially accompanied by aura. Neurovascular changes that occur during migraine attacks, are often accompanied by the increased blood viscosity, which mediates an even greater deterioration of the blood flow in the system of small vessels, and aggravates pathological processes [4, 5].

Several hypotheses explain the increased risk of ischemic stroke in the people with migraine. First, during the development of a migraine attack, there are neurovascular changes that can mediate the onset of a stroke. Second, these links are based on common pathophysiological aspects. Thus, the stroke can be the caused by pronounced hemorheological changes associated with hypercoagulation and increased blood viscosity (with low hematocrit and increased aggregation of erythrocytes), predisposing to the development of thrombosis in the cerebral arteries and the occurrence of “rheological stroke” [6, 7].

It is known that blood is a non-Newtonian fluid (its viscosity depends on the shear rate) and exhibits shear thinning (decreaseing in viscosity is accompanied by increaseing in the rate or shear stress), which determines the features of its effect on various parts of the vascular bed. It is customary to distinguish rheological parameters (viscosity of the whole blood, deformability and aggregation of erythrocytes) that determine the blood flow in the large vessels with a diameter of more than 200 μm, and in the microvasculature (less than 200 μm) [8].

It-has been registered that in small arteries, arterioles and venues, a 75% decrease in blood viscosity is determined by the processes of erythrocyte aggregation, and a 25% decrease – by cell deformability. At the same time, in exchange capillaries, the deformability of erythrocytes is an important microrheological parameter that ensures the efficient passage through the vessels, the diameter of which is smaller than the size of the cells, which is ensured by the membrane deformability.

Blood realizes its functions in the body through its fluidity property; its violation is manifested in an increase in blood viscosity, which is formed as a result of unidirectional shears in rheological parameters, including an increase in plasma viscosity, an increase in erythrocyte aggregation and a decrease in the deformability of their membranes. A “high blood viscosity syndrome” develops; it triggers a number of adverse hemodynamic consequences, such as slowing down a blood flow, increasing a total peripheral vascular resistance, increasing blood pressure and blood deposition in the venous bed. These processes lead to a violation of microcirculation and decreasing in the oxygen delivery to tissues and organs – and ischemia is developing. The development of a “high blood viscosity syndrome” and a serious impairment of rheological properties associated with it,

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are observed in many diseases, including the development of ischemic stroke in transient migraine disorders. Based on this, the problem of the migraine treatment, as well as the prevention of its comorbid conditions, remains relevant [9].

A subclass of anti-migraine drugs with a 5-HT_2-blocking mechanism of action is promising for the prevention of concomitant disorders in migraine attacks [10, 11]. In earlier preclinical studies (Project No.14.N08.11.0159), a new derivative of 2-methoxyphenyl-imidazobenzimidazole – 5-HT_{2A} antagonist – RU-31 was identified; it demonstrates anti-migraine properties and is positioned not only for migraine headaches treatment, but also for the attacks prevention [12, 13]. Activation of type 2 serotonin receptors causes constriction of cranial vessels, increases capillary permeability and alters platelet aggregation. It is believed that blockers of this receptor subtype, are capable of affecting cerebral microcirculation [14], and are also capable of inhibiting platelet and erythrocyte aggregation, thereby exerting a positive effect on the rheological properties of blood, improving microcirculation [15–17].

Thus, it is important to study 5-HT_{2A} antagonists as biologically active compounds capable of not only providing an anti-migraine effect, but also influencing the rheological characteristics of blood.

THE AIM of the research is to study and compare a hemorheological activity of anti-migraine drugs, antagonists of 5-HT2A receptors of cyproheptadine, and RU-31.

MATERIALS AND METHODS

Experimental animals

The experiments were performed on the blood samples taken from sexually mature male Chinchilla rabbits, in the amount of 6 animals (Breeding nursery of laboratory animals "Krolinfo" Ltd, Moscow region), weighing 4.0–4.4 kg. Before the start of the study, all the rabbits were adapted for 14 days, kept single in cages in the vivarium of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, the Ministry of Health of Russia. The clinical condition of the animals was monitored daily by visual inspection. The animals with abnormalities found out during the examination, were not included in the experimental studies.

The animals were kept in standard conditions in accordance with the Decree of 29 August, 2014 No.51 "On the approval of SP 2.2.1.3218-14" Sanitary and epidemiological requirements for the design, equipment and maintenance of experimental biological clinics (vivaria)", as well as in accordance with the directive of the European Parliament and the Council of the European Union 2010/63/EC dated 22 September, 2010 "On the protection of animals used for scientific purposes".

The animals were kept in controlled environmental conditions with the air temperature in the range of 20–22°C and the relative humidity of 30–70%. In the rooms for the animals, a twelve-hour lighting cycle was maintained, with a combined type of lighting – natural and fluorescent – during the daylight hours. The rabbits were kept in standard laboratory cages for large rodents with sawdust bedding and an access to drinking bowls and feeders *ad libitum*.

The study was complied with the ethical standards of the responsible committee of human experimentation (institutional and national) and the Declaration of Helsinki. The Regional Research Ethics Committee of the Volgograd Region (registration number IRB 00005839 IORG 0004900 (OHRP)) approved of the conduct of this experimental study – protocol No. 2032-2017 dated 26 June, 2017.

Investigated substances

The chemical substance RU-31 (1-(2-diethylaminoethyl)-2-(4-methoxyphenyl)imidazo[1,2-a]benzimidazole) [18], was synthesized at the Scientific Research Institute of Physical and Organic Chemistry of the Southern Federal University (Rostov-on-Don).

RU-31 was investigated in the concentration of 1 μM (an equimolar concentration to cyproheptadine with a serotonin-blocking effect [19, 20]). Testing was performed on each blood sample from every rabbit. The compounds were directly incubated *in vitro*.

Reference drugs

5-HT_2 antagonist – cyproheptadine hydrochloride (Merck, USA) and a microcirculation corrector – pentoxifylline (Merck, USA) were used as reference drugs.

For pentoxifylline, an effect on the rheological properties of blood have been justified in different *in vitro* studies. Pentoxifylline decreases blood viscosity, changes the microrheology of erythrocytes by decreasing their aggregation, and improves the deformability of their membranes. These characteristics make pentoxifylline possible to be used as the drug to confirm the validity of the selected test system [21].

Cyproheptadine hydrochloride was investigated in 1 μM. The reference drug pentoxifylline was used in the concentration of 100 μM (the concentration that affects the rheological properties of blood [22]).

For *in vitro* studies, weighed portions of the studied compound or reference drugs were dissolved in distilled water immediately before the experiment. The prepared solutions were instilled into the cuvette by laboratory dispensers for the *in vitro* incubation, and then the studies were carried out according to the design.

Study design

At the first stage, blood samples were collected from the marginal ear veins of the rabbits by the method of free-falling-mass. The blood was stabilized using an aqueous citrate solution (3.8%, pH 6.0) in the ratio of 9:1. To mix the blood with citrate, after filling, the closed tube was immediately gently inverted to the required volume. In this case, the formation of foam should be

prevented from.

At the second stage, the hemorheological activity of RU-31 and the reference drugs were studied, using an experimental model of rabbit blood hyperthermia *in vitro* [23].

For this, the following groups from the obtained samples of blood were formed: an intact control group (6 samples, and each was based on a blood sample of one rabbit); a control group (6 samples: warmed blood samples at 42°C, 1 hour,); there were also three experimental groups: pentoxifylline, RU-31 and cyproheptadine in the studied concentrations were introduced into the blood (6 samples in each group).

The experimental groups, as well as the control group, were subjected to hyperthermia (t=42°C, exposure – 1 hour), and the parameters of their blood viscosity, aggregation and deformability of erythrocytes were recorded for each sample.

To study the apparent viscosity, the required volume (2 ml) of blood samples were taken. Then the blood was centrifuged at 3000 rpm for 20 minutes to obtain platelet-poor plasma and erythrocyte mass, which were mixed to obtain blood with a standardized hematocrit of 45%. The hematocrit value was determined as the ratio of the length of the erythrocyte column to the plasma column during centrifugation of capillaries with blood samples (Elmi, Latvia) at 8000 rpm for 3 minutes.

The determination of the apparent viscosity of blood was carried out on a rotary blood analyzer (AKR-2, Russia) at shear rates from 300 s^{-1} to 10 s^{-1} (rotations per second). Based on the obtained data, the erythrocyte aggregation index (EAI, c.u.) was calculated as the ratio of blood viscosity at a shear rate of 10 s^{-1} (the change mainly depends on erythrocyte aggregation) to blood viscosity at 100 s^{-1} (the change of viscosity mainly depends on the deformability of erythrocytes) [24].

The degree of erythrocytes aggregation was determined using the method of optical microscopy (microscope “Biolam Lomo” (Russia). For this, erythrocytes were separated from plasma by centrifugation at 3000 rpm for 20 minutes. Erythrocytes were washed three times in saline and then suspended in autologous plasma. After video recording (digital camera for DCM500 microscope), the number of aggregated and non-aggregated erythrocytes was estimated, and the aggregation index (AI, c.u.) was calculated as the ratio of the number of aggregates to the number of non-aggregated cells [25].

Method for determining the deformability of erythrocytes

The deformability of erythrocytes was assessed by a viscometric method and by visualization in a flow-through microchamber.

The deformability of erythrocytes by a viscometric method, was assessed at a standardized hematocrit of 45%. The viscosity of erythrocyte suspension was measured on a rotary viscometer at shear rates of 300, 30, 3 s^{-1}.

The elongation of erythrocytes was visualized in the flow-through microchamber which was filled with a suspension of erythrocytes in an isotonic sodium chloride solution containing 0.1% albumin. The pressure applyed to the microchamber, thereby created a certain value of the shear stress (**τ**) in it, which was calculated according to the formula:

$$\tau = \frac{6\eta Q}{Wh2}$$

where η is the viscosity of the suspension (approximately 1.0 mPa s at 20°C), Q is the volumetric velocity in the microchamber, W is the width of the flow channel of the microchamber, h is the height of the channel equal to the thickness of the gasket.

The image of erythrocytes stretched by a flow of liquid, attached by a single point with the help of human albumin to the bottom of the microchamber, was transmitted from the microscope via a USB port to a computer using a digital eye-piece. After recording the image, it was analyzed in the Adobe Photoshop program (a trial version), where the length and width of the elongated erythrocytes were determined, and the elongation index (EI, c.u.) calculated as an indicator of deformation:

$$EI = \frac{L - W}{L + W}$$

where L is the length of the deformed cell, W is its width [26].

Statistical analysis methods

Statistical data processing was carried out using GraphPad Prism v.8.0 software and Microsoft Office Excel 16. The data are presented as M±m, where M is the mean values for the group, m is the standard error of the mean. The analysis of the intergroup differences was carried out using nonparametric Mann-Whitney U-test. The differences were determined at the 0.05 significance level.

RESULTS OF THE STUDY

Effect on the viscosity blood characteristics

Heating the control sample of the blood for an hour, led to a significant increase in the blood viscosity (Fig. 1).

Thus, a statistically significant increase in the viscosity of the heated blood samples was revealed in the entire range of the studied shear rates: at the shear rate of 300 s^{-1}, the blood viscosity increased by 10%; at 200 s^{-1} – by 12%, at 100 s^{-1} – by 20%, at 50 s^{-1} – by 19%, at 20 s^{-1} – by 22%, at 10 s^{-1} – by 27%.

When pentoxifylline in the concentration of 100 μM, RU-31 and cyproheptadine in the concentration of 1 μM were added to the blood samples and exposed to heat, the general tendency to decrease the blood viscosity was found out (Table 1).

Thus. when pentoxifylline in the concentration of

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100 μM was added to the treated blood samples. the general tendency for decreaseing in blood viscosity at high and low shear rates. was observed. At high shear rates from 300 s^{-1} to 100 s^{-1}. the decrease in blood viscosity in comparison with the control samples was 17% ($p<0.05$). In a lower range of shear rates. the decrease in blood viscosity was also observed. however. no significant differences in relation to control measurements were found when modeling pathology.

Effected by the action of RU-31 in the concentration of 1 μM. the blood viscosity indices were significantly lower in relation to the control sample at shear rates from 300 s^{-1} to 100 s^{-1}. In average. the decrease was 17% ($p<0.05$).

In the concentration of 1 μM. cyproheptadine also caused a general tendency for blood viscosity decrease at high shear rates.

Thus. it was revealed that pentoxifylline. the compound of RU-31 and cyproheptadine–demonstrate the ability to reduce the viscosity characteristics of blood over the entire range of shear rates. with the most significant differences in the range of shear rates from 300 s^{-1} to 100 s^{-1}. At the same time. in terms of the provided effect. RU-31 is not inferior to pentoxifylline and slightly exceeds the effect of cyproheptadine.

Effect on the parameters of erythrocyte aggregation

Modeling of hyperviscosity led to statistically significant increase in the indices of erythrocyte aggregation in autologous plasma by 54% compared to the intact samples (Fig. 2).

Pentoxifylline. RU-31 and cyproheptadinationse in the studied concentrations and exposed to heat. added to the blood samples. provide a general tendency to reduce the aggregation of erythrocytes.

Thus. pentoxifylline in the concentration of 100 μM. added to the blood samples subjected to hyperviscosity modeling. statistically significantly decreased in erythrocyte aggregation by 73%.

For the RU-31 compound in the concentration of 1 μM. a statistically significant decrease in this indicator by 70% was registered.

