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Assessment of the allergenic and immunotoxic properties of the recombinant non-immunogenic staphylokinase in preclinical and clinical trials

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The aim. To study possible allergenic and immunotoxic properties of the recombinant non-immunogenic staphylokinase molecule with the amino acids replacement Lys_{74}, Glu_{75} and Arg_{77} with alanine in preclinical and clinical studies.
Materials and methods. The allergenic and immunotoxic properties of the recombinant non-immunogenic staphylokinase drug were studied using standard methods in accordance with the Guidelines for the Preclinical Study of New Substances in guinea pigs (*n*=15) and mice (*n*=45) at doses 5, 10 and 20 times higher than therapeutic (for humans). A clinical study was conducted in 100 patients with acute ST-segment elevation myocardial infarction after a single intravenous injection of the drug. The study included the determination of titers of specific antibodies to recombinant non-immunogenic staphylokinase and the study of plasma neutralizing activity.
Results. During the complete set of preclinical studies, it was found that the drug does not affect the cellular and humoral immune response in guinea pigs and mice at doses many times higher than therapeutic doses for humans. It was found that the drug did not cause an immediate-type of hypersensitivity reaction (Weigle index 0) and a delayed type IV (0 points according to S.V. Suvorov) in guinea pigs, did not affect the cellular capacity of popliteal lymph nodes (reaction index 0.91), did not affect the number of nucleated and antibody-forming cells in the spleen of mice. As a result of a clinical study of recombinant non-immunogenic staphylokinase, no allergic reactions were registered. Assessment of the neutralizing activity of the plasma of patients who were administered recombinant non-immunogenic staphylokinase showed that 70% samples did not have neutralizing activity: 30% of the patients' samples were characterized by a minimum neutralizing activity of 0.33±0.02 μg/mL, which is 30–310 times lower than after the use of native staphylokinase. These values are 7.8 times lower than the determined concentration of recombinant non-immunogenic staphylokinase in the blood (2.59 μg/mL). Thus, the drug does not lead to the anti-staphylokinase neutralizing antibodies formation capable to neutralize its effect upon repeated administration.
Conclusion. According to the results of the trials, the absence of allergenic and immunotoxic properties of the recombinant non-immunogenic staphylokinase and its safety in relation to the immune system have been proven.
Keywords: thrombolysis; recombinant non-immunogenic staphylokinase; allergy; immunotoxic studies
Abbreviations: AFC — antibody-forming cells; IL — interleukin; STEMI — acute ST-segment elevation myocardial infarction; PE — pulmonary embolism; RE — ram erythrocytes.

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Оценка аллергизирующих и иммунотоксичных свойств рекомбинантной неиммуногенной стафилокиназы в доклинических и клинических исследованиях

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Цель. Изучить возможные аллергизирующие и иммунотоксичные свойства молекулы рекомбинантной неиммуногенной стафилокиназы с заменой аминокислот Lys_{74}, Glu_{75} и Arg_{77} на аланин в доклинических и клинических исследованиях.

Материалы и методы. Исследование аллергизирующих и иммунотоксических свойств препарата рекомбинантной неиммуногенной стафилокиназы проведено по стандартным методикам в соответствии с требованиями Руководства по экспериментальному (доклиническому) изучению новых фармакологических веществ на морских свинках (*n*=15) и мышах (*n*=45) в дозах, в 5, 10 и 20 раз превышающих терапевтические (для человека). Клиническое исследование проведено у 100 пациентов с острым инфарктом миокарда с подъёмом сегмента ST после однократного внутривенного введения препарата. Исследование включало определение титров специфических антител к рекомбинантной неиммуногенной стафилокиназе и оценку нейтрализующей активности плазмы.

Результаты. При проведении полного комплекса доклинических исследований было установлено, что препарат не влияет на клеточный и гуморальный иммунный ответ у морских свинок и мышей в дозах, кратно превышающих терапевтические для человека. Установлено, что препарат не вызывал реакцию гиперчувствительности немедленного типа (индекс по Weigle 0) и замедленного типа (0 баллов по С.В. Суворову) у морских свинок, а также не влиял на клеточность подколенных лимфоузлов (индекс реакции 0,91) и число ядросодержащих и антителообразующих клеток в селезёнке мышей. В результате клинического исследования рекомбинантной неиммуногенной стафилокиназы аллергических реакций не зарегистрировано. Оценка нейтрализующей активности плазмы крови пациентов, которым вводилась рекомбинантная неиммуногенная стафилокиназа, показало, что пробы 70% пациентов не обладают нейтрализующей активностью: 30% проб пациентов характеризовались минимальной нейтрализующей активностью 0,33±0,02 мкг/мл, что в 30–310 раз ниже, чем после применения нативной стафилокиназы. Эти значения в 7,8 раз ниже определяемой концентрации рекомбинантной неиммуногенной стафилокиназфы в крови (2,59 мкг/мл). Таким образом, препарат не приводит к образованию антител, способных нейтрализовать его действие при повторном введении.

