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EFFICACY AND SAFETY OF ORIGINAL DRUG BASED ON HEXAPEPTIDE SUCCINATE IN COMPLEX COVID-19 THERAPY IN ADULTS HOSPITALIZED PATIENTS

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Currently, there are data that that make it possible to speak about a high clinical efficacy of the use of succinic salt of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine (hexapeptide succinate) for the COVID-19 treatment. This article is devoted to the results of clinical trials of the original Russian drug based on it.

The aim of the study was to evaluate a clinical efficacy, safety and tolerability of intramuscular and inhalation use of hexapeptide succinate in complex therapy in comparison with standard therapy in patients with moderate COVID-19.

Materials and methods. The research was conducted from February 28, 2022 to November 22, 2022 based on 10 research centers in the Russian Federation. The study included hospitalized patients (n=312) over 18 years of age with moderate

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COVID-19 who had undergone a screening procedure and were randomized into 3 groups: group 1 received standard therapy in accordance with the Interim Guidelines in force at the time of the study, within 10 days; group 2 received hexapeptide succinate (Ambervin® Pulmo) intramuscularly at the dose of 1 mg once a day for 10 days; group 3 received hexapeptide succinate (Ambervin® Pulmo) 10 mg once a day by inhalation for 10 days.

Results. According to the results of the study, therapy with the drug hexapeptide succinate, both intramuscular and inhaled, provided an acceleration of recovery up to the complete absence of the disease signs in more than 80% of hospitalized COVID-19 patients. By the end of the therapy course with the drug, more than 60% of patients had met the criteria for discharge from hospital and could continue the treatment on an outpatient basis. About 70% of patients in the inhalation group and 80% in the intramuscular hexapeptide succinate injection group had concomitant diseases (hypertension – 28%, obesity – 14%), which indicates the effectiveness of this drug use in comorbid patients. The use of the drug contributed to the restoration of damaged lung tissues, normalization of oxygenation, the disappearance of shortness of breath and a decrease in the duration of the disease symptoms compared with standard therapy. As a result of a comparative analysis of adverse events in terms of their presence, severity, causal relationship with the therapy and outcome, there were no statistically significant differences between the treatment groups.

Conclusion. Thus, the results of the clinical study of the succinate hexapeptide efficacy and safety showed the feasibility of using the drug in pathogenetic therapy COVID-19 regimens.

Keywords: ambervine; hexapeptide succinate; acute respiratory distress syndrome; “cytokine storm”; COVID-19; tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine succinate

Abbreviations: AE – adverse events; SAE – serious adverse events; IG – Interim guidelines “Prevention, diagnosis and treatment of a new coronavirus infection”; ALT – alanine aminotransferase; AST – aspartate aminotransferase; LDH – lactate dehydrogenase; CRP – C-reactive protein; GFR – glomerular filtration rate; PIS – patient information sheet; HFO – high-flow oxygen; NIVL – non-invasive lung ventilation; ALV – artificial lung ventilation; ECMO – extracorporeal membrane oxygenation; ARDS – acute respiratory distress syndrome; SARS-CoV-2 – coronavirus, the causative agent of COVID-19; CTs – clinical trials, SD – standard deviation; LPO – lipid peroxidation; RR – respiratory rate.

ЭФФЕКТИВНОСТЬ И БЕЗОПАСНОСТЬ ОРИГИНАЛЬНОГО ПРЕПАРАТА НА ОСНОВЕ СУКЦИНАТА ГЕКСАПЕПТИДА В КОМПЛЕКСНОЙ ТЕРАПИИ COVID-19 У ВЗРОСЛЫХ ГОСПИТАЛИЗИРОВАННЫХ ПАЦИЕНТОВ

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На сегодняшний день имеются данные, позволяющие говорить о высокой клинической эффективности применения янтарнокислой соли тирозил-D-аланил-глицил-фенилаланил-лейцил-аргинина (гексапептида сукцинат) для лечения COVID-19. Настоящая статья посвящена результатам клинических исследований оригинального российского лекарственного препарата на его основе.

Цель. Оценить клиническую эффективность, безопасность и переносимость внутримышечного и ингаляционного применения препарата гексапептида сукцината в комплексной терапии в сравнении со стандартной терапией у пациентов со среднетяжелым течением COVID-19.

Материалы и методы. Исследование проводилось с 28 февраля 2022 г. по 22 ноября 2022 г. на базе 10 исследовательских центров на территории РФ. В исследование были включены госпитализированные пациенты (n=312) старше 18 лет со среднетяжелым течением COVID-19, которые прошли процедуру скрининга и были рандомизированы на 3 группы: группа 1 получала стандартную терапию в соответствии с Временными методическими рекомендациями, действующими на момент проведения исследования в течение 10 сут; группа 2 получала препарат гексапептида сукцинат (Амбервин® Пульмо) внутримышечно по 1 мг 1 раз/сут в течение 10 дней; группа 3 получала препарат гексапептида сукцинат (Амбервин® Пульмо) ингаляционно по 10 мг 1 раз/сут в течение 10 дней.

Результаты. По результатам исследования терапия лекарственным препаратом гексапептида сукцинат как при внутримышечном, так и при ингаляционном введении обеспечивала ускорение выздоровления вплоть до полного отсутствия признаков заболевания более, чем у 80% госпитализированных пациентов с COVID-19. К окончанию курса терапии препаратом более 60% пациентов соответствовали критериям выписки из стационара и могли продолжить лечение в амбулаторных условиях. Около 70% пациентов в группе ингаляционного введения и 80% в группе внутримышечного введения гексапептида сукцинат имели сопутствующие заболевания (гипертензию – 28%, ожирение – 14%), что говорит об эффективности применения указанного лекарственного препарата у коморбидных пациентов. Применение препарата способствовало восстановлению поврежденных тканей легких, нормализации оксигенации, исчезновению одышки и уменьшению продолжительности симптомов заболевания по сравнению со стандартной терапией. В результате сравнительного анализа нежелательных явлений по их наличию, степени тяжести, причинно-следственной связи с терапией и исходу не было выявлено статистически значимых различий между группами терапии.

Заключение. Таким образом, результаты проведенного клинического исследования эффективности и безопасности гексапептида сукцинат показали целесообразность применения препарата в схемах патогенетической терапии COVID-19.