When cyproheptadine was added to the blood samples in the concentration of 1 μM. a significant decrease in erythrocyte aggregation by 65% was observed.

Thus. in the concentration of 1 μM. RU-31 has a pronounced effect on the aggregation ability of erythrocytes in autologous plasma. The level of RU-31 activity is not inferior to pentoxifylline in the concentration of 100 μM and surpasses the reference drug cyproheptadine.

Effect on erythrocyte deformability

The assessment of the studied compounds effect on the deformability of erythrocytes. was carried out by measuring the viscosity of washed erythrocytes suspension as well as by measuring the degree of erythrocytes deformation in a flow-through microchamber.

When the syndrome of increased viscosity was modeled. a significant increase in the viscosity of a washed erythrocytes suspension was revealed in the entire range of shear rates. So. at the rate of 300 $s^{-1.}$ this indicator increased by 10%. at the rate of 30 s^{-1} – by 11%. at the rate of 3 s^{-1} – by 14% ($p<0.05$) (Table 2).

The addition of pentoxifylline in the concentration of 100 μM to the heated rabbits' blood samples. revealed a significant decrease in viscosity at shear rates of 300 s^{-1} and 3 s^{-1} by 10% and 14%. respectively. The RU-31 compound in the concentration of 1 μM mediated a statistically insignificant decrease in the viscosity parameters of the heated blood samples. For cyproheptadine in the concentration of 1 μM. a statistically significant decrease in viscosity was observed at shear rates of 300 s^{-1} and 3 s^{-1}.

When determining the elongation index of erythrocytes in a flow-through microchamber. it was found out that with the experimental syndrome of the increased blood viscosity. the elongation index of erythrocytes significantly decreased by 32%. The erythrocyte elongation index for pentoxifylline was 0.26±0.010. i.e. 37% higher than for the control group (modeling of hyperthermia). The compound RU-31 and cyproheptadine did not change the properties of erythrocytes membranes; no statistically significant change for elongation index was found out.

Thus. for RU-31. no statistically significant effects on the viscosity characteristics of the erythrocyte suspension and the structural and functional properties of their membranes was found out.

DISCUSSION

It is known that the rheological status of blood is determining by many factors. including the viscosity of the whole blood. deformability and aggregation of erythrocytes. The thermal action on the blood samples. led to a distinct change in these rheological parameters. As Fig. 1 shows. heating led to a statistically significant increase in the viscosity of the blood samples over the entire range of the studied shear rates. which is presumably associated with a decrease in the deformability of erythrocyte membranes and an increase in their aggregation in the vascular bed [27]. It was found out that the temperature effect on the blood samples contributed to the increase in the aggregation ability of rabbits' erythrocytes by more than twice. as well as to the decrease in the deformability of their membranes by almost 1.5 times. which indicates the decrease in the viscoelastic properties of the erythrocyte membrane.

The revealed changes in the rheological parameters of the blood during modeling the syndrome of increased viscosity. reflect the pathology that develops in the large-diameter vessels and the microvasculature bed [28].

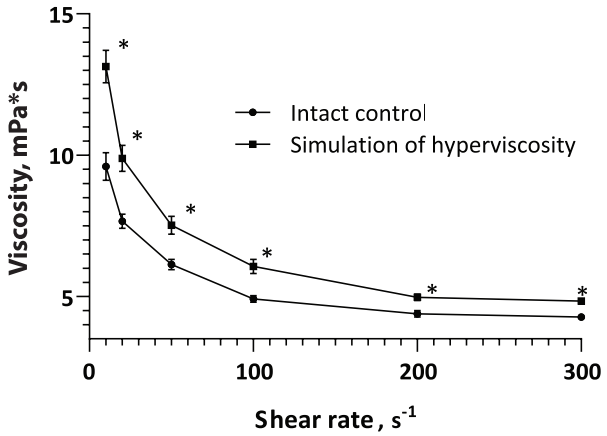

Figure 1 – Simulation of "high viscosity syndrome" (temperature – 42°C, incubation period – 1 hour)

Notes: s^{-1} – reciprocal seconds. The data are presented as M ± m (mean ± standard error), n = 6. * – the data are reliable in relation to intact blood, with a nonparametric distribution, Mann-Whitney test (p <0.05)

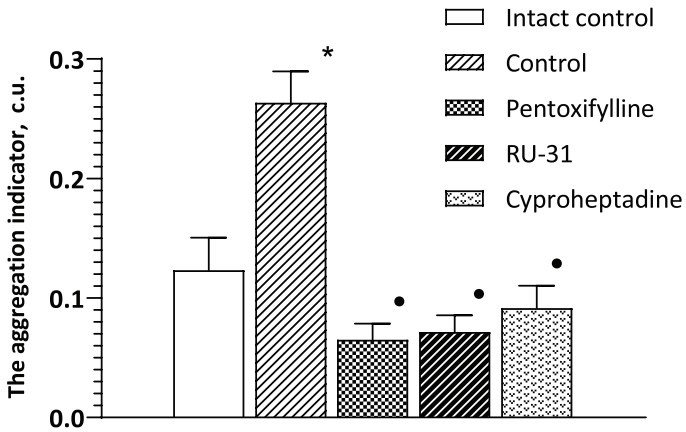

Figure 2 – Effect of pentoxifylline. compound RU-31 and cyproheptadine on aggregation of rabbit erythrocytes after heat exposure

Notes: the aggregation indicator is the ratio of the number of aggregates to the number of erythrocytes. Data are presented as M ± m (mean ± standard error). n = 6. * – the data are reliable in relation to intact blood. with a nonparametric distribution. Mann-Whitney test (p <0.05). ● – differences are significant relative to control measurements (hyperviscosity modeling). with nonparametric distribution. Mann-Whitney U-test (p <0.05)

Table 1 – Effect of pentoxifylline, RU-31 and cyproheptadine, on the blood viscosity of the rabbits exposed to heat, in order to simulate a "high viscosity syndrome" (temperature – 42°C, incubation period – 1 hour) *in vitro*

Group	mPa*s					
	300 s^{-1}	200 s^{-1}	100 s^{-1}	50 s^{-1}	20 s^{-1}	10 s^{-1}
Control	4.8±0.11	5.0±0.12	6.1±0.26	7.5±0.32	9.9±0.46	13.1±0.58
Pentoxifylline	4.0±0.21*	4.1±0.21*	5.1±0.30*	6.5±0.51	8.6±0.47	11.2±0.87
RU-31	4.1±0.23*	4.2±0.23*	4.9±0.35*	6.3±0.52	8.6±0.75	11.3±1.43
Cyproheptadine	4.2±0.13*	4.3±0.15*	5.2±0.17*	6.8±0.32	9.2±0.41	11.6±0.70

Notes: s^{-1} – reciprocal seconds. The data are presented as M ± m (mean ± standard error). n = 6. * – the data are reliable in relation to intact blood. with a nonparametric distribution. Mann-Whitney test (p <0.05)

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Table 2 – Influence of RU-31 and reference preparations on the viscosity of washed erythrocytes suspension of rabbits

Group	mPa*s		
	300 s^{-1}	30 s^{-1}	3 s^{-1}
Intact control	2.7±0.06	4.1±0.05	7.2±0.26
Control	3.0±0.02*	4.6±0.15*	8.4±0.18*
Pentoxifylline	2.7±0.10●	4.2±0.24	7.2±0.24●
RU-31	2.9±0.11	4.4±0.29	7.9±0.36
Cyproheptadine	2.8±0.05●	4.5±0.13	7.8±0.11●

Notes: s^{-1} – reciprocal seconds. The data are presented as M ± m (mean ± standard error). n = 6. * – the data are reliable in relation to intact blood. with a nonparametric distribution. Mann-Whitney U-test (p <0.05). ● – differences are significant relative to control measurements (in hyperviscosity modeling). with nonparametric distribution. Mann-Whitney U-test (p <0.05)

The experiment revealed that in the concentration of 100 μM. pentoxifylline reduced the blood viscosity in the entire range of the studied shear rates. The most pronounced changes were observed at high shear rates. which is characterized by changes in the components of microrheology.

This was confirmed by the significant decrease in the erythrocyte elongation index. as well as a significant decrease in the rate of their aggregation. The data obtained for the reference drug pentoxifylline. made it possible to confirm the performance of the selected test systems for studying the hemorheological activity of 5-HT_2-antagonists.

During the further work. the data on the effect of RU-31 in the concentration of 1 μM on the whole blood viscosity as well as the erythrocytes aggregation and deformation were obtained. Thus. RU-31 promoted the decrease in blood viscosity over the entire range of the studied shear rates. The most pronounced changes were observed at high shear rates. In addition. the pronounced decrease in the aggregation ability of erythrocytes was notified. The revealed changes in rheological parameters (viscosity of whole blood and erythrocyte aggregation) at high shear rates as well as the decrease in blood viscosity at low shear rates. indicate the ability of RU-31 to improve rheological properties in large-caliber vessels and microvasculature.

In terms of the activity level. RU-31 exceeded the reference drug cyproheptadine and was not significantly inferior to pentoxifylline.

As for the reference drug cyproheptadine in the concentration of 1 μM. it was also characterized by the ability to improve blood viscosity. The most significant changes were recorded at high shear rates. In addition. cyproheptadine slightly improved the aggregation characteristics of erythrocytes.

The ability of type 2A serotonin receptor antagonists to reduce blood viscosity is probably associated with a possible effect on the plasma component. This was confirmed by the fact that cyproheptadine and RU-31 did not have a pronounced effect on the viscosity of the washed erythrocyte suspension and their deformability. but significantly reduced the aggregation of washed erythrocytes in autologous plasma.

The revealed hemorheological activity of RU-31. comparable to the reference drug pentoxifylline. suggests that this compound is promising for the correction of rheological disorders that provoke a deterioration in cerebral blood flow. which can mediate a decrease in the risk of ischemic stroke in the persons with migraine pathology and stop the aggravation of the pathological processes.

CONCLUSION

Taking into account the data on the participation of 5-HT_2 receptors in the migraine pathogenesis as well as their involvement in the formation of comorbid conditions. it was reasonable to study the effect of type 2 blockers of serotonin receptors. on the viscosity characteristics of blood. aggregation of erythrocytes. as well as their membranes deformability.

The comparative study showed that 5-$HT_{2/2A}$-antagonists cyproheptadine and RU-31. are able of influencing the rheological properties of the animals' blood with the syndrome of increased blood viscosity *in vitro*.

It was also revealed that the RU-31 compound reduces blood viscosity with the experimental syndrome of increased blood viscosity *in vitro*. and it is practically not inferior to the reference drug pentoxifylline and surpassing the 5-HT_2 blocker cyproheptadine.

Thus. the data obtained in this research. indicate the presence of the ability of 5-$HT_{2/2A}$ antagonists cyproheptadine and RU-31. to influence the rheological properties of blood by reducing viscosity and erythrocytes aggregation. which can expand the range of application of the drugs of this group.

FUNDING

The study was partially carried out within the framework of an internal research grant funded from the funds of-Volgograd State Medical University of the Ministry of Health of Russia (Order 29-KO dated 2 Jun 2020).

AUTHORS' CONTRIBUTION

All authors contributed equally to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DOI: 10.19163/2307-9266-2020-8-5-345-353

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RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

PROBLEMS OF RESPONSIBLE SELF-MEDICATION OF ALLERGY SYMPTOMS AND FARMACEUTICAL COUNSELING

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Received 15 Feb 2020 Accepted 15 Aug 2020

Cure of allergy symptoms reported by a patient with the help of non-prescription antiallergic drugs, is one of the important aspects of a responsible self-medication.

The aim of the study is to assess the peculiarities of consumers' behavior as pharmacy visitors, when choosing non-prescription antiallergic drugs, and to identify potential problems of pharmaceutical counseling for allergy symptoms regarding the responsible self-medication.

Materials and methods. Sociological survey in the form of a questionnaire; graphoanalytical and comparative analyses.

Results. The profile of an over-the-counter antiallergic drugs buyer in the pharmacies of Kazan was assessed in the following way: it is a woman aged 18–44 of a middle level of income, having a family of 3–4 people, ready to spend from 101 to 500 rubles on the purchase of antiallergic drugs, buying anti-allergic drugs not for the first time. The main reasons to seek for treatment were skin rash, redness and itching, which had also been observed in the past. The allergic nature of the disease had already been confirmed by the doctor. For the average consumer of over-the-counter antiallergic drugs, the most important criteria for choosing a medicine were: efficiency, safety, the doctor's recommendations and price. The medicines were purchased for the visitors themselves or their children. The customers were satisfied with the choice of non-prescription antiallergic medicines available in the pharmacy. The visitors generally trusted the pharmacists' advice and recognized them as health professionals, but considered that the main goal was not only to provide a pharmaceutical care, but also to profit from the sale of the drugs. The following flaws of pharmaceutical counseling when dispensing non-prescription antiallergic drugs, were identified: an improper diagnosis by a pharmaceutical specialist, the lack of recommendations to consult a doctor, incomplete provision of information on the use, storage, and the possibility of interaction with other drugs and food.

Conclusion. Misdiagnosis and incomplete provision of information by pharmaceutical specialists on antiallergic the drugs that are approved for the over-the-counter dispensing, require the implementation of a pharmaceutical consultation algorithm for the visitors contacting a pharmacy with allergy symptoms.