Заключение. По результатам проведённых исследований доказано отсутствие аллергизирующих и иммунотоксичных свойств рекомбинантной неиммуногенной стафилокиназы и ее безопасность в отношении иммунной системы.

Ключевые слова: тромболизис; рекомбинантная неиммуногенная стафилокиназа; аллергические реакции; исследования иммунотоксичности

Список сокращений: АОК — антителообразующие клетки; ИЛ — интерлейкин; ОИМпST — острый инфаркт миокарда с подъёмом сегмента ST; ТЭЛА — тромбоэмболия легочной артерии; ЭБ — эритроциты барана.

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INTRODUCTION

Myocardial infarction, ischemic stroke, and pulmonary embolism (PE) continue to be leading causes of death among all cardiovascular diseases [1]. The prevalence of myocardial infarction (per 100,000 people) reaches 500 cases in men and 100 cases in women, ischemic stroke — 460–560 cases, PE — 35–40 cases [1]. Thrombolytic therapy is a pathogenetically sound method for treating acute ST-segment elevation myocardial infarction (STEMI), ischemic stroke, and massive PE, based on dissolving a fibrin clot (thrombus) and restoring blood flow in the occluded vessel. Thrombolysis can reduce the risk of disability and death [1].

Thrombolytic therapy is carried out using drugs based on recombinant proteins, which determines the need to assess their potential impact on the immune system. Staphylokinase is a unique thrombolytic agent with high biological activity and fibrinolytic properties [2]. As a plasminogen activator, staphylokinase initially reacts with a minimal content of plasmin (3 ppm) located on the fibrin clot, followed by activation of γ-plasminogen and the formation of a triple complex "staphylokinase–plasmin–plasminogen", which lyses fibrin clots. Simultaneously, the resulting plasmin enhances the fibrinolytic activity of staphylokinase, and its excess is rapidly inactivated by α_2-antiplasmin. When the "plasmin–staphylokinase" complex is inhibited by α_2-antiplasmin, an active staphylokinase molecule is released for subsequent recycles. The recirculation of staphylokinase helps to reduce the dose used in clinical practice compared to tissue plasminogen activators and makes it independent of the patient's body weight [3].

The inhibition of the "plasmin-staphylokinase" complex in plasma occurs more than 100 times faster compared to the same process in a thrombus. Thus, staphylokinase has high selectivity for fibrin, which prevents the formation of plasmin from plasminogen in the systemic circulation [4]. Due to the high fibrin selectivity of the drug, the use of staphylokinase is characterized by a minimal risk of developing hemorrhagic complications.

As a result of kinetic analysis of the interaction of staphylokinase with plasmin, it was found that the catalytic activity of staphylokinase is 1000 times higher than that of alteplase [5]. The high fibrin selectivity of staphylokinase made it a first-line drug for the treatment of STEMI and ischemic stroke back in the late last century. However, the presence of immunogenic properties of the native staphylokinase molecule hindered its introduction into clinical practice. To create a non-immunogenic staphylokinase, the amino acids Lys_{74}, Glu_{75}, and Arg_{77} were replaced with alanine in the desired molecule. Recombinant non-immunogenic staphylokinase is a single-chain molecule consisting of 138 amino acids, with a molecular weight of 15.5 kDa. It has been established that the fibrinolytic activity of recombinant non-immunogenic staphylokinase is 40% higher than that of the native staphylokinase molecule [6].

THE AIM of this work was to study the allergenic and immunotoxic properties of the recombinant non-immunogenic staphylokinase molecule in preclinical and clinical studies.

MATERIALS AND METHODS

Preclinical studies

The study of the allergenic, immunotoxic, and immunogenic properties of the recombinant non-immunogenic staphylokinase drug was carried out in accordance with the requirements of the Guidelines for Experimental (Preclinical) Study of New Pharmacological Substances (2005) in the Laboratory of Drug Toxicology of the National Medical Research Center of Cardiology named after Academician E.I. Chazov under the guidance of Prof. E.V. Arzamastsev in the period from September 2008 to January 2010. The studies were approved by the Ethics Committee of the National Medical Research Center of Cardiology named after Academician E.I. Chazov (Protocol No. 1 dated September 4, 2008).

Study of the effect of recombinant non-immunogenic staphylokinase on the immediate-type hypersensitivity reaction in guinea pigs

The studies were performed on 15 variegated male guinea pigs with an average body weight of 290±20 g, obtained from the Stolbovaya Laboratory Animal Nursery (Scientific Center for Biomedical Technologies). The animals were kept under standard vivarium conditions in ventilated cages with a 12-hour light/dark cycle, at an air temperature of +20°C and air humidity of 50–60%. The animals had free access to food and water.