Ключевые слова: амбервин; гексапептида сукцинат; острый респираторный дистресс-синдром; «цитокиновый шторм»; COVID-19; тирозил-D-аланил-глицил-фенилаланил-лейцил-аргинина сукцинат

Список сокращений: НЯ – нежелательные явления; СНЯ – серьёзные нежелательные явления; ВМР – Временные методические рекомендации «Профилактика, диагностика и лечение новой коронавирусной инфекции»; АЛТ – аланинаминотрансферазы; АСТ – аспартатаминотрансфераза; ЛДГ – лактатдегидрогеназа; СРБ – С-реактивный белок; СКФ – скорость клубочковой фильтрации; ИЛП – информационный листок пациента; ВПО – высокопоточная оксигенотерапия; НИВЛ – неинвазивная вентиляция легких; ИВЛ – искусственная вентиляция легких; ЭКМО – экстракорпоральная мембранная оксигенация; ОРДС – острый респираторный дистресс-синдром; SARS-CoV-2 – коронавирус, возбудитель COVID-19; КИ – клинические исследования, СО – стандартное отклонение; ПОЛ – перекисное окисление липидов.

INTRODUCTION

Since the mid-1970s, an era of research into endogenous substances that activate the same receptors as opiates began. Subsequently, these studies led to the discovery of the first endogenous opioid peptide. In 1975, two classes of endogenous peptides were discovered – methionine-enkephalin (met-enkephalin) and leucine-enkephalin (leu-enkephalin). Since then, more than 20 opioid peptides have been discovered. Each of these peptides binds with different affinity to three types of opioid receptors (μ, δ, or k) [1–5]. Currently, endogenous opioid peptides are divided into four families: enkephalins, dynorphins, endorphins, and nociceptin/orphanin FQ [6, 7].

Tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine is the world's first synthetic opioid peptide created on the basis of endogenous leucine-enkephalin by the standard replacement of Gly2 with D-Ala2 and the addition of a highly charged arginine residue to the C-terminal part of the molecule in order to obtain a peripheral effect and stability of the peptide. This modification of the leucine-enkephalin molecule contributed to the leveling of some side effects characteristic of other opiates: it did not cause addiction, physical dependence [8]. Tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine was previously used in the treatment of patients with peptic ulcer of the stomach and duodenum, resistant to the therapy and with an insufficient effect from the treatment with other drugs. Then the drug began to be used to treat acute and chronic pancreatitis [8]. Further studies revealed cardioprotective properties of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine in the patients operated on under cardiopulmonary bypass [9]. Subsequent studies have demonstrated a protective effect of the drug on the lungs [10].

Immune system cells are ones of the main opioid peptides targets due to the detection of the corresponding receptors on the surface of immunocytes of the lymph nodes, bone marrow, and spleen. Endorphins, dynorphins, and enkephalins are involved in the development and pathogenesis of a number of autoimmune disorders and, therefore, can alter the antiviral and antimicrobial response [11–14]. Taking into account a wide range of an opioid peptides therapeutic action, their high safety profile and good tolerability due to the fact that they are mainly composed of natural amino acids and have a high selectivity of action, no interest in them has faded, and the search for their possible use in various diseases continues [15, 16].

Enkephalins work as delta receptor agonists, suppressing excessive synthesis of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) and thus reducing the consequences of a systemic hyperimmune reaction (cytokine storm) [17–20]. Cytokine storm is the main cause of the acute respiratory distress syndrome (ARDS) development, in particular with COVID-19, which requires the transfer of a patient to the artificial lung ventilation due to severe hypoxia [21-23].

Delta receptor agonists, which include tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine, stimulate regeneration and healing processes; normalize microcirculation in the area of damage, contribute to the maintenance of structural homeostasis [13–15]. Hexapeptide has an immunomodulatory effect, regulates the activity of cells of innate and adaptive immunity, enhances the activity of the phagocytic link of immunity (macrophages and neutrophils). Tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine increases the activity of natural killer cells (NK cells), the availability of which decreases with severe infections caused by RNA viruses (influenza, Ebola virus, COVID-19, SARS, MERS). Hexapeptide stimulates the production of endogenous interferons, increases the body's resistance to viral infections [24, 25]. In completed preclinical studies was shown that tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine and his derivates have a positive effect on the course of acute respiratory distress syndrome (ARDS), significantly reducing animal mortality, inflammation and swelling of the lung tissue , as well as suppressing the "cytokine storm" [25].

The study by Ukrainskaya L.A. et al. (2002) showed that the use of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine succinate in experimental stress-induced lung alteration reduced lipid peroxidation (LPO) hyperactivation, surfactant breakdown, and the severity of edema and leukocyte infiltration of the alveoli and increased the gas exchange area. Limiting the altering stress effects by a hexapeptide administration has an effective pulmonoprotective action [26]. To date, a number of experimental studies have shown an immunomodulating effect of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine hexapeptide [24, 27].

In 2022, the drug Ambervin® Pulmo was developed and registered (RU No. LP-008604 dated October 07, 2022; Patent No. EA038010). It contained tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine succinate (hexapeptide succinate) in dosages of 1.16 mg and 5.8 mg. Ambervin® Pulmo has an anti-inflammatory effect by inhibiting the synthesis in the lungs and

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inhibiting the entry into the systemic circulation of one of the main pro-inflammatory mediators of the cytokine storm – IL-6, as well as other pro-inflammatory cytokines (in particular, IL-1, TNF-α, HMGB1). It also increases the formation of IL-10 and VEGF, which have an anti-inflammatory effect and increase the body's defenses. Being an analogue of leu-enkephalin, the drug[1] has a vasoprotective effect, reducing the permeability of the vascular wall and preventing the destruction of the endothelium, increases tolerance to hypoxia, prevents and reduces the severity of acute lung injury, reduces the risk of an oxygenation decrease and the development of secondary bacterial complications.

Due to the succinic acid fragment included in the structure of the hexapeptide, the drug[2] under consideration exhibits antioxidant, antihypoxic properties, including the ones in the alveolar cells of the lung tissue, in the epithelial cells of the middle and upper parts of the respiratory system. It inhibits lipid peroxidation, improves the structure and function of cell membranes, reduces the inhibition degree of oxidative processes in the Krebs cycle under hypoxic conditions, and increases the body's resistance to various damaging factors.