Keywords: consumer; pharmacy; antiallergic drugs; over-the-counter drugs; pharmaceutical counseling

ПРОБЛЕМЫ ОТВЕТСТВЕННОГО САМОЛЕЧЕНИЯ ПРИ СИМПТОМАХ АЛЛЕРГИИ И ФАРМАЦЕВТИЧЕСКОЕ КОНСУЛЬТИРОВАНИЕ

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Получена 15.02.2020 Принята к печати: 15.08.2020

For citation: V.A. Izrafilova, S.N. Egorova, G.Kh. Garifullina, T.A. Akhmetova. Problems of responsible self-medication of allergy symptoms and farmaceutical counceling. *Pharmacy & Pharmacology*. 2020;8(5):354-361. **DOI:** 10.19163/2307-9266-2020-8-5-354-361

Для цитирования: В.А. Израфилова, С.Н. Егорова, Г.Х. Гарифуллина, Т.А. Ахметова. Проблемы ответственного самолечения при симптомах аллергии и фармацевтическое консультирование. *Фармация и фармакология.* 2020;8(5):354-361. **DOI:** 10.19163/2307-9266-2020-8-5-354-361

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-5-354-361

Распознавание и устранение пациентом симптомов аллергии, с помощью противоаллергических лекарственных препаратов (ЛП), разрешенных к отпуску без рецепта врача, является одним из актуальных направлений ответственного самолечения.

Цель. Изучение особенностей потребительского поведения посетителей аптеки при выборе противоаллергических лекарственных препаратов безрецептурного отпуска и выявление проблем фармацевтического консультирования при симптомах аллергии в рамках ответственного самолечения.

Материалы и методы. Социологический опрос в форме анкетирования, графоаналитический, сравнительный анализ.

Результаты. Сформирован среднестатистический портрет покупателя противоаллергических лекарственных препаратов, разрешенных к отпуску без рецепта врача, в аптеках г. Казани: женщина в возрасте 18–44 года, имеет семью из 3–4 человек, среднего уровня финансового достатка, при этом готова потратить на покупку противоаллергических лекарственных препаратов от 101 до 500 рублей; покупает противоаллергические лекарственные препараты не в первый раз. Основным причинами обращения являются: сыпь на коже, покраснение и зуд, которые наблюдались ранее. Аллергическая природа заболевания была установлена врачом. Для среднестатистического потребителя противоаллергических лекарственных препаратов, разрешенных к отпуску без рецепта врача, важным критерием при выборе лекарства являются эффективность, безопасность, рекомендации врача и цена. Покупки совершаются для себя или ребенка. Аптечным ассортиментом противоаллергических лекарственных препаратов безрецептурного отпуска покупатели удовлетворены. Советам аптечного работника посетители аптек в основном доверяют и считают их работниками здравоохранения, однако, их главной целью считают не только оказание фармацевтической помощи, но и получение прибыли от продажи лекарств. Выявлены недостатки фармацевтического консультирования при отпуске противоаллергических лекарственных препаратов безрецептурного отпуска (ЛП БРО): неправомерное выставление диагноза фармацевтическим работником и отсутствие рекомендаций о необходимости консультации врача; неполное предоставление информации по вопросам применения, хранения, возможности взаимодействия с другими лекарствами и пищей.

Заключение. Неправомерное выставление диагноза и неполное предоставление информации фармацевтическим работником о противоаллергических лекарственных препаратах, разрешенных к отпуску без рецепта врача, требуют разработки алгоритма фармацевтического консультирования при обращении в аптеку с симптомами аллергии.

Ключевые слова: потребитель; аптека; противоаллергические лекарственные препараты; безрецептурный отпуск; фармацевтическое консультирование

INTRODUCTION

Research on medicines consumption is essential for the development of an integral component of the rational drug use system [1]. The issues of self-medication are of particular importance for both domestic and foreign health care systems. The current trend of the domestic pharmaceutical market is an over-the-counter (OTC) drugs sales increase [2], so the role and responsibility of the pharmaceutical personnel of pharmacies as health professionals will be growing [3–5].

According to the professional standard approved by the journal of «Pharmacist»[1], in the Russian Federation the activity of a pharmacist is a significant part of preventive work with the population («outreach and awareness-raising work to promote a healthy lifestyle»), i. e. ensuring the rational use of both prescription and non-prescription drugs within the framework of responsible self-medication («qualified pharmaceutical assistance to the population and patients of medical organizations», «informing the population and medical specialists about medicines and other goods in the pharmacy range»). Herewith, the Professional Standard stipulates the need for «keeping to moral and ethical standards of professional activities». It determines the social responsibility of the pharmacist in maintaining and strengthening the health of the population, the development of a healthy lifestyle, and is especially important for the reasonable, effective and safe use of OTC drugs abreast of the growth of their active advertising and other methods of promotion [6, 7].

Allergic diseases remain a relevant world health problem [8, 9], while the cure of various allergy symptoms, recognized by the patient, with the help of non-prescription antiallergic drugs is one of the topical areas of responsible self-medication [10, 11].

Foreign standardized protocols of a senior pharmacist (dispenser) on advising OTC drugs for symptomatic treatment of allergies, are not fully applicable to the realities of domestic pharmaceutical practice due to differences in the range of drugs and in the order of their dispensing from pharmacies [12, 13].

Regarding the marketing research, the study of medicines consumers would help to determine the behavior model, form a rational assortment of OTC drugs in a pharmacy, as well as to identify the problems associated with consumers' dissatisfaction with the service, the affordability of the necessary drugs and the quality of pharmaceutical care [14, 15]. A wide range of antiallergic OTC drugs is presented on the Russian pharmaceutical market [16]. Portraying the profile of an OTC antiallergic drugs consumer and detecting the problems in pharmaceutical counseling for allergy symptoms, is an important step to fulfil the concept of responsible self-medication and pharmaceutical care improvement.

[1] Order of the Ministry of Labor and Social Protection of the Russian Federation, March 9, 2016 No. 91n. On the approval of the professional standard "Pharmacist", URL: http://www.consultant.ru/document/cons_doc_LAW_196697/

THE AIM of the study is to assess the peculiarities of consumers' behavior as pharmacy visitors, when choosing non-prescription antiallergic drugs, and to identify potential problems of pharmaceutical counseling for allergy symptoms regarding the responsible self-medication.

MATERIALS AND METHODS

To achieve this aim, a questionnaire for pharmacy visitors who had contacted pharmaceutical workers regarding the purchase and / or use of OTC antiallergic drugs, was developed by the authors. The questionnaire included 18 questions, clustered into 5 blocks: socio-demographic characteristics of a pharmacy visitor – a consumer of OTC anti-allergic drugs; self-reported health status regarding the visitors' allergy symptoms; purchase characteristics; behavioral characteristics; assessment of the pharmaceutical care quality.

The survey was carried out at the pharmacies of the «MegaPharm Kazan» trading network (Kazan) from February to December 2018; a total of 100 respondents were interviewed. The data processing was performed using the Microsoft Excel software.

RESULTS AND DISCUSSION

The study of socio-demographic characteristics has shown that among the visitors who contacted a pharmaceutical worker for the purchase and/or use of OTC antiallergic drugs for allergy symptoms, were women (67% of the total number of respondents). Most of the respondents were from 18 to 44 years old (43%).

The largest group (32%) of OTC antiallergic drugs consumers had a family of 4 or more people, 31% – of 3, 20% – of 2, 17% of the consumers said they were single.

Important socio-demographic parameters are income and monthly expenses for the purchase of OTC anti-allergic drugs. These data make it possible to assess the respondent's standard of living, which has a significant impact on the choice and consumption of the studied group of drugs.

Out of 100 respondents, 43% characterized themselves as middle-income people, 37% – as low-income ones, 13% – as rather well-to-do, 7% reported their incomes as "upper-middle". Only 22% of the consumers were ready to purchase OTC anti-allergic drugs for a price higher than 500 rubles; 43% were ready to spend from 101 to 500 rubles, and 35% were ready to spend up to 100 rubles.

Based on the frequency of purchases of OTC anti-allergic drugs, the respondents were distributed as follows: permanent customers – 22%, rare customers – 49%, the customers who made their purchase for the first time – 29%.

Further, the predominant allergy symptoms, which had most often been the reason for the independent choice and purchase of OTC antiallergic drugs, were identified (Fig. 1).

Most of the respondents – the consumers of OTC antiallergic drugs (45%) – went to a pharmacy with a predominant symptom of «skin rash». Redness and itching of the skin and mucous membranes were the reason for the purchase of OTC antiallergic drugs in 27% of patients. Symptoms of allergic rhinitis, sneezing and coughing, lacrimation, were the reasons to seek for a pharmacist's advice in 8%, 10% and 5% of patients, respectively. 5% of respondents applied to a pharmacy for OTC antiallergic drugs due to the symptoms of shortness of breath and difficulty in breathing (Fig. 1).

The above listed symptoms were first noticed by 35% of the respondents, and 65% of the visitors had observed them before. Half of the respondents (51%) were diagnosed with an allergic disease earlier by a doctor; 15% of respondents consulted a senior pharmacist in a pharmacy and suggested an allergic nature of the symptoms that bother them; the same number of respondents (15%) concluded themselves that they had an allergy. 19% of the respondents decided that they had manifestations of allergy, based on the Internet resources (Fig. 2).

The most important criteria for the respondents when choosing the OTC antiallergic drugs were efficiency (90%), safety (89%) and doctors' recommendations (87%). The price of a medicine turned out to be slightly less important for the respondents (70%). The dosage form was important for 50% of the respondents and 60% also noted the importance of OTC drug usage frequency. Such characteristics of OTC medicines as the manufacturer and originality (a reference or generic drug) turned out to be important for 35% and 25% of the respondents, respectively (Fig. 3).

The surveyed pharmacy visitors usually bought antiallergic OTC drugs for themselves (45%) and for their children (30%). However, 9% of the respondents purchased antiallergic OTC drugs "for emergency" in their home first-aid kit, and 16% – for other adult family members.

The vast majority of respondents rated the assortment of OTC antiallergic medicines in the pharmacy organizations of «MegaPharm» LLC as wide – 78%, but 22% of the visitors found it insufficient.

When making a choice of OTC anti-allergic drugs, the largest number of the respondents focused on the doctors' recommendations (66%), the smallest (1%) – on their friends advice. 15% of the respondents chose OTC antiallergic drugs at their own discretion, 10% based on a pharmaceutical specialist's advice; 8% acted under the influence of advertising (Fig. 4).

It was found out that 90% of the people who looked for OTC anti-allergic drugs, trusted pharmacy specialists' advice, and 10% of the respondents reported their distrust of them.

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DOI: 10.19163/2307-9266-2020-8-5-354-361

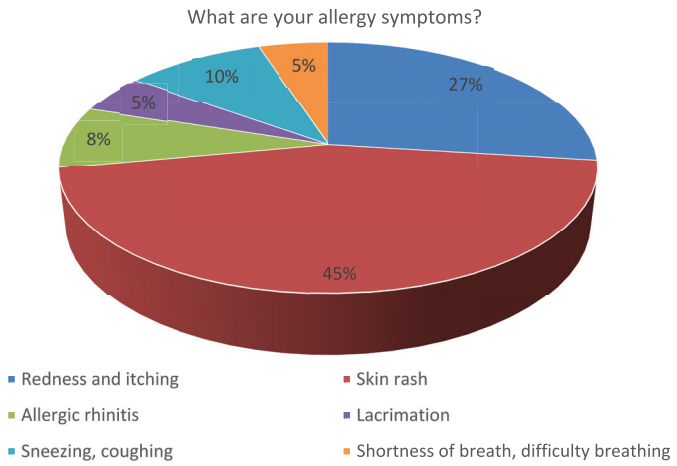

Figure 1 – The symptoms that were the reason for going to the pharmacy for non-prescription antiallergic drugs

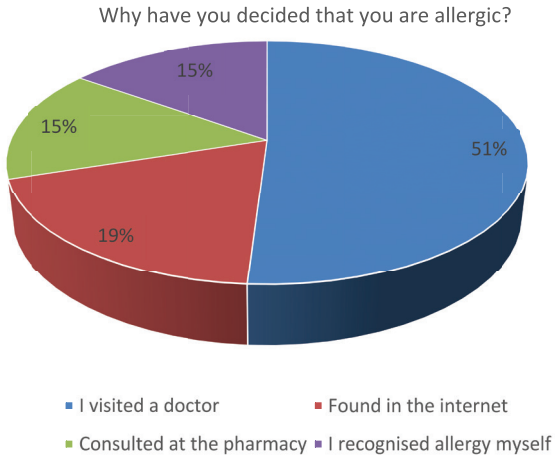

Figure 2 – Sources of information about the allergic nature of symptoms – the reasons for going to the pharmacy