Guinea pigs were divided into three groups of 5 animals each: Control — physiological saline, Group 1 — recombinant non-immunogenic staphylokinase at a dose of 0.665 mg/kg, Group 2 — recombinant non-immunogenic staphylokinase at a dose

of 1.33 mg/kg. The doses of the drug used corresponded to 5 and 10 times the highest daily dose recommended for humans (10 mg for humans or 0.133 mg/kg) [2]. The drug was administered intravenously at a dose of 0.665 mg/kg twice a day every other day. The permissive dose was administered intravenously to guinea pigs 14 and 21 days after sensitization. The intensity of anaphylactic shock was assessed using the Weigle index [8].

Study of the effect of recombinant non-immunogenic staphylokinase on delayed-type hypersensitivity reaction in guinea pigs

The studies were performed on 15 variegated male guinea pigs with an average body weight of 290±20 g, which were divided into three groups of 5 animals each. The groups and doses were similar to the previous experiment. The animals were sensitized by 5-fold intramuscular administration of recombinant non-immunogenic staphylokinase at 5-day intervals. On the 10th day after the last sensitization, the animals had their back hair shaved on a 3×3 cm area and were injected intradermally with 0.1 mL of recombinant non-immunogenic staphylokinase solution, and the same volume of saline was injected into another point. The reaction was assessed visually according to the S.V. Suvorov scale of skin tests (1974) in points 4 and 24 hours after intradermal injection of the permissive dose.

Study of the effect of recombinant non-immunogenic staphylokinase on the cellularity of the popliteal lymph node in mice

The studies were performed on 10 male F_1(CBAxC$_{57}$Bl$_6$) hybrid mice with an average body weight of 18±2 g, obtained from the Stolbovaya Laboratory Animal Nursery (Scientific Center for Biomedical Technologies). The animals were kept under standard vivarium conditions in ventilated cages with a 12-hour light/dark cycle, at an air temperature of +20°C and air humidity of 50–60%. The animals had free access to food and water. Mice were injected with 50 µl of recombinant non-immunogenic staphylokinase at a dose of 1.33 mg/kg into the pad of the left hind paw, and saline into the pad of the right hind paw. After 7 days, the cellularity of the left and right popliteal lymph nodes was determined in mice, and then the relative index was calculated by dividing the indicators of the left lymph node by similar indicators of the right lymph node [8].

Study of the effect of recombinant non-immunogenic staphylokinase on the number of nucleated and antibody-forming cells in the spleen of mice

The experiment is based on determining the number of nucleated and antibody-forming cells (AFC) in the spleen according to Jerne in accordance with generally accepted methods [9]. The studies were performed on 35 male F_1(CBAxC$_{57}$Bl$_6$) hybrid mice with an average body weight of 18±2 g, which were divided into 7 groups of 5 animals each. The mice were immunized by intravenous administration of ram erythrocytes (RE) (Microgen, Russia) at a dose of 5×108 cells/mouse. Animals of groups 1 and 2 received recombinant non-immunogenic staphylokinase intraperitoneally at doses of 1.33 and 2.66 mg/kg, respectively, one day before immunization with RE (day -1), groups 3 and 4 — at the same doses 1 h after immunization (day 0), groups 5 and 6 — 24 hours after immunization with RE (day +1). Control mice were injected with saline on “day +1”. On the 5th day after immunization, the spleen was removed from the mice, which was disintegrated in Hanks’ solution (pH=7.4). The cell suspension was separated from the stroma elements by filtration through a two-layer nylon filter, then washed 3 times and centrifuged (ELMI CM-50, Latvia) at 200 g for 5 min. After lysis of erythroid cells with 3% acetic acid, the number of karyocytes was counted in the resulting suspension on a cell counter (Picoscale PS-4M, Hungary).

Study of the effect of recombinant non-immunogenic staphylokinase on delayed-type hypersensitivity reaction in mice

The studies were performed on 35 male F_1(CBAxC$_{57}$Bl$_6$) hybrid mice with an average body weight of 18±2 g, which were divided into 7 groups of 5 animals each. The mice were immunized by subcutaneous injection of RE into the interscapular region at a dose of 2×108 cells/mouse. The scheme of administration of recombinant non-immunogenic staphylokinase and distribution by groups was similar to the previous experiment. On the 5th day after immunization, all animals received a resolving injection of RE into the left hind paw at a dose of 1×108 cells/mouse in a volume of 50 µl (“experimental paw”). Saline solution was injected into the pad of the contralateral paw (“control paw”). The reaction results were recorded after 24 hours by

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weighing the “control” and “experimental” paws. The reaction index (R.M. Khaitov, 2000) was calculated as the ratio of the difference in mass between the “experimental” and “control” paws to the mass of the “control” paw.

Statistical analysis

Statistical analysis was performed using R 4.2 (R Foundation, USA). For continuous variables, the mean and standard deviation (M±SD) are given. Comparison of distributions in independent groups for continuous parameters was made using the Mann–Whitney test, for discrete parameters — using Fisher’s exact test. Differences were considered statistically significant at p <0.05.