Hexapeptide succinate[3] stimulates regeneration and healing processes, promotes the damaged tissues restoration. It includes alveolar epithelial cells, reduces the severity of interstitial edema in the lower respiratory tract (alveoli, bronchi, bronchioles), normalizes microcirculation in the area of damage, helps maintain structural homeostasis, has anti-inflammatory, detoxification, antioxidant, reparative and immunomodulatory effects, increasing the effectiveness of ongoing antiviral and antibacterial therapy.

This article is devoted to the clinical study results of this drug use in the treatment of COVID-19 patients.

THE AIM of the study was to evaluate a clinical efficacy, safety and tolerability of intramuscular and inhalation use of hexapeptide succinate in complex therapy in comparison with standard therapy in patients with moderate COVID-19.

MATERIALS AND METHODS

The efficacy, safety, and tolerability of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine succinate, or succinate hexapeptide, compared with standard therapy in patients hospitalized with COVID-19 was studied in an open-label, randomized, multicenter, comparative, phase III clinical trial (CCT the Ministry of Health No. 100, dated 2022 Feb 14).

The research was conducted from February 28, 2022 to November 22, 2022 on the basis of 10 research centers in the Russian Federation:

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2. Regional Clinical Hospital;
3. Municipal clinical hospital No. 24, Moscow City Health Department
4. Voronezh Regional Clinical Hospital No. 1;
5. Ryazan State Medical University named after academician I.P. Pavlov;
6. City Clinical Hospital named after S.I. Spasokukotsky, Moscow City Health Department;
7. Smolensk Clinical Hospital No. 1;
8. Infectious Clinical Hospital No. 1, Moscow City Health Department;
9. City Hospital No. 40, St. Petersburg, Kurortny District;
10. Emergency Hospital, Cheboksary, Chuvash Republic.

Study design

The hospitalized male and female patients (n=313) aged 18 to 80 years inclusive, with moderate COVID-19, were screened and randomized into 3 groups in a 1:1:1 ratio. The drug choice for patients was carried out in accordance with the randomization number assigned to patients at the time of randomization.

Randomization of study subjects into groups

Male and female patients (at least 312 people) aged 18 to 80 years inclusive, hospitalized with COVID-19, meeting the inclusion criteria and not meeting the exclusion criteria, were randomized into 3 groups in a 1:1:1 ratio (Fig. 1).

The randomization was carried out according to the following algorithm: each patient who had met all the inclusion criteria and had not meet any of the exclusion criteria, was assigned a three-digit randomization number using the IWRS system. A patient's randomization number and other relevant data were entered by the investigator into the Subject Screening/Randomization Journal. If a patient discontinued participation in the

[1] Russian State Register of Medicines. Instructions for Ambervin® Pulmo. Available from: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=1f912539-dd59-4a95-adeb-31621b26fb0b
[2] Ibid.
[3] Ibid.

study prematurely, their randomization number was not reused.

This study was open, so both the patient and the investigator knew what therapy the patient was receiving.

Group 1 (n=104) received standard therapy in accordance with the BMPs[4] in force at the time of the study for 10 days;

Group 2 (n=104) received hexapeptide succinate (Ambervin® Pulmo, PROMOMED RUS LLC) intramuscularly at the dose of 1.16 mg once a day for 10 days;

Group 3 (n=104) received hexapeptide succinate (Ambervin® Pulmo, PROMOMED RUS LLC) by inhalation using a nebulizer, 11.6 mg once a day for 10 days.

As concomitant therapy, patients in groups 2 and 3 received standard therapy, presented in the BMPs, valid at the time of the study. Intramuscular and inhalation uses of the study drug was carried out in a hospital setting. The design of the study is shown in Fig. 2. The total duration of a patient's participation in the study was no more than 30 days.

Selection of subjects for analysis

Primary and secondary efficacy outcomes were analyzed using a dataset of study participants selected according to the protocol compliance, i.e. all the patients who had completed the study in accordance with the Study Protocol. A participant was excluded from the data set if they had met the exclusion criteria.

The safety data set included all randomized patients who had been exposed to the study drug, regardless of the degree of adherence to the Protocol during the study.

Inclusion criteria

Availability of a signed and dated Informed Consent Form (ICF) by the patient, male and female, aged 18 to 80 years inclusive at the time of signing the ICF; a confirmed case of COVID-19 at the time of screening based on the results of the analysis for the determination of SARS-CoV-2 RNA by the nucleic acid amplification method (NAAM); hospitalization due to the COVID-19 disease; a moderate course of SARS-CoV-2 infection (presence of at least 2 of the following criteria: body temperature >38°C; respiratory rate (RR) >22/min; dyspnea on exertion; changes on computed tomography (CT), typical for viral damage; SpO_2<95%; Serum C-reactive protein (CRP)>10 mg/l.); the volume of the lungs damage is minimal or medium (CT 1-2); a patient's consent to use reliable methods of contraception throughout the study and for 3 weeks after the end of the study. The reliable means of contraception are sexual abstinence, the use of a condom in combination with spermicide. The study could also include women who are unable to bear children (history: hysterectomy, tubal ligation, infertility, menopause for more than 2 years), as well as men with infertility or a history of vasectomy.

Noninclusion criteria

Noninclusion criteria are as follows: hypersensitivity to the components of the study drug; obstacles or inability to perform intramuscular injections and/or inhalations; the inability to perform a CT procedure (for example, a plaster cast or metal structures in the study area); arterial hypotension (a decrease in blood pressure (BP) below 100/60 mm Hg) at the time of screening and/or a history of hypotensive crises; the need to use drugs from the list of prohibited therapies; the presence of criteria for severe and extremely severe course of the disease at the time of screening; the presence of a probable or confirmed case of COVID-19 moderate course within 6 months prior to screening; the presence of a probable or confirmed case of severe and extremely severe COVID-19 in history; vaccination less than 4 weeks prior to screening; the need for treatment in the intensive care unit at the time of screening. There are some more noninclusion criteria: an abnormal liver function (AST and / or ALT ≥3 ULN and/or total bilirubin ≥1.5 ULN) at the time of screening; an impaired renal function (GFR<60 ml/min) at the time of screening; positive for HIV, syphilis, hepatitis B and/or C at the time of screening; a chronic heart failure of FC III–IV according to the functional classification of the New York Heart Association (NYHA); a history of malignant neoplasms, except in patients who have not been observed for the disease within the last 5 years, patients with completely healed basal cell skin cancer or completely healed carcinoma *in situ*; a history of alcohol, pharmacological and/or drug dependence and/or at the time of screening; a history of epilepsy; schizophrenia, schizoaffective disorder, bipolar disorder, or other psychiatric disorder in history or suspected of having them at the time of screening; severe, decompensated or unstable somatic diseases (any diseases or conditions that threaten