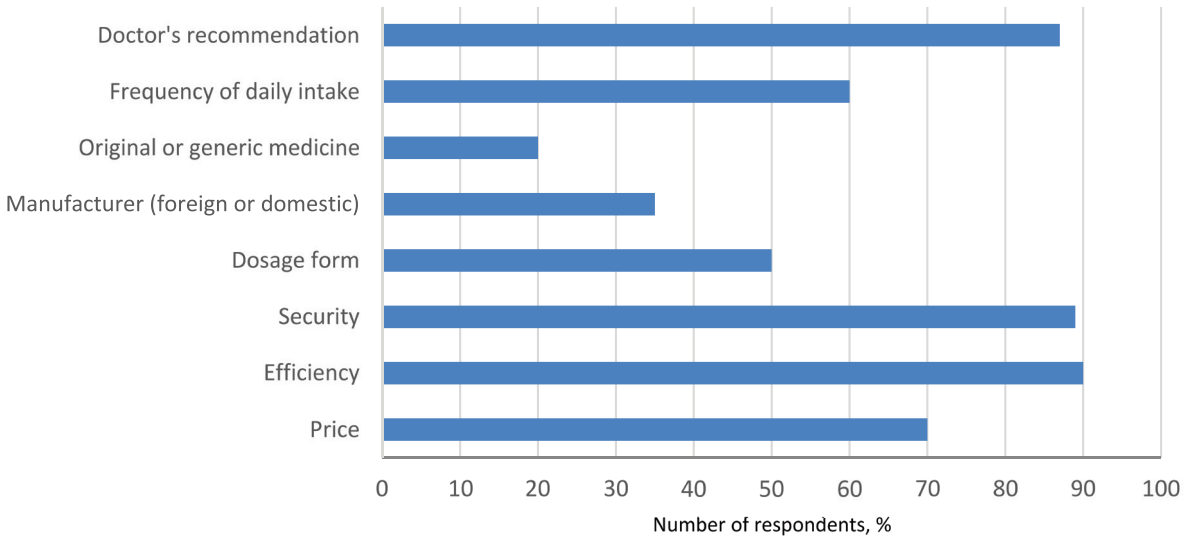

Figure 3 – Criteria for choosing over-the-counter antiallergic drugs by buyers

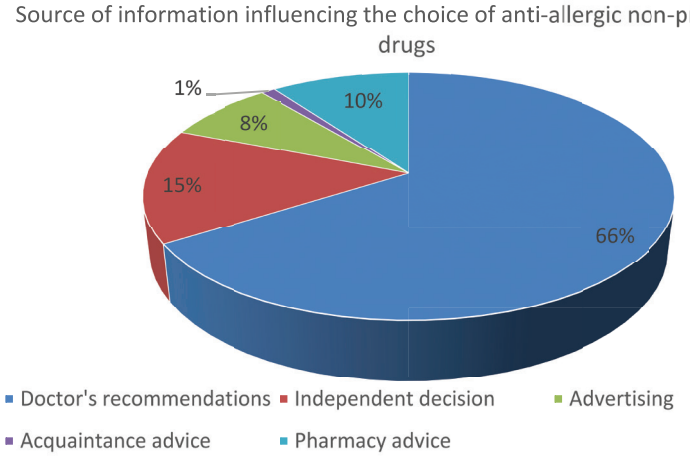

Figure 4 – Sources of information affecting the choice of non-prescription antiallergic drugs

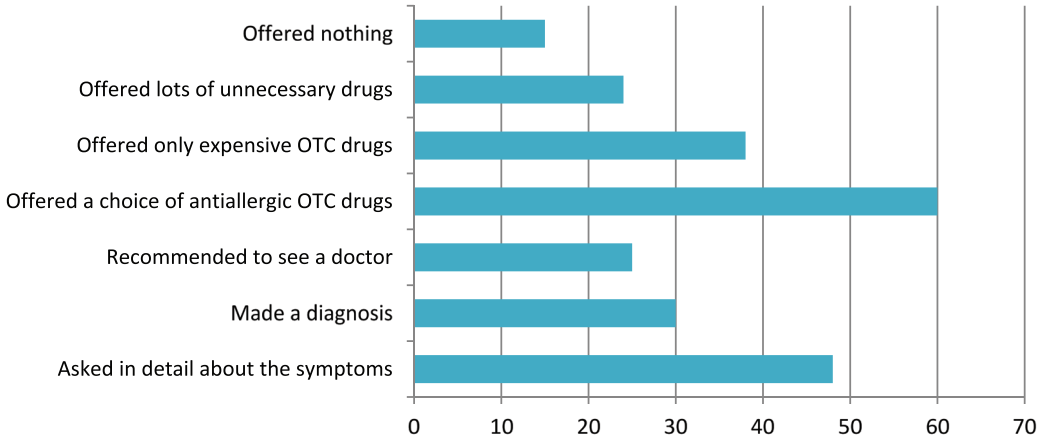

Figure 5 – Actions by a pharmacist in case of a visitor with allergy symptoms

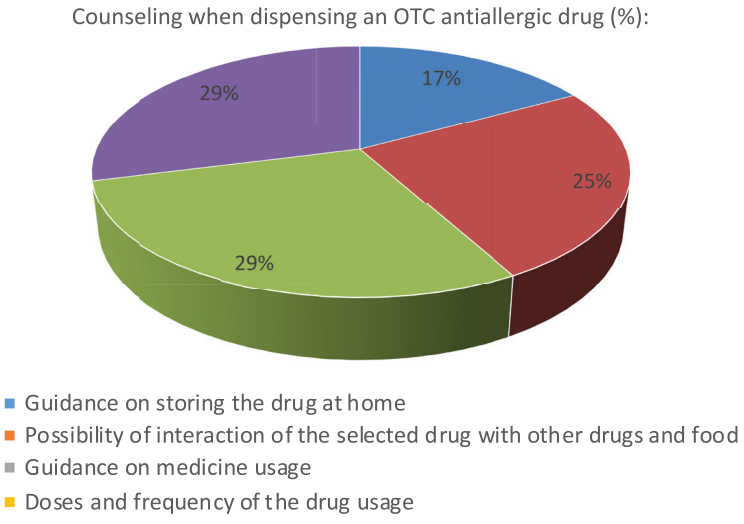

Figure 6 – Counseling when dispensing non-prescription antiallergic drugs

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DOI: 10.19163/2307-9266-2020-8-5-354-361

The next set of questions was focused on the quality of pharmaceutical consulting, i.e. the compliance of pharmacy specialists with the requirements of Order of the Ministry of Health of the Russian Federation dated 31 August, 2016, No. 647n «On approval of the rules for good pharmacy practice of drugs for medical use»[2]. The analysis of pharmaceutical specialists' actions showed that in case consumers with allergy symptoms were visiting a pharmacy, specialists most often (in 60% of cases) offered a choice of OTC anti-allergic drugs of different price categories. 38% of the respondents believed that they were offered only expensive drugs, and 24% – unnecessary ones. According to 48% of the respondents, a pharmaceutical specialist asked them in detail about the symptoms – the reasons for going to the pharmacy as a part of responsible self-medication, and 30% even made a diagnosis. Only 25% of the surveyed indicated that a pharmacist recommended seeing a doctor. 15% of the consumers evaluated the pharmacists' actions as «offering nothing» (Fig. 5)

When answering a question of evaluating a pharmaceutical specialist's actions when dealing with a visitor complaining of the allergy symptoms, 29% of the respondents notified that when dispensing OTC antiallergic drugs, pharmacists drew their attention to the method of usage of the purchased drug, the dose and the frequency of administration; 25% focused on possible drug and food interactions; 17% concentrated their attention on the peculiarities of OTC medicines storage at home (Fig. 6).

It should be notified that the approval of the professional standard represented in «Pharmacist» and Order of the Ministry of Health of the Russian Federation dated 31 August, 2016, No.647n «On the approval of the Rules of Good Pharmacy Practice of Medicines for Medical Use», increased the quality of information and consulting services: the study by O.A. Ryzhova, T.L. Moroz (2016) revealed that 50% of pharmaceutical specialists do not provide any information when dispensing medicines [17]. However, current recommendations for pharmaceutical consulting lack specificities [18,19].

The analysis of the consumers' opinions regarding the functions of pharmaceutical specialists, revealed that 60% of buyers of antiallergic OTC drugs identified pharmacy specialists with health professionals. Pharmacy specialists were recognized as trade workers by 22% of the respondents, and 18% of the respondents found it difficult to answer.

Answering the question about the goals of pharmacy specialists, only 57% of the respondents chose "the provision of pharmaceutical care" as pharmacists' main priority; 27% marked "making a profit from the sales of medicines", and 16% of the respondents believed that a pharmacy specialist is interested in both profit and providing pharmaceutical assistance".

Thus, as representatives of the health care system, pharmaceutical specialists are highly reputed by pharmacy visitors. An increasingly larger role of pharmaceutical specialists as health professionals, is notified by the World Health Organization (WHO), the International Pharmaceutical Federation (IPF), in publications of domestic and foreign scientists [20–23]. At the same time, in contrast to doctors, there are no standardized and approved protocols of pharmaceutical counseling in the framework of responsible self-medication for pharmaceutical specialists in Russia.

Performing medical functions – asking a pharmacy visitor about allergy symptoms and even making a diagnosis (Fig. 5), pharmacists rely on their professional experience without any standardized guidance on pharmaceutical counseling for symptoms – the most common reasons for going to a pharmacy for responsible self-medication.

There is international experience in the use of the pharmacists' (dispensers') protocols when selling industrially manufactured OTC drugs, in particular for the symptomatic treatment of allergies [24]. The protocols are designed to provide informational support for dispensing-medicinal products when patients or their representatives visit a pharmacy organization without a doctor's prescription. Protocols contain a list of standardized questions. The key points for the visitors contacting a pharmacy with allergy symptoms are:

1. Who has got a problem (a patient, family members, acquaintances – children or adults);
2. When the symptoms appeared and how long they have lasted;
3. What measures had been taken before contacting the pharmacy;
4. What medications have already been taken to relieve the conditions.

For the timely provision of medical care, it is vital to include a list of symptoms and life threatening conditions for allergies in the protocols. They are: general weakness, drop of blood pressure, suffocation, signs of laryngeal edema (a hoarse voice, a «barking» cough), swelling of the upper half of the face, bloody nasal discharge, neurological symptoms (anxiety, fear, increased physical activity), nausea, vomiting, abdominal pain. In such cases, a patient needs to seek for urgent medical attention. The protocols should also indicate the need to consult a doctor if allergy manifestations persist or recur sporadically when taking OTC drugs during the day, or new allergy symptoms appear during the treatment. The algorithms provide a list of questions regarding the presence of allergy symptoms: whether a diagnosis had been made by a doctor, whether there are the symptoms associated with possible contacts with allergens (pet hair, a plant blooming period, contact with chemicals, insect bites, etc.).

[2] Order of the Ministry of Health of the Russian Federation, August 31, 2016 No. 647n "On approval of the rules for good pharmacy practice of drugs for medical use" URL: http://www.consultant.ru/document/cons_doc_LAW_210618/

The protocols provide a list of OTC drugs for symptomatic treatment of allergies, recommendations regarding the dosage regimen of drugs, the conditions of use, the treatment period, storage rules, warnings about possible side effects, interactions, contraindications, as well as advice to eliminate contact with possible allergens, keep to hygienic measures and diet. Similar protocols for pharmaceutical consulting should be developed in each country, taking into account the legal regulation of the dispensing drugs procedure, the range of OTC drugs, the local specifics of allergic diseases, hygienic traditions and standards [13, 25, 12].

The survey of the pharmacies' visitors, helped to detect certain flaws in pharmaceutical counseling for allergy. It was found out that pharmaceutical specialists offered lots of unnecessary as well only expensive drugs, tried to diagnosticate, did not provide information about the need to consult a physician or the frequency and peculiarities of OTC drugs use. Despite the requirements of Order of the Ministry of Health of the Russian Federation No. 647n "On approval of the rules of good pharmacy practice for drugs for medical use", only 25% of pharmaceutical specialists drew the visitors' attention to possible drug and food interactions, and 17% paid attention to the peculiarities of OTC medicines storage at home.

The development of pharmaceutical consulting protocols for dispensing of non-prescription drugs, will help to improve the quality of service in pharmacy organizations as a component of the health care system in the Russian Federation.

CONCLUSION

The profile of an over-the-counter antiallergic drugs buyer in the pharmacies of Kazan was assessed in the following way: it is a woman aged 18–44 of a middle level income, having a family of 3–4 people, ready to spend from 101 to 500 rubles on the purchase of antiallergic drugs, buying anti-allergic drugs not for the first time. The main reasons to seek for treatment were skin rash, redness and itching, which had also been observed in the past. The allergic nature of the disease had already been confirmed by the doctor. For the average consumer of over-the-counter antiallergic drugs, the most important criteria for choosing a medicine were as follows: efficiency, safety, the doctor's recommendations and price. The medicines were purchased for the visitors themselves or their children. The buyers were satisfied with the range of antiallergic OTC drugs. Pharmacy specialists' advice were generally trusted. The visitors recognized pharmacists as health professionals but the respondents believed that a pharmacy specialist is interested in both profit from the sale of drugs and providing pharmaceutical assistance. The following isadvantages of pharmaceutical counseling when dispensing antiallergic OTC medicines were detected: improper diagnoses by a pharmaceutical specialist and lack of recommendations to consult a doctor, incomplete provision of information on the use, storage, and the possibility of interaction with other drugs and food.

The need for domestic standardized algorithm of pharmaceutical counseling for the visitors contacting a pharmacy with allergy symptoms in the framework of responsible self-medication, has been demonstrated.

FUNDING

This study did not receive any support from external organizations.