Clinical studies

In accordance with the permission of the Ministry of Health of Russia No. 261 dated May 16, 2014, a multicenter open randomized comparative trial of the efficacy and safety of a single bolus administration of recombinant staphylokinase (15 mg) and tissue plasminogen activator tenecteplase (30–50 mg) in patients with STEMI was conducted from October 2014 to August 2016 at 11 leading medical institutions in Russia [10, 11]. Before inclusion in the study, all patients signed an informed consent. The study was approved by the Ethics Council of the Ministry of Health of Russia (Protocol No. 81 dated April 15, 2014) and Local Ethics Committees at research centers.

Clinical study of the effect of recombinant non-immunogenic staphylokinase on the antibodies formation

382 patients participated in the study. *Inclusion criteria:* diagnosis of STEMI with ST segment elevation of more than 1 mm in two or more consecutive limb leads and/or more than 2 mm in chest leads in the first 12 h from the onset of the disease. *Exclusion criteria:* bleeding, hemorrhagic stroke, ischemic stroke in the preceding 6 months, diseases with an increased risk of bleeding. A complete list of inclusion and exclusion criteria, as well as criteria for evaluating the effectiveness and safety of thrombolytic therapy, have been published previously [10, 11].

Patients were randomized into two groups (n=191 each) to receive recombinant non-immunogenic staphylokinase (Fortelyzin®, SupraGen LLC, Russia) or tenecteplase (Metalyse®, Boehringer Ingelheim International, Germany). Randomization was carried out by the envelope method, in blocks of 4 drugs (2 — recombinant non-immunogenic staphylokinase and 2 — tenecteplase). The sequence of randomization numbers was generated by an independent biostatistician. Recombinant non-immunogenic staphylokinase was administered at a dose of 15 mg, regardless of body weight, as a bolus over 10–15 seconds, tenecteplase — as a bolus at a dose of 30–50 mg, depending on body weight, according to the instructions for medical use[1]. Blood was collected from 100 patients from the recombinant non-immunogenic staphylokinase group to determine the antibody titer of to recombinant non-immunogenic staphylokinase and the neutralizing activity of plasma before its administration, as well as on days 7, 14 and 30 after.

The antibody titer was determined at the Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences using a solid-phase indirect enzyme immunoassay according to the standard method [12]. The studied plasma samples obtained from patients after a single intravenous administration of the drug were diluted with a buffer solution (20 mM Tris-HCl, 150 mM NaCl, 0.005% Tween-20, pH=8.0) 1:100, 1:400, 1:1600, 1:6400, 1:25600, 1:102400. 100 µl of dilutions of the test samples were added to the wells. The plate was incubated for 60 min at 37°C. After incubation, the liquid was removed and the wells were washed three times with a buffer solution (20 mM Tris-HCl, 150 mM NaCl, 0.005% Tween-20, pH=8.0). 100 µl of rabbit secondary antibody solution to human IgG labeled with horseradish peroxidase (1:1000, pH=8.0) (Sigma, USA) was added to each well and incubated for 60 min at 37°C. After incubation, the wells were washed again with a buffer solution. 100 µl of substrate (0.07% orthophenylenediamine, 0.06% H_2O_2, pH=5.0) was added to each well. Incubated at room temperature for 3–5 min until staining appeared in the negative control sample. 50 µl of stop reagent (10% sulfuric acid solution) was added to each well. 15 min after the addition of the stop reagent, the optical density in each well was measured at 490 nm on an ImmunoChem-2100 microplate photometer (USA). The maximum dilution of the sample was determined, at which the optical density value of the positive wells exceeded the corresponding value of the negative wells by a factor of three. The antibody titer to recombinant non-immunogenic staphylokinase was calculated using the formula:

[1] Metalyse®. The State Register of Medicines of the Russian Federation. Available from: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=123f0609-001e-4e9c-8830-34e99ca499df

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$$T = \frac{1}{P_{max}},$$

where P_{max} — the maximum dilution at which the optical density value in positive wells exceeds the optical density value in negative wells by a factor of three.

Clinical study of the neutralizing activity of plasma in patients after administration of recombinant non-immunogenic staphylokinase

The neutralizing activity was determined in accordance with the method of D. Collen [13]. Dilutions of the test samples were prepared similarly to the previous experiment. 5 µl of the test sample and 5 µl of the drug solution in the concentration range of 0.2–100 µg/mL were added to microcentrifuge tubes, mixed and 10 µl of thrombin solution (5 NIH units/mL, pH=7.4) was added. The samples were incubated for 20 min at 37°C. The concentration range of recombinant non-immunogenic staphylokinase at which the moment of thrombus lysis and time were observed was determined. For each test sample, a graph of the dependence of thrombus lysis time (min) on the concentration of the drug (µg/mL) was plotted. Using the graphs, the neutralizing activity was determined — the concentration of the drug at which the thrombus lysis time was 20 min.