[4] Interim guidelines "Prevention, diagnosis and treatment of novel coronavirus infection (COVID-19)". Version 16 (2022 Aug 18). Available from: https://static-0.minzdrav.gov.ru/system/attachments/attaches/000/060/193/original/%D0%92%D0%9C%D0%A0_COVID-19_V16.pdf

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the patient's life or worsen the patient's prognosis, and also make it impossible for him to participate in a clinical trial); any history data that, in the opinion of the investigator, may complicate the interpretation of the results of the study or create additional risks for the patient as a result of his participation in the study; unwillingness or inability of the patient to comply with the procedures of the Protocol (in the opinion of the investigator); pregnant or lactating women, or women planning a pregnancy; participation in another clinical trial within 3 months prior to the enrollment in the study; other conditions that, in the opinion of the investigator, prevent the inclusion of a patient in the study.

Exclusion criteria

A decision to exclude a subject from the study was made by the investigator.

A patient was withdrawn from the study immediately if any of the following situations had occurred:

1. Negative SARS-CoV-2 RNA NAAT selected at screening (for patients with a probable case of COVID-19 at the time of screening).
2. The appearance of any diseases or conditions that worsen the patient's prognosis, and also make it impossible for the patient to continue participating in the clinical trial during the study.

If it was necessary to transfer the patient to high-flow oxygen (HFO), non-invasive lung ventilation (NILV), the therapy provided for by the Protocol continued, the patient was not excluded from the study. The inhalation use of the hexapeptide succinate preparation was carried out through the apparatus circuit while maintaining the specified oxygenation parameters.

If it was necessary to transfer a patient to the artificial lung ventilation (ALV), extracorporeal membrane oxygenation (ECMO), the patient was excluded from the study and prescribed therapy in accordance with the clinical practice of the research center.

3. Taking drugs of prohibited therapy or the need to prescribe them.
4. Pregnancy of a patient.
5. Erroneous inclusion of a patient who does not meet the inclusion criteria and/or meets the non-inclusion criteria.
6. Other violations of the Protocol, which, in the opinion of the investigator, are significant.
7. Patient refusal to participate in the study.
8. Other administrative reasons.

Criteria for efficacy evaluation

Primary criteria for efficacy:

- Frequency of achieving category 0-1 on the categorical ordinal scale of clinical improvement at Visit 4 (Table 1).

Secondary criteria for efficacy:

- Frequency of patients with clinical status fewer than 4 points on the categorical ordinal scale of clinical improvement at Visits 3 and 4;
- Frequency of improvement in clinical status on the categorical ordinal clinical improvement scale of 2 or more categories at Visits 3 and 4;
- Time (in days) to improve clinical status on a categorical ordinal scale of clinical improvement by ≥1 point;
- The rate of patients meeting discharge criteria for a continued outpatient treatment according to IGs at Visits 2 and 3.

Discharge criteria (meeting all the criteria, however, a patient could continue to stay in hospital after reaching the discharge criteria if the investigator considered it necessary or it was required for social reasons):

- persistent improvement of the clinical picture;
- level of blood oxygen saturation in air ≥95%;
- body temperature <37.5℃;
- CRP level <10 mg/l;
- level of blood lymphocytes >1.2×109/l.

- Rate of patients with RR <22/min at Visits 2 and 3. The evaluation was performed only for patients who had a RR >22/min at Visit 1;
- Incidence of patients with CRP levels <10 mg/l at Visits 2 and 3;
- Evaluation was performed only for patients who had a CRP level >10 mg/l at Visit 0;
- Incidence of patients with blood lymphocytes >1.2×109/l at Visits 2 and 3. The evaluation was limited to the patients who had a blood lymphocyte count <1.2×109/l at Visit 0;
- Assessment of the lung damage degree according to CT data for Visit 4;
- Incidence of patients with SpO_2≥95% for 2 consecutive days at Visits 2, 3 and 4. The evaluation was performed only for patients who had an SpO_2 <95% at Visit 1;
- The frequency of transfers of patients to the intensive care unit;
- The frequency of cases of the use of HFO, NIVL, ALV, ECMO;
- Incidence of acute respiratory distress syndrome (ARDS);
- Incidence of patient deaths.

Additional research parameters

- Frequency of patients reaching reference levels at Visits 2, 3 for each of the following: IL 6, D dimer, ferritin, fibrinogen, CRP, lymphocytes, leukocytes, platelets, triglycerides, LDH;
- Change (%) to Visits 2, 3 for each of the following: IL-6, D-dimer, ferritin, fibrinogen, CRP, lymphocytes, leukocytes, platelets, triglycerides, LDH.

Criteria for safety assessment

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

Statistical analysis

For a statistical analysis, software with validated algorithms for performing statistical analyzes and a proper documentation was used (StatSoft Statistica 10.0., IBM SPSS Statistics 22 (current version, GPL-2/GPL-3 license).

Continuous (quantitative) data are presented using the number of observations, arithmetic mean, 95% confidence interval (CI) for the mean, standard deviation, median, interquartile range (25^{th} and 75^{th} centiles), minimum and maximum.

Qualitative data (ordinal, nominal) are presented using absolute frequencies (a number of observations), relative frequencies (percentage) and 95% CI.

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

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

For the analysis of the primary criterion for efficacy, it is assumed to use an intergroup comparison of shares using a one-sided version of Fisher's exact test or $\chi 2$ (the chi-square) test, if all the expected values in the cells of the contingency table for this analysis are 5 or more. The proportion of patients achieving grade 0–1 on a categorical ordinal scale of the clinical improvement at Visit 4 is presented with a two-sided 95% confidence interval (CI) by treatment groups. The difference in proportions between the treatment groups and the 95% two-sided CI for the difference in proportions calculated by the Newcomb-Wilson method, are shown. Secondary criteria for efficacy and additional study parameters are presented descriptively for each group.