CONTRIBUTION OF AUTHORS

All authors contributed equally to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

ANALYSIS OF INDUSTRIAL PRACTICE OF DRUG QUALITY RISK MANAGEMENT IN RUSSIAN PHARMACEUTICAL ENTERPRISES

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Received 11 Sep 2020 Accepted 30 Oct 2020

The aim of the research was to study the current industrial practice of drug quality risk management in Russian pharmaceutical enterprises, including the assessment of the main problems during the implementation of the risk management system and its compliance with the accepted international approaches.
Materials and methods. In the period from 6 April to 10 May 2020, an online survey of the leading employees in the field of quality assurance of Russian manufacturers was conducted. In the survey, the questionnaire was based on the results of the authors' analysis of the national regulatory legal acts of the Russian Federation, the European Union countries, international guidelines of the EAEU, ICH and WHO in this area. 111 people took part in the survey, the return of questionnaires was 11.5%.
Results. The data obtained indicate the prevalence of a superficial approach to quality risk management in the Russian pharmaceutical industry, the presence of objective and subjective reasons that hinder the effective implementation of these methods, the fragmentation of the systems used and, in most cases, their ineffective use. The respondents believe that the most significant reasons for the difficulties in implementing this methodology, are the lack of recommendations from the Ministry of Industry and Trade of Russia on creating an effective quality risk management system and a shortage of the specialists who are ready to work in the area of this industry. The survey revealed rather large gaps in the deployment of a risk management system at the enterprise and separation from the established international practice.
Conclusions. The data obtained indicate the extreme urgency of developing recommendations for a quality risk management system, which should be based upon and supported by Russian regulatory legal acts and international experience in this area. The authors propose highlights for these recommendations.
Keywords: quality risks; drugs; Russian pharmaceutical industry
Abbreviations: EU – European Union; EAEU – Eurasian Economic Union; ICH – The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use; SOP – Standard Operating Procedures; GMP – Good Manufacturing Practice; FMEA – Failure Mode and Effects Analysis; HACCP – Hazard Analysis and Critical Control Points; FMECA – Failure Mode, Effects and Criticality Analysis; PHA – Preliminary Hazard Analysis; FTA – Fault tree analysis; MHRA – Medicines and Healthcare products Regulatory Agency; PDA – Parenteral Drug Association; ISPE – International Society of Pharmaceutical Engineering; ASTM – American Society for Testing and Methodology

АНАЛИЗ ОТРАСЛЕВОЙ ПРАКТИКИ ПО УПРАВЛЕНИЮ РИСКАМИ ДЛЯ КАЧЕСТВА ЛЕКАРСТВЕННЫХ СРЕДСТВ НА РОССИЙСКИХ ФАРМАЦЕВТИЧЕСКИХ ПРЕДПРИЯТИЯХ

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Получено 11.09.2020 Принята к печати 30.10.2020

For citation: A.B. Kashirina, Zh.I. Aladysheva, N.V. Pyatigorskaya, V.V. Belyaev, V.V. Beregovykh. Analysis of industrial practice of drug quality risk management in russian pharmaceutical enterprises. *Pharmacy & Pharmacology*. 2020;8(5):362-376. **DOI:** 10.19163/2307-9266-2020-8-5-362-376

Для цитирования: А.Б. Каширина, Ж.И. Аладышева, Н.В. Пятигорская, В.В. Беляев, В.В. Береговых. Анализ отраслевой практики по управлению рисками для качества лекарственных средств на российских фармацевтических предприятиях. *Фармация и фармакология.* 2020;8(5):362-376. **DOI:** 10.19163/2307-9266-2020-8-5-362-376

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-5-362-376

Цель работы: изучение текущей отраслевой практики по управлению рисками для качества лекарственных средств на фармацевтических предприятиях России, включая основные проблемы при внедрении системы управления рисками и соответствие общепринятым международным подходам.

Материалы и методы. В период с 6 апреля по 10 мая 2020 года был проведен онлайн-опрос ведущих сотрудников в области обеспечения качества российских производителей. Анкета, использованная при опросе, разработана по результатам анализа национальных нормативных правовых актов Российской Федерации, стран Европейского Союза, международных руководств ЕАЭС, ICH и ВОЗ в данной области. В опросе приняли участие 111 человек, возврат анкет составил 11,5%.

Результаты. Полученные данные свидетельствуют о превалировании в российской фармацевтической отрасли поверхностного подхода к управлению рисками для качества, наличии объективных и субъективных причин, мешающих эффективному внедрению этих методов, фрагментарности используемых систем и, в большинстве случаев, их неэффективному использованию. Наиболее значимыми причинами сложностей при внедрении этой методологии респонденты считают отсутствие рекомендаций Минпромторга России по созданию эффективной системы управления рисками для качества и дефицит в отрасли специалистов, готовых к проведению работ в этой области. Опрос выявил достаточно большие пробелы российских предприятий в развертывании системы управления рисками на предприятии и разрыв с устоявшейся международной практикой.

Заключение. Полученные данные свидетельствуют о крайней актуальности разработки рекомендаций по системе управления рисками для качества, опирающихся на положения российских нормативных правовых актов и международный опыт в этой области. Авторами предложены тезисы для этих рекомендаций.

Ключевые слова: риски для качества; лекарственные средства; российская фармацевтическая отрасль

Список сокращений: ЕС – Европейский союз; ЕАЭС – Евразийский экономический союз; ICH (The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) – Международный совет по гармонизации технических требований к регистрации лекарственных препаратов для медицинского применения; СОП – стандартные операционные процедуры; GMP (Good Manufacturing Practice) – надлежащая производственная практика; FMEA (Failure Mode and Effects Analysis) – анализ видов и последствий отказов; HACCP (Hazard Analysis and Critical Control Points) – анализ рисков и критических контрольных точек; FMECA (Failure Mode, Effects and Criticality Analysis) – анализ видов, последствий и критичности отказов; PHA (Preliminary Hazard Analysis) – метод предварительного анализа опасностей; FTA (Fault tree analysis) – Анализ дерева отказов; MHRA (Medicines and Healthcare products Regulatory Agency) – Агентство Великобритании по контролю оборота лекарств и медицинских товаров; PDA (Parenteral Drug Association) – Ассоциация парентеральных препаратов; ISPE (International Society of Pharmaceutical Engineering) – Международное общество фармацевтического инжиниринга; ASTM (American Society for Testing and Methodology) – Американское общество по испытанию материалов

INTRODUCTION

The quality risk management system is a part of enterprises' quality management system in different industry sectors. Such industries include, for example, food industry, production of medical devices, car manufacturing, aircraft engineering and others[1]. Risks can be present at all stages of the product life cycle. The risk-based approach contributes to ensuring the quality of products, achieving control of technological processes, and a proper allocation of resources [1, 2]. Herewith, it is only in the pharmaceutical industry that a quality risk management, is a mandatory element of the pharmaceutical quality system at any enterprise, specified by good manufacturing practices (GMP), and included in licensing requirements[2]. Requirements for the proper use of drug quality risk management, are specified by regulatory authorities in many countries, as well as international organizations [3–5]. The fundamental principles of a quality risk management at Russian pharmaceutical enterprises, are reported in the Rules of Good Manufacturing Practice, approved by Order of the Ministry of Industry and Trade of the Russian Federation dated June 14, 2013, No.916. A systematic approach to the quality risk management, aimed at improving the efficiency of the application of the Good Manufacturing Practice Rules, is reported in Order of the Ministry of Industry and Trade of the Russian Federation dated 12

December, 2013 No.1997 "On the approval of Recommendations for the organization of production and quality control of medicines". GMP rules of the Eurasian Economic Union, approved by the Resolution of the Council of the Eurasian Economic Commission dated 3 November, 2016 No.77, in relation to the quality risk management, do not differ from the Russian ones: Part 3 (chapter "Quality risk management") provides

[1] 1. GOST R 51705.1-2001. Quality systems. HACCP principles for food products quality management. General requirements. 2. GOST R 54617.1-2011. Risk management in nanoindustry. General principles. 3. GOST R 54617.2-2011. Risk management in nanoindustry. Identification of hazards. 4. GOST R 54762-2011. Prerequisite programmes on food safety. Part 1. Food manufacturing. 5. GOST R 58045-2017. Aircraft equipment. Risk management for quality assurance through life cycle stages. Risk assessment methods and acceptability criteria. 6. GOST R 58050-2017. Aircraft equipment. Risk management for quality assurance through life cycle stages. Areas of uncertainty classification. 7. GOST R 58139-2018. Quality management systems. Requirements for automotive organizations. 8. GOST R ISO 17666-2006. Risk management. Space systems. 9. GOST R ISO 17776-2012. Petroleum and natural gas industries. Offshore production installations. Techniques and methods for hazard identification and risk assessment. Basic principles.

[2] clause 5 of the Rules of Good Manufacturing Practice (approved by Order of the Ministry of Industry and Trade No. 916 of June 14, 2013).

similar recommendations on the organization of risk management activities; part 3 is non-regulatory[3]. Most countries, including the EU, EAEU countries and the Russian Federation, apply the risk management procedure given in the ICH Q9 guideline and presented in the international standard ISO 31000 (GOST R ISO 31000).

However, the application of the risk management system causes difficulties for manufacturers [6-10]. For example, in the FDA (Food and Drug Administration) warning letters database, the issues regarding the risk management system, are posted quite often[4] [11]. In the official statistics of Good Manufacturing Practice (GMP) inspection deficiencies published by United Kingdom's Medicines and Healthcare products Regulatory Agency (MHRA UK), in 2018 solely [5], there are 74 non-conformities in the quality risk management, 54 (almost 73%) of which were assessed as critical or significant. The similar data on the results of GMP inspections in the Russian Federation are not publicly available. Implementing a quality risk management system into an enterprise's quality management system can be challenging. Enterprises have a wide range of products, different medicines are produced in different conditions: from non-sterile to aseptic, production processes have different stages and methods of control. Each enterprise needs to make its own individual choice of risk assessment methods, methods of risk communication, working out its documentation, etc., taking into account the peculiarities of its production and quality system [12–16]. The carried out search and analysis of the literature showed that the national and international regulatory documents contain only guidelines to quality risk management, while there are no explanatory methodological materials containing specific examples of possible approaches to the quality risk management, including specific industries.

The importance of applying the quality risk management in pharmaceutical production, is due to several reasons: first, the risk management makes it possible to ensure the acceptable product quality, and therefore, to reduce risks to patients' healths. Second, it allows the company's management to focus on the issues associated with the highest risks for patients, therefore, it affords a more efficient allocation of resources. Third, it helps with making the most well-argued decisions regarding the development, quality control, production of medicines, etc. Fourth, the application of the risk management is the fulfillment of the requirements of regulatory authorities [1, 8, 9, 17, 18].

There are no publications on the state of this issue at domestic pharmaceutical enterprises. All of the above indicates the relevance of studying the current industry practice of drug quality risk management at Russian pharmaceutical enterprises, including main problems during the implementation of a risk management system and compliance with currently accepted international approaches.

THE AIM of the research was to study the current industrial practice of drug quality risk management at Russian pharmaceutical enterprises, including the assessment of the main problems during the implementation of the risk management system and its compliance with the accepted international approaches.

MATERIALS AND METHODS

To obtain information about the existing approaches to drug quality risk management, a questionnaire method was chosen. The questionnaire was developed on the basis of the analysis of the requirements and recommendations for the quality risk management specified in national and international regulatory documents and guidelines[6].

RESULTS AND DISCUSSION

The following essential requirements for the quality risk management system, accepted in the international pharmaceutical industry, have been selected by the authors and used in their questionnaire [10, 19–22].

[3] Rules of Good Manufacturing Practice of the Eurasian Economic Union approved by the Resolution of the Council of the Eurasian Economic Commission No. 77 dated 3 November 2016

[4] https://www.fda.gov/inspections-compliance-enforcement-and criminal-investigations/compliance-actions-and-activities/warning-letters

[5] https://www.gov.uk/government/statistics/good-manufacturingpractice-inspection-deficiencies

[6] 1. Good Manufacturing Practice Rules (approved by Order of the Ministry of Industry and Trade No. 916 of June 14, 2013). 2. Rules of Good Manufacturing Practice of the Eurasian Economic Union approved by the Resolution of the Council of the Eurasian Economic Commission No. 77 dated 3 November 2016. 3. Order of the Ministry of Industry and Trade of the Russian Federation of December 12, 2013 N 1997 On the approval of the Recommendations on the organization of production and quality control of medicines. Recommendations for the preparation of Site Master File, quality risk management, pharmaceutical quality system, batch certification (part III). 4. Decision of the Board of the Eurasian Economic Commission No.1 "On Approval of the Guidelines for establishing acceptable limits for health effects in order to identify risks in the production of medicines on common production (technological) lines" dated January 14, 2020. 5. Department of Health and Human Services, U.S. Food and Drug Administration, Guidance for Industry, Q9 Quality Risk Management, 2006. 6. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Q8 (R1), Pharmaceutical Development, 2008. 7. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Q9, Quality Risk Management, 2005. 8. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Q10, Pharmaceutical Quality System, 2008. 9. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Q11, Development and manufacture of drug substances (chemical entities and biotechnological/biological entities), 2012. 10. MHRA Good Manufacturing Practice (GMP) – Quality Risk Management: Frequently asked questions, Available at http://www.mhra.gov.uk. 11. World Health Organization WHO Guideline on Quality Risk Management, Working document QAS/10.376/Rev.1 Draft for discussion, August 2011, pp. 9–10, Available at http://www.who.int.

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DOI: 10.19163/2307-9266-2020-8-5-362-376

Essential requirements for the quality risk management system accepted in the international pharmaceutical industry

1. An enterprise should have a high-level document (SOP, an enterprise standard, policy) that regulates its quality risk management system and approaches used to manage risks.

2. The areas of application of the risk management system within the pharmaceutical quality system, should be determined.