Statistical analysis

Statistical analysis was performed using R 4.2 (R Foundation, USA). Continuous variables are described as mean and standard deviation (M±SD) or median and quartiles (Me [Q1; Q3]). Categorical variables are represented by absolute and relative frequencies. The Mann-Whitney *U*-test was used to compare continuous variables, and the two-sided Fisher's exact test was used to compare categorical variables. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Preclinical studies

Study of the effect of recombinant non-immunogenic staphylokinase on the immediate-type hypersensitivity reaction (anaphylactic shock) in guinea pigs

It was found that after a double intraperitoneal administration to guinea pigs on the 14th day of sensitization with recombinant non-immunogenic staphylokinase at a dose of 1.33 mg/kg (10 times the highest daily therapeutic dose for humans), anaphylactic shock was not induced (Table 1). No changes in the behavior of animals — their general condition, indicators of vital functions compared with control animals receiving saline — were revealed (Weigle index is 0). Thus, recombinant non-immunogenic staphylokinase did not cause anaphylactic shock in guinea pigs.

Study of the effect of recombinant non-immunogenic staphylokinase on the delayed-type hypersensitivity reaction in guinea pigs

After sensitization of guinea pigs with recombinant non-immunogenic staphylokinase for 25 days of intradermal injection of the drug, no cases of hyperemia, signs of edema or inflammation were found in any of the animals in the experimental groups (0 points on the S.V. Suvorov scale). The behavior and condition of the animals corresponded to those of the control group.

Study of the effect of recombinant non-immunogenic staphylokinase on the cell content of the popliteal lymph node in mice

7 days after the administration of recombinant non-immunogenic staphylokinase into the pad of the left hind paw of a mouse at a 10-fold higher daily dose recommended for humans, the cell content of the right and left popliteal lymph nodes was determined in comparison with the control. The calculation results are presented in Table 2. No significant differences were found.

The cell count index of the popliteal lymph nodes of the "experimental" and "control" paws was 0.91[2]. Thus, recombinant non-immunogenic staphylokinase did not affect the cell count of popliteal lymph nodes and did not have allergenic properties.

Study of the effect of recombinant non-immunogenic staphylokinase on the number of nucleated and antibody-forming cells in the spleen of mice

The drug was administered to mice 1 day before, on the day of RE administration, or on the day RE immunization in doses 10 and 20 times higher than the highest daily dose for humans. The results of the study are presented in Table 3.

The data presented in Table 3 show that a single intraperitoneal administration of recombinant non-immunogenic staphylokinase to mice did not affect the cell count of the spleen.

[2] The index of 1.00 indicates the equality of the cellular parameters of the right and left lymph nodes. The smaller it is, the greater the difference.

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From the results of counting the number of AFCs in the spleen of mice on day 5 after immunization, presented in Table 4, it is clear that there were no significant differences in the number of AFCs between the groups of animals that received recombinant non-immunogenic staphylokinase in various doses at different times relative to RE immunization and the control animals.

Thus, the drug did not affect the number of nucleated cells and AFCs in the spleen of mice immunized with RE, and, therefore, did not affect the cellular and humoral immune response.

Study of the effect of recombinant non-immunogenic staphylokinase on the delayed-type hypersensitivity reaction in mice

After immunization of mice with RE and recombinant non-immunogenic staphylokinase, a permissive injection of RE was administered into the left hind paw, and physiological saline was administered into the pad of the contralateral paw. The reaction index of animals in different groups is presented in Table 5.

Analysis of the data presented in Table 5 indicates that the drug, at the tested doses of 1.33 and 2.66 mg/kg and sensitization regimens, did not affect the formation of cellular immunity.

Thus, during preclinical studies of the allergenic and immunotoxic properties of recombinant non-immunogenic staphylokinase, it was found that the drug, when administered to guinea pigs and mice at doses 5, 10, and 20 times higher than the therapeutic doses for humans, does not affect cellular and humoral immunity. A complete set of preclinical studies of recombinant non-immunogenic staphylokinase, including the assessment of acute and subacute (subchronic) toxicity, mutagenicity, genotoxicity, embryotoxicity, reprotoxicity, and teratogenicity, also demonstrated its safety and absence of toxic properties [15], which allowed obtaining permission for clinical trials of the drug as a fibrinolytic agent in patients with STEMI.

Clinical studies

Clinical study of the effect of recombinant non-immunogenic staphylokinase on the antibodies formation and neutralizing activity of patient plasma

The study included 100 patients with STEMI who received a single bolus dose of 15 mg of recombinant non-immunogenic staphylokinase. Demographic, anthropometric, medical history data, clinical characteristics, and time intervals are presented in Table 6.

76% of the study participants were male. The average age was 58.9±9.9 years. The proportion of patients with arterial hypertension was 75%, previous myocardial infarction — 12%, lipid metabolism disorders — 86%. Inferior myocardial infarctions predominated in the study population (56%).

Assessment of the effectiveness and safety of thrombolytic therapy is presented in Table 7.