Safety population: the patients who received at least one dose of the study drug and for whom there is an assessment of the condition and/or AE for at least one time point after application. If the study drug was not taken by the volunteer/patient, their data were not included in the statistical analysis, but were presented in the final report of the study.

RESULTS AND DISCUSSION

Baseline Patient Characteristics

313 patients underwent the screening and randomization procedure, 312 were included in the study, one patient was excluded from the study before taking the drug due to meeting the exclusion criterion "Patient refusal to participate in the study": 104 patients received standard therapy in accordance with current IGs, 104 patients – hexapeptide succinate intramuscularly (IM) and 104 patients – hexapeptide succinate by inhalation. The groups were comparable in terms of demographic, anthropometric, and clinical characteristics (Table 2).

The average age of all the patients included in the study was 58.21 years (from 18 to 80 years), the number of women was slightly more – 53.21% (n=166) than men – 46.79% (n=146). The average body mass index (BMI) was 27.55 kg/m^2 (from 15.30 to 51.42 kg/m^2), which corresponds to the overweight according to the WHO classification. In 242 patients (77.56%), comorbidities were identified. The most common comorbidities were hypertension 28% (n=173) and obesity 14% (n=88). Other comorbidities/conditions that occurred with a frequency of 2 to 5% were atrial fibrillation (3.2%), chronic heart failure (2.4%), myocardial ischemia (2.6%), angina pectoris (2.6%), osteochondrosis (2.1%), type 2 diabetes mellitus (4.8%), menopause (2.4%). In 163 (52.24%) patients, ECG abnormalities were detected. The groups were comparable in terms of sex, age and comorbid status of patients.

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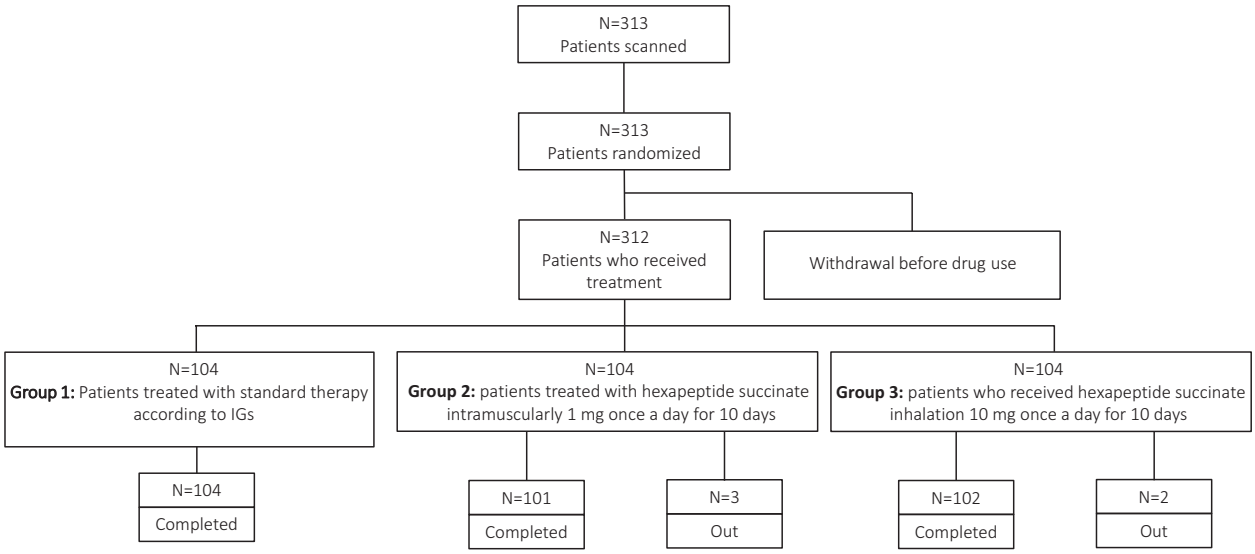

Figure 1 – Groups' allocation

Note: IGs – Interim guidelines "Prevention, diagnosis and treatment of novel coronavirus infection (COVID-19)". Version 16 dated 2022 Aug 18 (here and Fig. 2).