3. The following papers should be documented:

– commitment of the management to the principles of the system;

– responsibilities and functions of the key personnel in this system;

– scope of application, planning and scheduling;

– monitoring of work and evaluation of its efficiency and progress;

– the approval procedure of work/jobs and the information distribution;

– personnel training programs that include information about a risk management system;

– training requirements for teaching the personnel actually performing the work related to the quality risk management;

– the risk assessment tools and methods used at the enterprise;

– the inclusion of the risk management in the pharmaceutical quality system;

– application of the change management procedure during risk management activities;

– cross-references to the risk management system in the main control procedures of the enterprise.

4. Risk analysis and assessment:

– should be carried out by experienced specialists, including the involvement of third-party consultants, with due regard to modern scientific knowledge;

– should be documented;

– should be subjected to the agreement/approval;

– should be based on the systematic risk identification;

– should be carried out using both qualitative and quantitative methods and tools,

– the results of the assessment should be regularly revised;

– the decisions based on the results of the risk assessment should not contradict GMP rules and regulatory requirements;

– the level of formality and documentation should be appropriate to the degree of the risk to the patient.

5. There should be a risk register containing a refreshable list of the main identified risks, a list of risk assessments carried out or a link to this list, a brief description of the measures to mitigate the main identified risks, and a justification for reassessing risks.

6. The effectiveness of the risk management system should be regularly evaluated. The procedure for assessing the effectiveness of the risk management system and the effectiveness of the risk management plans, should include:

– frequency of assessment;

– responsibility of performers;

– a formal list of the documentation to be inspected during the assessment;

– the ways of information distribution on the results of the assessment;

– the procedure for developing recommendations for improvement;

– the procedure for implementing the subsequent actions and their verification.

The questionnaire contains 25 questions, some of which correlate to each other. The clarity of the questions was tested by 42 people from the pharmaceutical industry.

The survey was conducted online by Sechenov University in cooperation with the National Chamber of Pharmacy from 6 April to 10 May, 2020. The letter with a link to the electronic questionnaire, was sent to e-mails of the qualified persons who had been trained and certified at Sechenov University (981 people, 48 constituent entities of the Russian Federation, more than 300 enterprises).

111 specialists of the pharmaceutical industry took part in the survey, the return of the questionnaires was 11.3%. About a third of the respondents (35%) were under 40 years old, 15 percent were over 55. More than half of the respondents work at medium and large pharmaceutical enterprises (45% and 13%, respectively), the rest – at small enterprises and in micro organizations. This distribution generally reflects the structure of the Russian pharmaceutical industry (Fig. 1).

Most of the respondents (63%) work at the enterprises producing drugs of the chemical origin and have more than 10 years of professional experience at a pharmaceutical enterprise (67%) (Fig. 2).

The majority of respondents (87.4%) work at the enterprises with a cyclical turnaround; the collected information also includes the respondents from market authorization holders that use contract manufacturing sites (4.5%).

As Fig. 3 shows, all major dosage forms produced in Russia, were covered with this questionnaire, including those requiring and not requiring isolation. Based on the above data, the authors arrived at the conclusion that the sample obtained was sufficiently representative.

In the first part of the survey, general approaches and problems of domestic pharmaceutical enterprises in implementing the systems of drug quality risk management were studied.

In the Russian pharmaceutical industry, the quality risk management is applied at various stages of the product life cycle: most of the enterprises apply risk management at the stage of the industrial production (95%), and only 28% of enterprises apply a risk-based approach when scaling the process (Fig. 4).

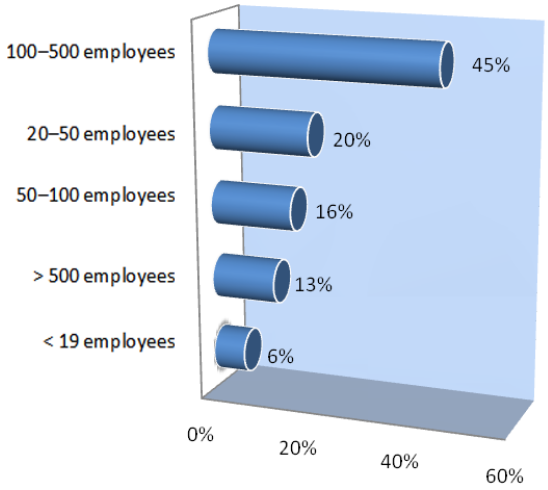

Figure 1 – Distribution of respondents by the size of their enterprise

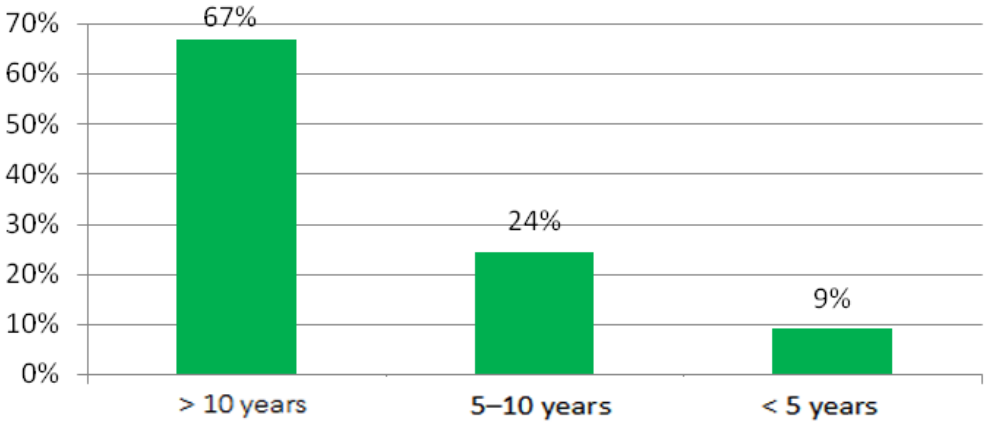

Figure 2 – Distribution of respondents by work experience in the pharmaceutical industry

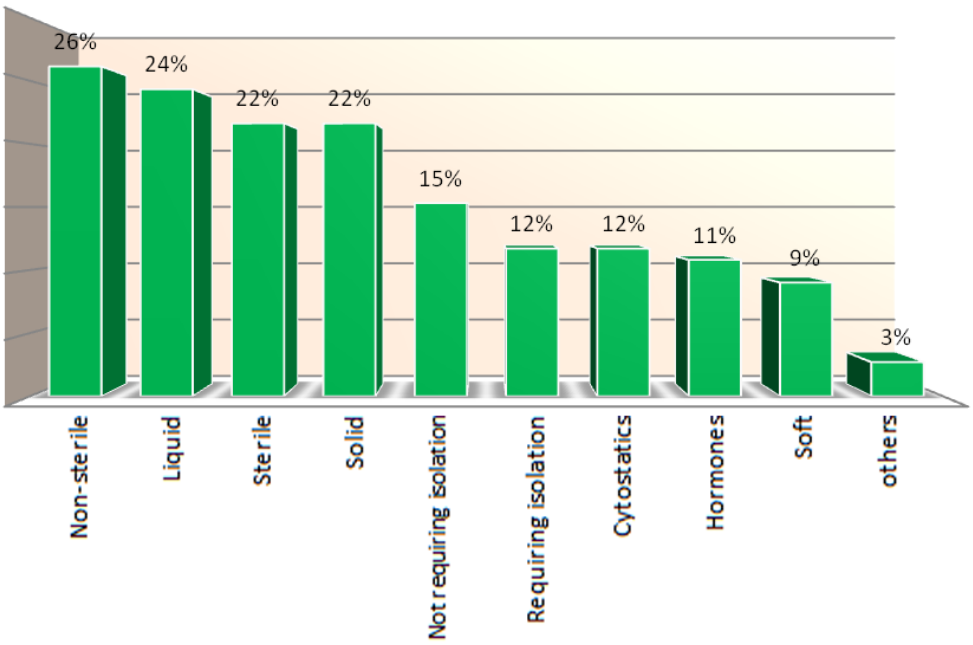

Figure 3 – Manufactured dosage forms

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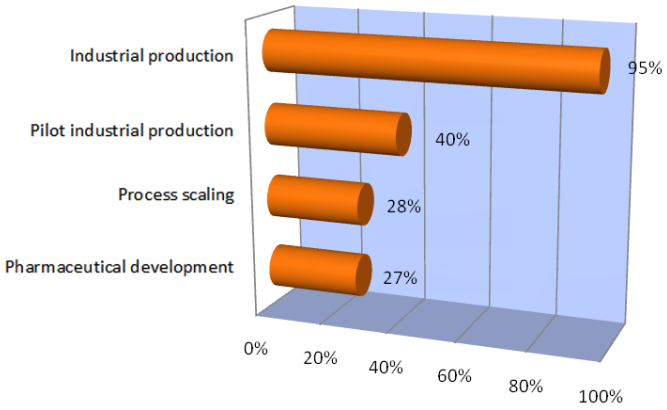

Figure 4 – Stages of product life cycle at which quality risk management is applied, in Russian pharmaceutical industry

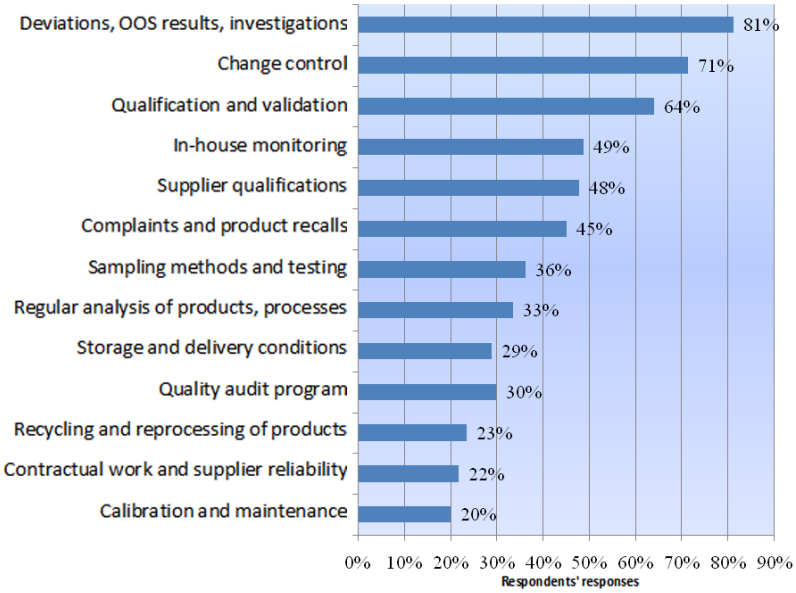

Figure 5 – Areas where quality risk management is most commonly used

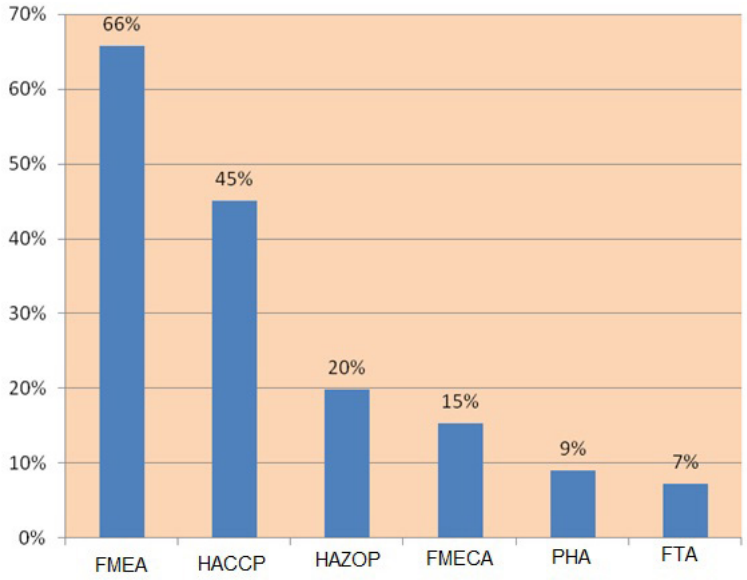

Figure 6 – The most commonly used risk assessment tools and methods

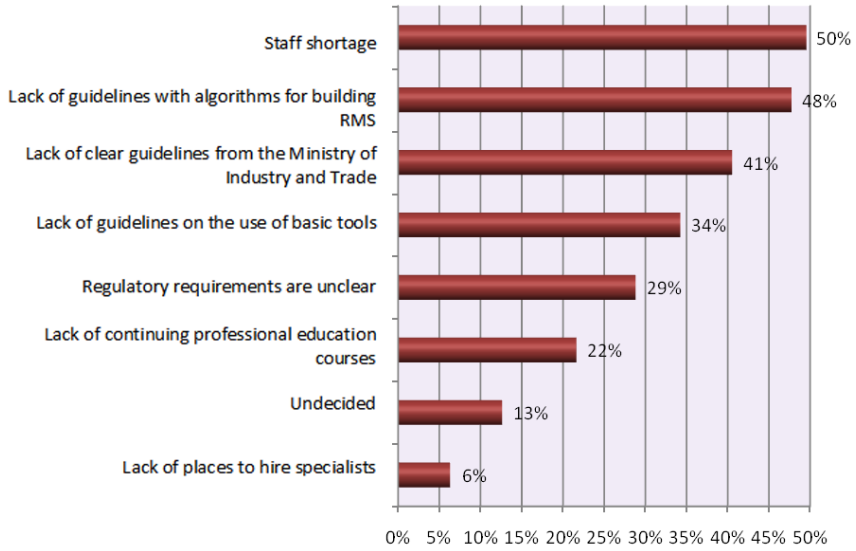

Figure 7 – The main difficulties faced by enterprises during the implementation of a quality risk management system