ST segment reduction by 50% from baseline after 90 min was observed in 80% of patients. Restoration of coronary blood flow according to TIMI 2+TIMI 3 criteria was observed in 70%. All-cause mortality was 3%. No intracranial hemorrhages were reported with the use of recombinant non-immunogenic staphylokinase. No major bleeding or hemorrhagic stroke was observed in any patient with cardiogenic shock in the groups. No allergic reactions were reported as a result of the study.

In a study of specific antibody titers in patients with STEMI who were administered recombinant non-immunogenic staphylokinase, it was found that 30 (30%) patients had no detectable specific antibodies. In 70 (70%) patients, specific antibodies were detected with a low titer — in the range of 1/100–1/800.

When determining the neutralizing activity of blood plasma in patients with STEMI who were administered recombinant non-immunogenic staphylokinase, it was found that samples from 70 (70%) patients did not have neutralizing activity. Samples from the remaining 30 (30%) patients were characterized by neutralizing activity at a dose of 0.33±0.02 μg/mL. It has previously been shown that the average values of neutralizing activity of native staphylokinase were many times higher, in the range of 9–93 μg/mL [14–16].

Thus, the average value of the neutralizing activity of blood plasma of patients after administration of recombinant non-immunogenic staphylokinase is 30–310 times lower than after administration of native staphylokinase.

Considering that the determined concentration of recombinant non-immunogenic staphylokinase in the blood with a single bolus administration at a dose of 15 mg is 2.5 μg/mL, which is 7.8 times higher than the neutralizing activity of blood plasma samples (0.33 μg/mL), observed only in 30% of patients, it can be concluded that the administration of the drug does not lead to the formation of antibodies capable of neutralizing its effect upon repeated administration.

Table 1 – Effect of recombinant non-immunogenic staphylokinase on the immediate-type hypersensitivity reaction in guinea pigs

Animal groups, doses	Weigle, M±SD	*p*
Control	0.0±0.00	–
Group 1, 0.665 mg/kg	0.0±0.00	1.00
Group 2, 1.33 mg/kg	0.0±0.00	1.00

Table 2 – Effect of recombinant non-immunogenic staphylokinase on the cell count of popliteal lymph nodes in $F_1(CBAxC_{57}Bl_6)$ hybrid mice

Animal groups, doses	Cell count of popliteal lymph nodes, million/mL, M±SD	*p*
Control	0.78±0.07	–
Group 1, 1.33 mg/kg	0.71±0.07	0.95

Table 3 – Effect of recombinant non-immunogenic staphylokinase on the cell count of the spleen of $F_1(CBAxC_{57}Bl_6)$ mice immunized with ram erythrocytes

Animal groups, doses	Number of karyocytes, 10^7/spleen, M±SD	*p*
Control	26.88±1.67	–
Group 1, 1.33 mg/kg «day -1»	25.64±0.93	0.89
Group 2, 2.66 mg/kg «day -1»	26.62±2.77	0.98
Group 3, 1.33 mg/kg «day 0»	28.44±1.84	0.91
Group 4, 2.66 mg/kg «day 0»	24.48±2.96	0.82
Group 5, 1.33 mg/kg «day +1»	27.71±1.45	0.85
Group 6, 2.66 mg/kg «day +1»	28.48±1.81	0.95

Note: Animals in groups 1 and 2 received recombinant non-immunogenic staphylokinase intraperitoneally at doses of 1.33 and 2.66 mg/kg, respectively, one day before ram erythrocytes immunization ("day -1"), groups 3 and 4 — at the same doses 1 hour after immunization ("day 0"), groups 5 and 6 — 24 hours after ram erythrocytes immunization ("day +1").

Table 4 – Effect of recombinant non-immunogenic staphylokinase on the number of antibody-forming cells in $F_1(CBAxC_{57}Bl_6)$ mice immunized with ram erythrocytes

Animal groups, doses	Number of antibody-forming cells in the spleen, 1×104, M±SD	*p*
Control	8.66±1.73	–
Group 1, 1.33 mg/kg «day -1»	10.29±1.32	0.72
Group 2, 2.66 mg/kg «day -1»	8.65±2.22	0.95
Group 3, 1.33 mg/kg «day 0»	9.39±1.41	0.80
Group 4, 2.66 mg/kg «day 0»	9.03±1.14	0.82
Group 5, 1.33 mg/kg «day +1»	9.96±1.95	0.78
Group 6, 2.66 mg/kg «day +1»	10.12±0.68	0.75

Note: Animals in groups 1 and 2 received recombinant non-immunogenic staphylokinase intraperitoneally at doses of 1.33 and 2.66 mg/kg, respectively, one day before ram erythrocytes immunization ("day -1"), groups 3 and 4 — at the same doses 1 hour after immunization ("day 0"), groups 5 and 6 — 24 hours after ram erythrocytes immunization ("day +1").