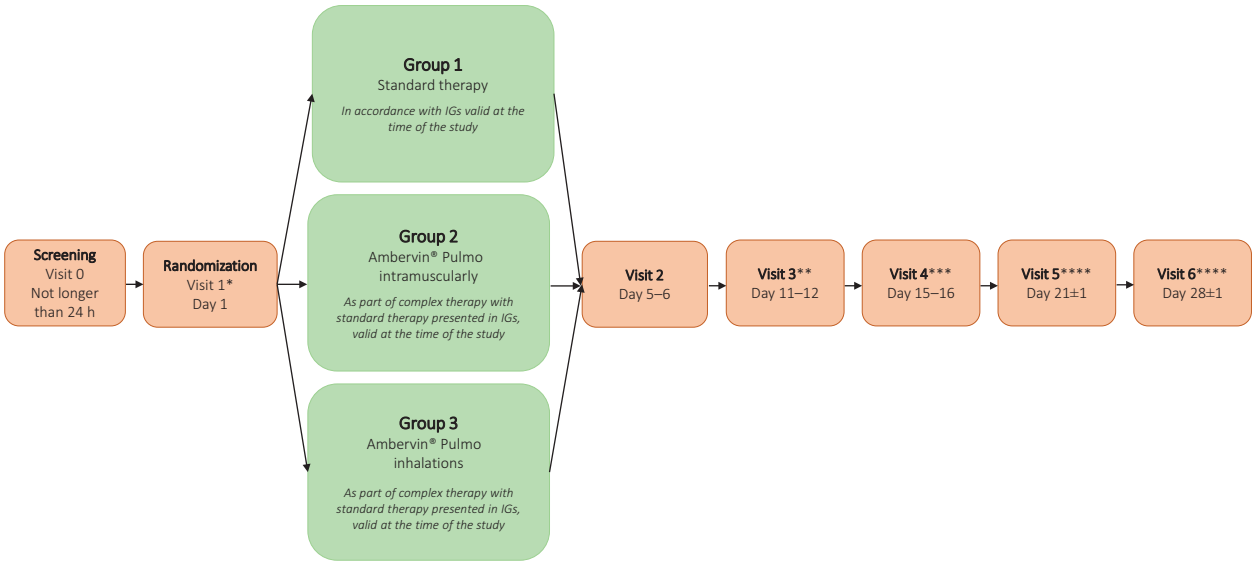

Figure 2 – Study design

Note: *Visit 1 could coincide with Visit 0. If Visit 1 and Visit 0 were the same, then a physical examination, vital signs assessment, registration of concomitant therapy, pulse oximetry with SpO_2 measurement were not repeated, evaluation of inclusion and non-inclusion criteria was performed immediately before randomization, and exclusion criteria were assessed after drug use. **For patients in group 1: if a patient was discharged from hospital earlier, at the time of discharge the patient was undergoing procedures of Visit 3, CT of the lungs and the assessment of changes in the lungs using an "empirical" visual scale (according to CT of the lungs). If discharge from hospital was carried out earlier than day 7, then CT of the lungs and the assessment of changes in the lungs using an "empirical" visual scale (according to CT of the lungs) was carried out at the discretion of the researcher. *** If discharge from hospital was carried out on the 13th or 14th day from the therapy start, at the time of discharge, a carried out visit corresponded to the volume of procedures provided for an in-person Visit. All patients who were discharged earlier than day 15 received a Visit on days 15–16 corresponding to the scope of procedures provided for a Visit conducted by a phone call. ****If a discharge the hospital was carried out earlier, then, instead of a face-to-face Visit, it was made *via* a phone call.

Table 1 – Categorical ordinal scale for clinical improvement in COVID-19

Patient Status	Description	Category
Outpatient	No clinical and virological signs of infection	0
	No activity restrictions	1
	Activity restrictions	2
Hospitalized:	Hospitalized, no oxygen therapy	3
– mild disease	Oxygenation with a mask or nasal cannula	4
– severe disease course	Non-invasive ventilation or high-flow oxygenation	5
	Intubation or artificial lung ventilation	6
	Ventilation + additional organ support – vasopressors, renal replacement therapy, extracorporeal membrane oxygenation (ECMO)	7
Dead	Death	8

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

Characteristics	Standard therapy, n=104	Hexapeptide succinate IM, n=104)	Hexapeptide succinate inhalation, n=104
Age, years (M ±SD)	57.64±16.44	57.54±16.02	59.46±16.46
Male, n (%)	50 (48.08)	53 (50.96)	43 (41.35)
BMI, kg/m^2 (M ±SD)	27.87±5.72	26.91±5.90	27.86±5.30
Following comorbidities/conditions *			
Hypertension, n (% of all FCs)	60 (29.56)	49 (25.13)	64 (29.22)
Obesity, n (% of all FCs)	34 (16.75)	21 (10.77)	33 (15.07)

Note: *in addition to those indicated in the table, the following comorbidities/conditions (FCs) were identified with a frequency of 5% or less: atrial fibrillation, chronic heart failure, myocardial ischemia, angina pectoris, osteochondrosis, type 2 diabetes mellitus, menopause.

Table 3 – Summarized data on comparative evaluation for hexapeptide succinate efficacy

Check point	Groups		
	Standard therapy	Hexapeptide succinate (IM)	Hexapeptide succinate (inhalation)
Primary criterion			
Achievement of category 0–1			
Visit 4 (Day 15)	66.35% (69/104)	85.15% (86/101)	83.33% (85/102)
Secondary criteria for efficacy			
Clinical status fewer than 4 points			
Visit 3 (Day 11)	69.23% (72/104)	87.13% (88/101)	83.33% (85/102)
Visit 4 (Day 15)	94.23% (98/104)	99.01% (100/101)	99.02% (101/102)
Improvement in clinical status by 2 or more categories			
Visit 3 (Day 11)	52.88% (55/104)	58.42% (59/101)	59.80% (61/102)
Visit 4 (Day 15)	90.38% (94/104)	98.02% (99/101)	96.08% (98/102)
Time till improvement in clinical status by ≥1 point			
Median time, days	7	6	6
Eligibility for discharge to continue treatment on outpatient basis in accordance with the IGs			
Visit 2 (Day 5)	13.46% (14/104)	16.83% (17/101)	17.65% (18/102)
Visit 3 (Day 11)	52.88% (55/104)	67.33% (68/101)	67.65% (69/102)
RR<22/min			
Visit 2 (Day 5)	60.98% (25/41)	71.43% (25/35)	85.71% (36/42)
Visit 3 (Day 11)	92.68% (38/41)	100.00% (35/35)	100.00% (42/42)
CRP<10 mg/l			
Visit 2 (Day 5)	52.78% (38/72)	59.46% (44/74)	55.88% (38/68)
Visit 3 (Day 11)	79.17% (57/72)	83.78% (62/74)	92.65% (63/68)
Blood lymphocytes >1.2×109/l at Visits 2 and 3			
Visit 2 (Day 5)	55.26% (21/38)	67.86% (19/28)	69.23% (27/39)
Visit 3 (Day 11)	71.05% (27/38)	75.00% (21/28)	76.92% (30/39)
Lung damage degree according to CT			
Visit 4 (Day 15) CT-0	30,77% (28/91)	33,33% (33/99)	33,33% (34/102)
CT-2	9,89% (9/91)	6,06% (6/99)	5,88% (6/102)
SpO_2≥95% for 2 consecutive days			
Visit 2 (Day 5)	64,29% (36/56)	72,41% (42/58)	74,14% (43/58)
Visit 3 (Day 11)	87,50% (49/56)	96,55% (56/58)	96,55% (56/58)
Visit 4 (Day 15)	91,07% (51/56)	100,00% (58/58)	98,28% (57/58)

Note: RR – respiratory rate; CRP – C-reactive protein; CT – computer tomography.