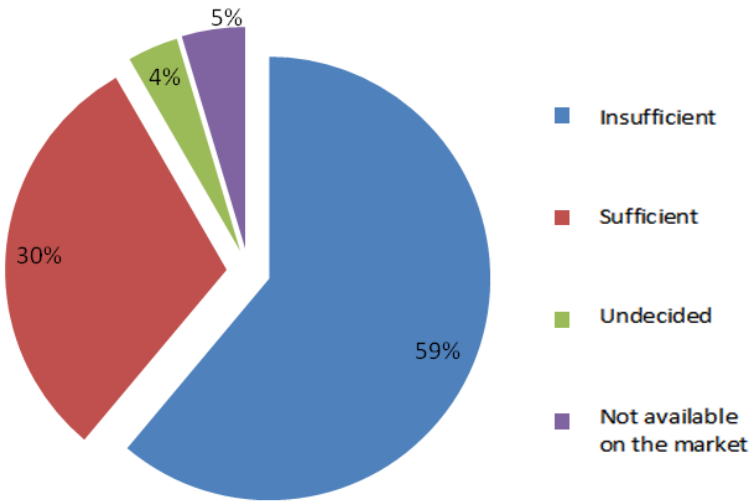

Figure 8 – Sufficiency of the number of employees with the necessary knowledge and experience in risk assessments

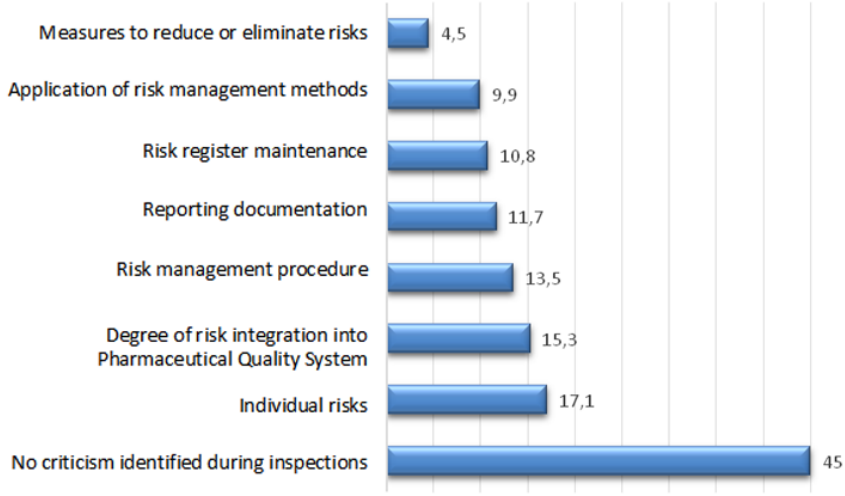

Figure 9 –Criticism of quality risk management system identified during external inspections

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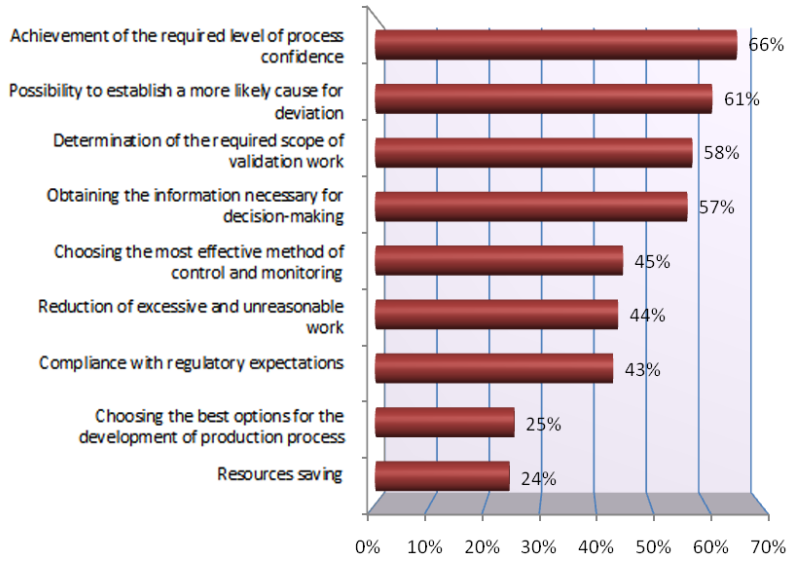

Figure 10 – Positive effects of the quality risk management implementation at the enterprise

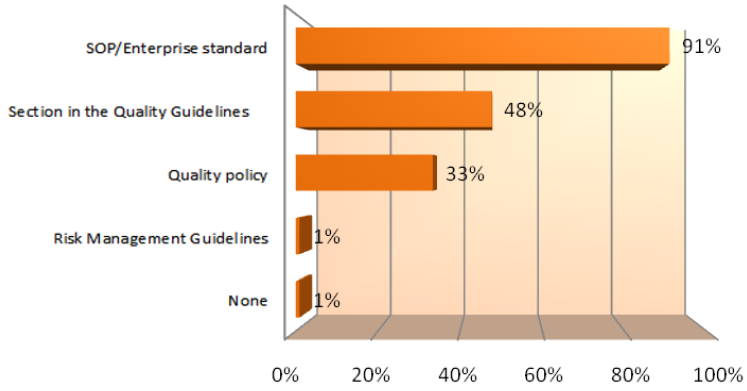

Figure 11 – Available high-level documents regulating the quality risk management system

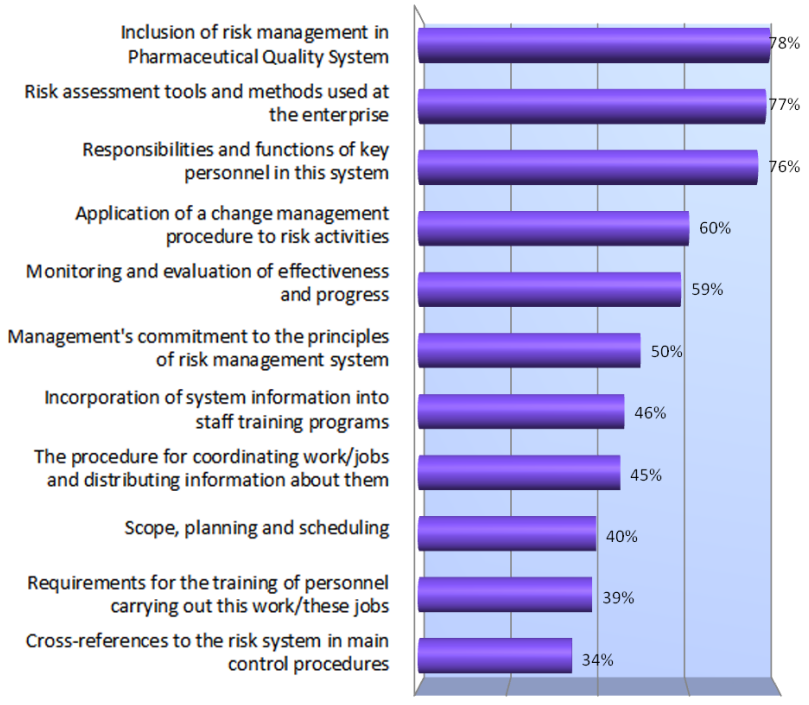

Figure 12 – Approaches to formalization of the quality risk management system

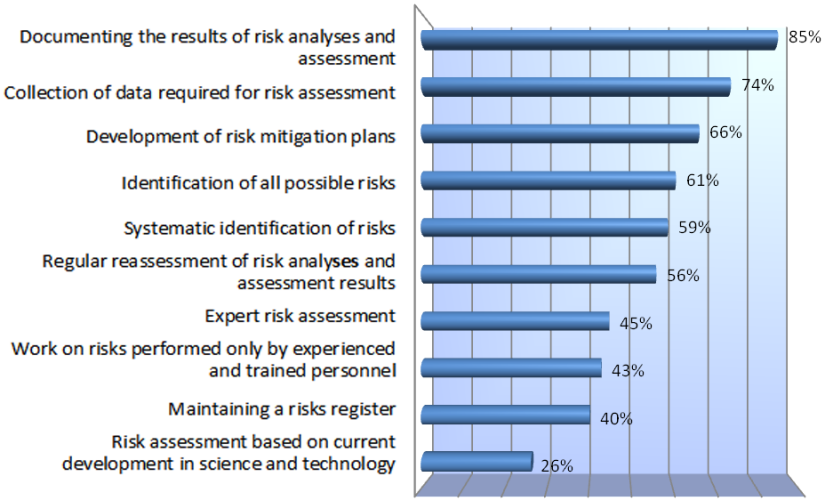

Figure 13 – Actions in relation to the quality risks

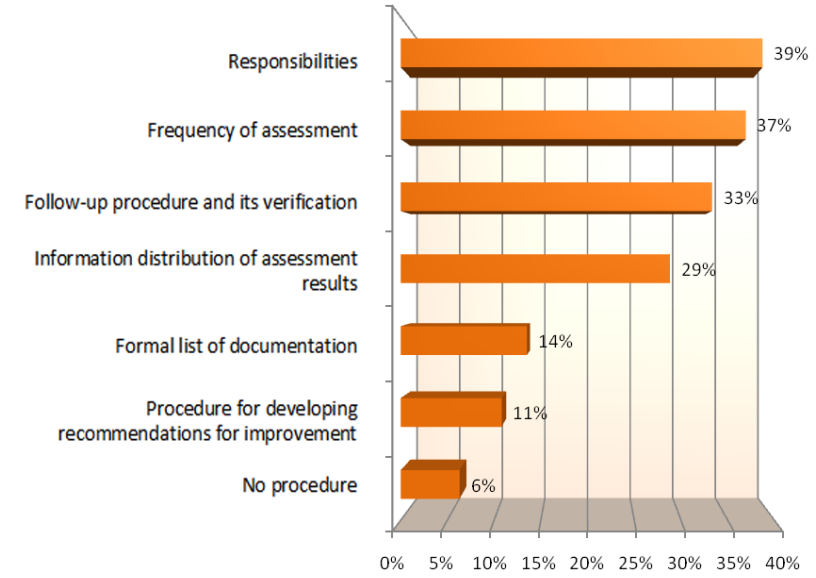

Figure 14 – Contents of the quality risk register

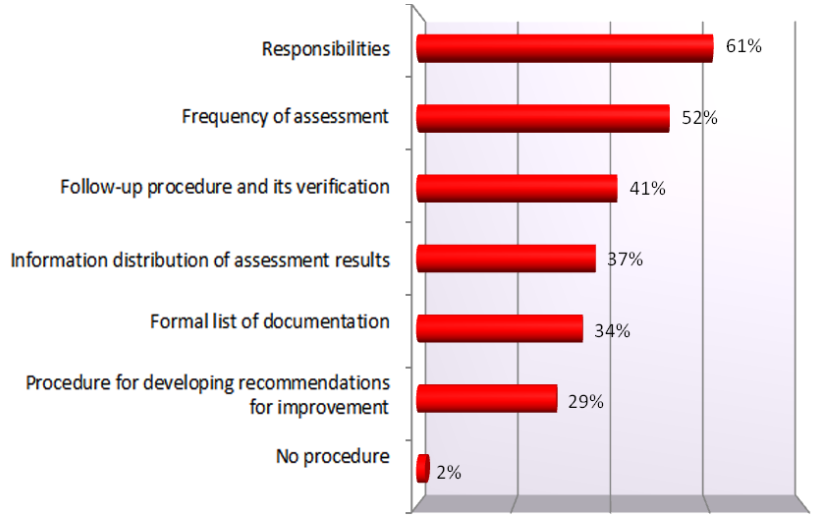

Figure 15 -Information in the Procedure for assessing effectiveness of risk management system and risk management plans

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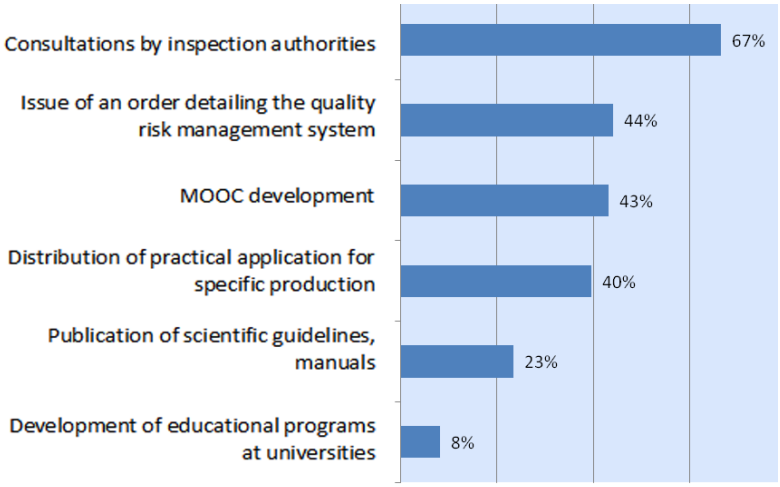

Figure 16 – Measures that can help improve the effectiveness of quality risk management in Russian pharmaceutical industry

Fig. 5 shows the areas where the quality risk management is most commonly used. In general, the figure shows that they coincide with the pharmaceutical enterprises' areas of activities, proposed in Order of the Ministry of Industry and Trade of the Russian Federation No.1997 "On approval of recommendations for the organization of production and quality control of drugs", where the quality risk management is applicable. Noteworthy is the small percentage of the enterprises using this methodology for organizing calibration and maintenance work (20%), during product processing and reprocessing (23%), and during storage and delivery (29%).