Table 5 – Effect of recombinant non-immunogenic staphylokinase on the development of delayed-type hypersensitivity reaction in $F_1(CBAxC_{57}Bl_6)$ mice

Animal groups, doses	Reaction index, M±SD	*p*
Control	19.81±2.43	–
Group 1, 1.33 mg/kg «day -1»	17.96±2.64	0.75
Group 2, 2.66 mg/kg «day -1»	19.91±2.59	0.96
Group 3, 1.33 mg/kg «day 0»	24.52±3.85	0.12
Group 4, 2.66 mg/kg «day 0»	23.48±2.77	0.35
Group 5, 1.33 mg/kg «day +1»	22.16±1.62	0.59
Group 6, 2.66 mg/kg «day +1»	20.46±0.94	0.79

Note: Animals in groups 1 and 2 received recombinant non-immunogenic staphylokinase intraperitoneally at doses of 1.33 and 2.66 mg/kg, respectively, one day before ram erythrocytes immunization ("day -1"), groups 3 and 4 — at the same doses 1 hour after immunization ("day 0"), groups 5 and 6 — 24 hours after ram erythrocytes immunization ("day +1").

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Table 6 – Baseline characteristics of patients

Characteristics	Indicator (*n*=100)
Gender, male / female	76/23 (76%/23%)
Age, years	58.9±9.9
Patients older than 75 years	6 (6%)
Weight, kg	83.8±14.2
Body mass index, kg/m^2	28.5±4.5
Myocardial infarction	12 (12%)
Arterial hypertension	75 (75%)
Diabetes mellitus Type II	14 (14%)
Dyslipidemia	86 (86%)
Smoking	39 (39%)
STEMI, mm	3.58±1.96
SBP, mm Hg .	118.6±8.2
DBP, mm Hg	74.7±7.2
HR, bpm	75.9±14.7
STEMI localization:	
anterior	42 (42%)
inferior	56 (56%)
other	2 (2%)
Type of heart failure according to Killip:	
I	87 (87%)
II	8 (8%)
III	3 (3%)
IV	2 (2%)

Table 7 – Assessment of the effectiveness and safety of thrombolytic therapy

Criterion	Indicator (*n*=100), abs. (%)
ST segment reduction by 50% after 90 min	80 (80%)
Restoration of coronary blood flow according to TIMI criteria:	
0	24 (24%)
1	6 (6%)
2	32 (32%)
3	38 (38%)
2+3	70 (70%)
Death from all causes	3 (3%)
Cardiogenic shock	4 (4%)
Recurrent myocardial infarction	4 (4%)
Major bleeding	1 (1%)
Intracranial hemorrhage	0 (0%)
Minor bleeding	3 (3%)
Allergic reactions	0 (0%)

DISCUSSION

In accordance with the Rules of Good Clinical Practice approved by the Eurasian Economic Commission, the assessment of allergenic, immunotoxic, and immunogenic properties of original molecules is one of the most important stages of research necessary for further clinical trials and registration of a medicinal product. Immunogenicity can provoke such serious side effects from the immune system as anaphylactic shock and Quincke's edema. According to the Clinical Guidelines of the Ministry of Health of Russia "Anaphylactic Shock" (2020)[3], anaphylactic shock is understood as "acute circulatory failure, manifested by a decrease in systolic blood pressure below 90 mm Hg. or by 30% from the working level and leading to hypoxia of vital organs." It should be emphasized that such a

[3] Clinical Guidelines of the Russian Ministry of Health «Anaphylactic shock» (2020). Available from: https://cr.minzdrav.gov.ru/view-cr/263_2

diagnosis without pronounced hemodynamic disorders is illegal and should be considered as anaphylaxis. Anaphylactic shock refers to a type I hypersensitivity reaction that occurs with the participation of class E immunoglobulins fixed on the surface of basophil and mast cell membranes[4]. Anaphylactic shock should be distinguished from angioedema (Quincke's edema) — a localized transient acute edema of the skin or mucous membranes, as well as urticaria — a group of diseases characterized by the development of itchy blisters or angioedema[5].

This article presents the results of preclinical and clinical studies of the allergenic, immunotoxic, and immunogenic properties of the recombinant non-immunogenic staphylokinase molecule. During a set of preclinical studies, it was found that the drug does not affect the cellular and humoral immune response in guinea pigs and mice at doses 5, 10, and 20 times higher than therapeutic doses for humans. Recombinant non-immunogenic staphylokinase does not cause anaphylactic shock and the development of edema in experimental animals with intravenous, intramuscular, and subcutaneous administration. In experiments on inhalation administration of recombinant non-immunogenic staphylokinase to mice with acute respiratory distress syndrome [17], it was found that the drug also did not cause allergic reactions and did not have an irritating effect on the respiratory tract, reduced the deposition of fibrinogen in the lungs and had a normalizing effect on the concentration of pro-inflammatory cytokines — IL-1α, IL-17A, IL-6.