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Table 4 – Description of total number of AEs registered in patients in study groups AE (RT according to MeDRA)*

AE (RT according to MeDRA)*	Number of AEs, absolute value (% of AEs total number)			
	Hexapeptide succinate (IM) group, n=104	Hexapeptide succinate (IM) inhalation group, n=104	Standard therapy group, n=104	Total, n=312
Arrhythmia	0 (0%)	0 (0%)	1 (8.33%)	1 (3.33%)
Hyperglycemia	0 (0%)	0 (0%)	1(8.33%)	1(3.33%)
Headache	0 (0%)	1 (12.5%)	0 (0%)	1 (3.33%)
Diarrhea	0 (0%)	0 (0%)	1 (8.33%)	1 (3.33%)
Respiratory failure	1 (10%)	2 (25%)	0 (0%)	3 (30%)
Urinary tract infection	1 (10%)	0 (0%)	0 (0%)	1 (3.33%)
Concrement in urinary tract	0 (0%)	1 (12.5%)	0 (0%)	1 (3.33%)
Increase in ALT level	3 (30%)	1 (12.5%)	2 (16.67%)	6 (20%)
Increase in AST level	1 (10%)	2 (25.%)	2 (16.67%)	5 (16.67%)
Increase in blood glucose	2 (20%)	1 (12.5%)	1 (8.33%)	4 (13.33%)
Increase in blood creatinine level	1 (10%)	0 (0%)	0 (0%)	1 (3.33%)
Nausea	0 (0%)	0 (0%)	1 (8.33%)	1 (3.33%)
Prolongation of activated partial thromboplastin time	0 (0%)	0 (0%)	1 (8.33%)	1 (3.33%)
Prolongation of prothrombin time	0 (0%)	0 (0%)	2 (16.67%)	2 (6.67%)
Heart failure	1 (10%)	0 (0%)	0 (0%)	1 (3.33%)
Total:	10 (100%)	8 (100%)	12 (100%)	30 (10%)

Note: *PT (preferterm) – the level of the international dictionary of medical and therapeutic terms MeDRA; ALT – alanine aminotransferase; AST – aspartate aminotransferase.

Results of efficacy evaluation

Summarized comparative analysis data on efficacy criteria are presented in Table 3.

Primary criterion for efficacy

In the succinate hexapeptide intramuscular group, the proportion of patients who achieved category 0–1 on the categorical ordinal scale of the clinical improvement at Visit 4 was 85.15% (86/101), in the succinate hexapeptide inhalation group it was 83.33% (85/102), in the standard therapy group – 66.35% (69/104). The 95% CI for the proportion of patients achieving category 0–1 on the categorical ordinal scale of the clinical improvement at Visit 4 was 95% CI [0.7637; 0.9118] for hexapeptide succinate intramuscularly, and 95% for hexapeptide succinate inhalation CI [0.7437; 0.8972], in the standard therapy group – 95% CI [0.5634; 0.7514]. The difference in proportions between the succinate hexapeptide intramuscular group and the standard therapy group was 0.188 (18.80%), a 95% CI for the difference in proportions between the groups was -95% CI [0.0638; 0.3049]. The difference in proportions between the succinate hexapeptide inhalation group and the standard therapy group was 0.1699 (16.99%), a 95% CI for the difference in proportions between the groups was -95% CI [0.0443; 0.2886].

As a result of the analysis, statistically significant differences were found in the frequency of achieving category 0–1 on the categorical ordinal scale of the clinical improvement by Visit 4 both between the group of the drug hexapeptide succinate, intramuscular administration, and the standard therapy group (p=0.0017), and between the hexapeptide succinate group, the inhalation administration, and the standard therapy group (p=0.0050).

Thus, it was shown that, in contrast to standard hexapeptide therapy, succinate, both intramuscular and inhaled, provided an acceleration of recovery up to the complete absence of signs of the disease in more than 80% of hospitalized COVID-19 patients.

Moreover, since there were patients with concomitant diseases among the study participants, it can be concluded that hexapeptide succinate therapy is highly effective both in patients without concomitant diseases and in patients with comorbid pathology who have risk factors for the progression of COVID-19 to a severe course, regarding the acceleration of recovery and discharge from hospital, as well as reducing the risk of a aggravated course of COVID-19 and transfer to the ICU, which confirms the clinical efficacy and pharmacoeconomic feasibility of using the studied treatment regimens.

The course of therapy with the drug hexapeptide succinate helped to accelerate the recovery and discharge from hospital, prevent the progression of COVID-19 to a severer course, which indicates a high efficacy and substantiates the introduction of studied therapy regimens into the clinical practice.

Secondary criteria for efficacy

At Visit 3, as a result of a comparative analysis of the patients' frequency with a clinical status of fewer than 4 points on a categorical ordinal scale of clinical improvement, statistically significant differences were revealed between the succinate hexapeptide group (IM) and the standard therapy group (p=0.0020), and also between the succinate hexapeptide group (inhalation) and the standard therapy group (p=0.0175). The data obtained indicate a more effective, compared with standard therapy, effect of succinate hexapeptide on the dynamics of symptoms in COVID-19 patients, leading to a pronounced improvement in the clinical condition of patients. The treatment with succinate hexapeptide, both intramuscularly and by inhalation, by the end of therapy, 10 days after its start, ensured the absence of restrictions on daily activities in more than 80% of patients with a coronavirus infection. These data confirm the efficacy of therapy in relation to the course of the disease, improving the quality of life of patients.

As a result of a comparative frequency analysis of the improvement in a clinical status on a categorical ordinal scale of a clinical improvement by 2 or more categories, statistically significant differences were found between the hexapeptide succinate (IM) group and the standard therapy group at Visit 4 (p=0.0334). Thus, it has been shown that, compared with standard therapy, the use of hexapeptide succinate leads to a more pronounced, rapid and significant improvement in the condition of COVID-19 patients.

As a result of a comparative frequency analysis of the of patients meeting the criteria for discharge to continue treatment on an outpatient basis in accordance with the BMRs, there were statistically significant differences between the succinate hexapeptide (inhalation) group and the standard therapy group at Visit 3 (p=0.0305), and between the hexapeptide succinate (IM) group and the standard therapy group (p=0.0348). Thus, it was shown that, in contrast to the standard therapy in the main group, by the end of therapy with hexapeptide succinate, both intramuscularly and inhaled, more than 60% of patients met the discharge criteria and could continue treatment on an outpatient basis, which reduces the burden on the healthcare system and indicates the appropriateness of the study therapy.