In most of the cases, domestic enterprises use quantitative methods to assess and analyze risks (FMEA, HACCP, FMECA, PHA, FTA, etc.). The most frequently used methods of the 7 ones, specified in the ICH Q9 guideline and in Order of the Ministry of Industry and Trade of the Russian Federation No.1997 "On approval of recommendations for the organization of production and quality control of drugs", are the following: FMEA (66%), HACCP (45%), HAZOP (20%), FMECA (15%) (Fig. 6).

The implementation of the quality risk management methodology is difficult in practice for various reasons (Fig. 7).

According to the respondents, the main difficulties are: staff shortage (50%), lack of guidelines and manuals with algorithms for decision-making and building a risk management system (48%), lack of clear guidelines from the Ministry of Industry and Trade (41%), lack of guidelines on the use of the basic risk analysis tools (34%). When summed up, the lack of additional guidelines from the Ministry of Industry and Trade of Russia, is the prevailing reason for the complexity of the implementation of the quality risk management system. An extended analysis of the responses regarding staff shortages, revealed approximately the equal percentage of responses among the employees of small and micro enterprises: the problem of staff shortages was notified by 59% of respondents working at the enterprises with 20 to 50 employees, 53% – from 50 to 100 employees, 50% – from 100 to 500 employees, 43% – above 500 employees.

The problem of a shortage of personnel with the necessary knowledge and skills in the field of quality risk management, was also revealed when analyzing the answers about the sufficiency of such employees at the enterprise: 59% of the respondents indicated this shortage (Fig. 8).

Herewith, 88% of respondents stated that external consultants are not involved in dealing with risks at their enterprise.

Some interesting data were obtained when analyzing the issues of the drug quality risk management system discovered by external auditors (Fig. 9). Just over half of the enterprises received comments on their risk management systems. At the same time, auditors' reports on various aspects of the risk management contained approximately the same number of issues, except risk mitigation and preventive measures of identified quality risks.

In general, the respondents positively assessed the impact of the risk management system on the company's activities (Fig.10). More than half of the respondents notified that the risk management provides the necessary level of confidence in the processes (66%), the ability to determine the most likely cause of deviations (61%), determining the required amount of validation work (58%), obtaining the information necessary for decision-making (57%). Attention should be paid to a rather low percentage of the enterprises, which conducted an economic assessment of the application of the risk management system – 24%, and used a risk methodology for a pharmaceutical development – 25%

(see also Fig. 4, where 27% of respondents apply this methodology in pharmaceutical development). A fairly large number of respondents (57%) who do not consider these works mandatory for compliance with the established regulatory requirements is noteworthy, although this requirement came into force almost 7 years ago. In the authors'opinions, the number of enterprises (57%) which use a risk-based approach for decision making, is also too few.

A coincidence of the data obtained from different questions, indicates the validity of the data.

The results obtained, made it possible for the authors to conclude that a superficial approach to the quality risk management prevails in the Russian pharmaceutical industry, and this is due to a number of objective and subjective reasons. Among the most significant ones are the lack of recommendations from the Ministry of Industry and Trade of the Russian Federation on creating an effective quality risk management system and the shortage of specialists who are ready to work in this area.

In the second part of the survey, the authors examined the way the Russian enterprises implement various elements of the risk management system and their compliance with essential requirements for the quality risk management system in the international pharmaceutical industry (see above).

91% of the respondents confirmed the existence of the document regulating the quality risk management system at the enterprise, and 33% notified the existence of a policy in the field of the risk management system (Fig. 11). Almost half of the respondents (48%) reported that the risk management system is included in the quality guidelines.

The approaches used in the Russian pharmaceutical industry to formalize the risk management system, are shown in Fig. 12. As can be seen from the above data, all international criteria are met with, but the degree of their implementation varies from 78% to 34% of cases. It should be notified that external auditors do not attract manufacturers' attention to the absence of such important aspects as the adherence of the management to the principles of the system, the responsibility and functions of the key personnel in this system, the procedure for coordinating work and distribution of information about them, applying the change management procedure to the risks management, cross-linking to the risks system in the main control procedures of the enterprise (see Fig. 9). A significant number of enterprises do not pay due attention to personnel training in the field of the risk management system (54%), to the establishment of requirements for personnel training (61%), and to the importance of ensuring information flows in the risk management system (55%). With close reference to the data shown in Fig. 10, the lack of formalized confirmation, in other words, commitment, adherence to the principles of the risk management system, indicates a low awareness and interest of the top management in more than half of pharmaceutical enterprises in Russia in the risk management system and its business opportunities.

Approximately the same data were obtained for the work/jobs that constitute the quality risk management (Fig. 13): compliance with international criteria ranges from 85% to 26%. 15% of the respondents notified that they do not document the results of the risk analysis and assessment. The further research showed that such an informal approach is more often observed at small enterprises (with 20 to 50 employees). The data obtained, also indicate a lack of the systematic quality risks management in more than half of the surveyed pharmaceutical companies. A low percentage of the enterprises that use a scientific approach to work with risks (only 26%), should be also notified.

A very big gap was identified in the maintenance of the quality risk register (Fig.14). First, only about 40% of the respondents answered that their company has a quality risk register (Fig. 13 and Fig. 14). Second, the content of the risk register of a Russian manufacturer also differs from the international practice. Thus, only 39% of the enterprises include a list of the conducted risk assessments or links to this list in the register; only 33% of the enterprises have a refreshable list of the identified key risks. It should be notified that there is a very low percentage of the enterprises that include justifications in the register for risks reassessment and for establishing the frequency of reassessment (14% and 6%, respectively), as well as the data on the residual risks (11%).

The procedure for assessing the effectiveness of the risk management system and the effectiveness of the risk management plans in the Russian pharmaceutical industry, also differs from the international approaches (Fig. 15). Despite the fact that the absence of this procedure was confirmed by only 2% of respondents, 61% of the respondents confirmed that it contains a description of responsibilities, 52% confirmed the established frequency of the assessments, 41% – the procedure for implementing subsequent actions and their verification, 37% – the ways of sharing information about the results of the evaluation, 34% – a formal list of documentation, 29% – the procedure for developing recommendations for its improvement.

The size of the enterprise (by the number of employees) or its products did not influence the distribution of the respondents' answers.

It should be notified that many disadvantages of the implementation of the quality risk management system

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at Russian enterprises are typical and are also encountered by foreign manufacturers, including ineffective application of the risk management methods, the absence of periodic reassessment of identified risks, and the lack of credibility of the decisions taken [1, 9, 10, 17, 18, 23]. Therefore, foreign experience could be useful for developing measures to improve the current situation.

The opinions of the surveyed pharmaceutical industry employees on the measures that can help their companies implement the effective quality risk management, are shown in Fig. 16. The most popular response was to consult with the inspection authorities (67%), but this type of measure is not used in any country with a developed pharmaceutical industry. In terms of the number of pharmaceutical companies in the country, Russia is comparable to the United Kingdom, France and Germany [24, 25].

Based on the authors' experience and observations, foreign inspection bodies (as well as Russian ones) do not give direct individual consultations. It is a common practice to organize seminars: independent seminars on behalf of the regulatory body, and as parts of congresses and conferences [8, 18, 26]. Such events also take place in our country. The practice of creating massive open online courses (MOOC) by regulators or with their participation, is not yet.

No regulatory legal acts by foreign regulators with a detailed description of the quality risk management system, have been found by the authors. The quality risk management guidelines have only been issued by the MHRA (UK) and are not legally binding. There are recommendations from professional communities: PDA (Parenteral Drug Association) and ISPE (International Society of Pharmaceutical Engineering). A risk-based approach to an equipment validation is described in ASTM (American Society for Testing and Methodology) standard manual E2500-13[7].

A possible contribution to improving the situation by universities and research institutes, was rated by the respondents as extremely low. Abroad, on the contrary, universities, often with the assistance of regulators and with grants from regulators, summarize various data, develop and distribute various scientifically based recommendations for the pharmaceutical industry.

CONCLUSION

In the course of the study, the authors analyzed the requirements for the risk management in the pharmaceutical industry, and identified the essential elements of an effective drug quality risk management system. The survey provided valid information about the industry practice of implementing the risk management system at Russian enterprises, the difficulties faced by pharmaceutical companies during this process, and the opinions of specialists on the measures to support the application of risk system. The results of the analysis of the current industry practice of the quality risk management at Russian pharmaceutical enterprises, revealed the prevalence of a superficial approach to the quality risk management, the presence of objective and subjective reasons that hinder the effective implementation of the quality risk management systems, the fragmentation of the quality risk management systems used and, in most cases, their ineffective use. The main problems of implementing a quality risk management system are: an oversimplified description of the regulator's requirements for a quality risk management system and the lack of explanatory methodological materials containing specific examples of possible methodological approaches to the quality risk management, including the ones for specific productions. It should be emphasized that the situation is systemic in nature, and is the same in all the studied segments.

Considering the above, the development of recommendations for a quality risk management system is of a great current interest.

Based on the general requirements for the quality risk management system in the pharmaceutical industry, the authors formulated 17 steps to implement the quality risk management system in pharmaceutical companies. Their development takes into account the results of the industry practice analysis on drug quality risk management in the Russian Federation. Particular attention was paid to the description of the elements of the risk management system that are practically absent at many Russian enterprises. Closer attention was paid to the issues of the risk management system (the requirements for them had not been determined by the regulatory legal acts of the Russian Federation).

Recommendations for quality risk management at pharmaceutical enterprises

1. Develop a high-level document (a standard operating procedure, an enterprise standard, policy) that regulates the quality risk management system at the enterprise, and the approaches used to manage risks. In the document, specify the following:

– the areas of application of the risk management system;

– the persons responsible for decision-making;

– the responsibilities and functions of the key personnel in the risk management system;

– the responsibilities of both managers and employ-

[7] ASTM E2500-13. Standard Guide for Specification, Design, and Verification of Pharmaceutical and Biopharmaceutical Manufacturing Systems and Equipment.

ees involved in the risk analysis and assessment;

– the applied methods and tools for the risk analysis;

– recommendations on the risk classification and documentation of the processes;

– the information on the training of employees, participating in the risk analysis and assessment.

2. Include the risk management system in the the quality guidelines.

3. Create a risk register, including the following:

– a list of conducted risk assessments or a link to this list;

– a list of key identified risks;

– a brief description of the measures to mitigate the identified risks;

– justifications for reassessment of risks and for a specified frequency of reassessment;

– the data on residual risks.

4. Regularly review the results of the quality risk management process, taking into account new knowledge and experience, since earlier decisions may have been based on unreliable data; an earlier identified risk may have been underestimated or exaggerated, and earlier developed mitigation measures may have been underresourced. The frequency of revision should be determined taking into account the priority of risk.

5. Update the risk register in a timely manner, considering the results of risk assessments and revision of the results of the quality risk management process.

6. Include references to the risk system in the main control procedures of the enterprise.

7. Develop risk communication mechanisms. Establish a documented procedure for the information distribution on the works performed for the quality risk management.

8. Consider the possibility of applying the quality risk management system in the areas of work where such methods and tools are not currently used, for example, in the change control, supplier qualifications, qualification and validation, processing and reprocessing of products, in-house monitoring, calibration and maintenance, developing a quality audit program, evaluating storage and shipping conditions.

For a self-assessment on the coverage of the areas of work, use Order of the Ministry of Industry and Trade of the Russian Federation dated 12 December, 2013 No. 1997 “On the approval of the Recommendations for the organization of production and quality control of medicines.”

9. Consider the possibility of applying the risk management system at the stages of pharmaceutical development, pilot production and process scaling.

10. Create an enterprise working group to conduct a risk analysis and assessment. This group should be as multidisciplinary as possible and have narrowly focused specialists, so that the risk assessment could be carried out from different points of view, and a constructive exchange of information about the identified risks would be ensured. Assign the responsibility for the organization of risk analyses and the assessment to individual experts who are well versed in the risk assessment methodology, methods and tools for risk analyses.

11. Include information about the risk management system in the company’s personnel training programs. Document training requirements for the personnel directly involved in the quality risk management. Develop procedures for training personnel in methods and tools for the risk analysis and assessment.

12. Document the possibility of attracting external consultants for analysis and risk assessment when there is an insufficient number of employees with the necessary knowledge and experience to carry out these works.

13. Develop standard operating procedures containing a description of procedures for control, agreement and approval of the results of the risk assessment and reassessment, as well as the forms of protocols for the risk analyses and assessment.

14. Document all the analyses and risk assessments performed.

15. Based on the results of the conducted risk assessments, develop work plans to reduce significant risks.

16. Develop a procedure for evaluating the effectiveness of the risk management system and the risk management plans, specifying the following:

– frequency of evaluation;

– responsibility of performers;

– a formal list of documentation to be verified during assessment;

– the ways of information distribution of the assessment results;

– procedure for developing recommendations for the improvement;

– procedure for subsequent actions and their verification.

17. Document the application of the change management procedure to the risk management.

FUNDING

This study did not receive any support from external organizations.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHORS' CONTRIBUTION

A.B. Kashirina – literature analysis, article writing, research planning; conducting all stages of the research, formalization of the list of references;
Zh.I. Aladysheva – idea, research design development, consultations on all stages of the research, article writing;
N.V. Pyatigorskaya – research planning; consultation on all stages of the research, article writing;
V.V. Belyaev – literature analysis, article writing, consultations on research planning and data processing, data processing, formalization of the list of references;
V.V. Beregovykh – consultations on the individual stages of the study.

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ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

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