As a result of a comparative frequency analysis of patients with a RR<22/min by the end of the therapy, statistically significant differences (p=0.01) were revealed between the group of the patients who had received hexapeptide succinate (the inhalation administration) and standard therapy: 85.7% (36/42) and 60.9% (25/41), respectively. That indicates an improvement in the condition of patients, the disappearance of shortness of breath and a respiratory failure, which helps to reduce the risk of developing COVID-19 complications.

As a result of a comparative frequency analysis of patients with a level of CRP<10 mg/l at Visits 2 and 3, there were no statistically significant differences between the study groups. It should be notified that, in contrast to the patients receiving standard therapy, more than 50% of the patients treated with hexapeptide succinate showed a decrease in CRP<10 mg/l by the 5^{th} day of therapy. It should be emphasized that by the end of therapy, more than 90% of patients who had received the drug in the inhalation form, achieved a decrease in CRP to normal values. That indicates the anti-inflammatory effect of the drug, reducing the consequences of a systemic hyperimmune reaction, reducing the severity of the acute tissue damage, and reducing the risks of developing COVID-19 complications and improved the disease prognosis.

According to the CT data in the succinate hexapeptide (IM) group at Visit 4, the mean value (Mean±SD) of the lung injury degree was 0.73±0.57; in the group of hexapeptide succinate (inhalation) – 0.73±0.57; in the standard therapy group – 0.79±0.61.

According to the CT data, the assessment of the lung damage degree showed that therapy with hexapeptide succinate leads to a significant improvement in the condition of the lungs up to a complete disappearance of the disease symptoms. It should be notified that, according to the results of the intragroup analysis of the lung damage degree, in contrast to the standard therapy, in both succinate hexapeptide groups, a statistically significant difference was found out between the moment of screening patients and days 15-16 of therapy (p<0.0001). That indicates the presence of positive

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dynamics in the course of the disease – a decrease in the lung damage degree in the study drug group, both with the intramuscular and inhalation administrations. Therefore, the study drug use contributes to the restoration of damaged lung tissues, including alveolar epithelial cells.

As a result of a comparative frequency analysis of the patients with SpO_2≥95% for 2 consecutive days before Visit 4, statistically significant differences were found between the hexapeptide succinate (IM) group and the standard therapy group (p=0.0260). It should be notified that by the end of therapy, in the study drug group, more than 90% achieved normalization of the oxygenation index, which indicates a decrease in the risk of developing COVID-19 complications and an improvement in prognosis. Thus, the use of hexapeptide succinate reduces the severity of diffuse alveolar damage to the lung tissue, which helps prevent the development of pulmonary fibrosis and normalizes a ventilation lungs function.

Additional research parameters

As a result of comparing the biochemical blood test parameters, statistically significant differences were revealed between groups 1 and 3 at Visit 2 in terms of "LDH" (p=0.016).

In the group of the studied drug, a decrease in LDH was observed in the hexapeptide succinate inhalation administration at Visit 2, and the values of this enzyme were lower compared to the standard therapy group. That may indicate a more damage reduction and recovery, restoration of damaged tissues, including alveolar epithelial cells, improving energy metabolism in the cells and the function of cell membranes. In addition, in the groups treated with the test compound, there was a decrease in such indicators as ESR, CRP, IL-6, D-dimer, lactate, triglycerides. These factors also confirm its anti-inflammatory effect.

Safety assessment

The frequency of patients with reported cases of AE/SAE was 7.69% (24/312). A total of 24 patients had 30 AEs (Table 4).

A comparative analysis in terms of their presence, severity, causal relationship with therapy and the outcome, no statistically significant differences were found out between the treatment groups. In the study drug groups, the majority of AEs were transient, and there were no cases of discontinuation of therapy or dose changes due to the development of AEs in the study drug groups. The study physicians assessed that the study drug had been well tolerated by the patients.

As a result of a comparative frequency analysis of the patients' transfer cases to the intensive care unit, the use of HPE, NIVL, ALV, ECMO, the development of ARDS, no statistically significant differences were found out between the study groups.

There were no serious adverse events associated with the study drug. Thus, the assessment of the ongoing therapy safety indicates a positive benefit/risk profile in relation to the drug Ambervin® Pulmo.

CONCLUSION

Thus, the results of the clinical study "Open randomized multicenter comparative study to assess the efficacy, safety and tolerability of the use of Ambervin® Pulmo, a lyophilisate for the preparation of a solution for intramuscular injection and a solution for inhalation in patients hospitalized with COVID-19" showed that therapy study drug, both intramuscular and inhaled, provided an acceleration of recovery up to the complete absence of signs of the disease in more than 80% of hospitalized COVID-19 patients. By the end of the therapy course with hexapeptide succinate, more than 60% of the patients met the criteria for discharge from hospital and could continue treatment on an outpatient basis, which reduces the burden on the healthcare system and confirms the feasibility of using the study therapy. It is important to notify that 70% of patients in the inhalation group and 80% in the intramuscular group of the study drug had comorbidities (mainly hypertension and obesity), which are risk factors for the progression of COVID-19 to a severe course. The use of the drug contributed to the restoration of damaged lung tissues, including alveolar epithelial cells, the normalization of oxygenation, the disappearance of shortness of breath and a decrease in the duration of symptoms of the disease compared with standard therapy. As a result of a comparative analysis of adverse events in terms of their presence, severity, causal relationship with therapy and the outcome, there were no statistically significant differences between the treatment groups. According to the investigators, the study drug is characterized by a high safety profile and good tolerability.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Larisa A. Balykova – development and implementation of research design, text writing and editing; Olga A. Radaeva – study design development, results analysis, text editing; Kira Ya. Zaslavskaya – research design development, text editing, analysis of literary sources; Petr A. Bely – study design development, results analysis, text editing; Vera F. Pavelkina – sources collecting, data processing, article writing; Nikolai A. Pyataev – sources collecting, data processing, article writing; Anastasia Yu. Ivanova – study design implementation, data processing; Grigory V. Rodoman – study design implementation, data processing; Natalya E. Kostina – study design implementation, data processing; Viktor B. Filimonov – research design implementation, data processing; Elena N. Simakina – study design implementation, data processing; Dmitry A. Bystritsky – study design implementation, data processing; Alina S. Agafyina – study design implementation, data processing; Ksenia N. Koryanova – sources collecting, data processing, article writing; Dmitry Yu. Pushkar – development and implementation of research design, data processing

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