A clinical study of the neutralizing activity of blood plasma of patients who were injected with recombinant non-immunogenic staphylokinase showed that samples taken from 70% of patients with STEMI do not have neutralizing activity against the drug. In the remaining 30% of patients, the average values of neutralizing activity of blood plasma after a single administration of the drug did not exceed 0.3 μg/mL, which is 30–310 times lower than after the administration of the native staphylokinase molecule. These values are significantly lower than the concentration of the drug in the blood, so they are not able to neutralize its effect in case of repeated administration.

In 2012, based on the results of preclinical and clinical studies, the Ministry of Health of Russia registered the original thrombolytic drug of recombinant protein containing the amino acid sequence of staphylokinase — Fortelyzin® (produced by SuperGene LLC, Russia; registration certificate No. LP-001941 dated December 18, 2012) for the treatment of patients with STEMI.

According to the safety and efficacy monitoring registers of recombinant non-immunogenic staphylokinase, since 2012, the drug has been used in more than 50 thousand patients with STEMI [18] and in more than 20 thousand with ischemic stroke. According to IMS Health as of March 2025, the recombinant non-immunogenic staphylokinase drug has been used in more than 200 thousand patients. During this period, the automated information system of Roszdravnadzor registered 18 (0.009%) reports of the development of anaphylactic shock after using the drug. This is reflected in the "Side effect"[6] section in the instructions for medical use of the drug, which indicates that such disorders of the immune system as anaphylactoid reactions are very rare (<1 in 10 thousand cases). Thus, the absence of immunogenic properties of recombinant non-immunogenic staphylokinase and its high safety with respect to the immune system are confirmed by many years of experience in use in a wide range of patients.

In clinical studies of recombinant non-immunogenic staphylokinase, no serious adverse events such as anaphylactic shock, urticaria, or Quincke's edema were reported.

In the FRIDOM1 trials [10, 11], recombinant non-immunogenic staphylokinase at a dose of 15 mg was no less effective than tenecteplase in terms of restoring coronary blood flow according to coronary angiography (70 *vs* 71%, p=0.76) and ECG (8% *vs* 80%, p=0.81); the absence of intracranial hemorrhages and the high efficacy of recombinant non-immunogenic staphylokinase allowed it to be registered for the treatment of patients with STEMI. Currently, recombinant non-immunogenic staphylokinase is included in the Clinical Guidelines and standards of treatment for patients with STEMI. The Eurasian Clinical Guidelines for the diagnosis and treatment of acute coronary syndrome with ST-segment elevation specifically mention the absence of antigenicity as an advantage of the drug. By Order of the Ministry of Health of Russia No. 1165n dated October 28, 2020, a recombinant protein containing

[4] Ibid.
[5] Ibid.
[6] Fortelizin®. The State Register of Medicines of the Russian Federation. Available from: https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=9721d84a-5efa-4a24-b940-d711798bd51c

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the amino acid sequence of staphylokinase is included in the mandatory requirements for the equipment of medicines and medical devices for emergency medical care kits and sets.

According to the REGION-IM register of the National Medical Research Center of Cardiology named after Academician E.I. Chazov, 36% of patients with STEMI receive thrombolytic therapy using recombinant non-immunogenic staphylokinase, and at the prehospital stage, this figure reaches 42%. The frequency of using recombinant non-immunogenic staphylokinase in primary vascular departments reaches 51% [19].

The FRIDA study showed that recombinant non-immunogenic staphylokinase is an effective and safe thrombolytic agent for treating patients with ischemic stroke within 4.5 h of the onset of the first symptoms [20, 21]. In terms of the number of patients with good functional recovery (0–1 points on the modified Rankin scale), the drug was at least as effective as alteplase (50 *vs* 41%, p=0.10). Recombinant non-immunogenic staphylokinase is used as a rapid single bolus (10 seconds) at a single dose of 10 mg in patients with ischemic stroke of any body weight. In 2024, the recombinant non-immunogenic staphylokinase drug was included in the Clinical Guidelines for the treatment of ischemic stroke in the first 4.5 hours from the onset of the disease.

The FORPE trials presents the results of using recombinant non-immunogenic staphylokinase in patients with massive PE [22, 23]. Recombinant staphylokinase is not inferior to alteplase in terms of the primary efficacy endpoint “all-cause mortality within 7 days” (2 *vs* 3%, p=1.00) and has a high safety profile. The use of recombinant non-immunogenic staphylokinase was not accompanied by the development of major bleeding, which occurred with the use of alteplase (0 *vs* 3%, p=0.06) and hemorrhagic stroke (0 *vs* 2%, p=0.25). According to CTPA with contrast enhancement of the pulmonary arteries, a significant decrease in thrombotic masses (65.8 *vs* 47.4%, p <0.001) and a reduction in the size of the right ventricle (50 *vs* 39 mm, p <0.001) were shown 24 hours after thrombolysis with recombinant non-immunogenic staphylokinase. Currently, a double-blind placebo-controlled clinical trial of recombinant non-immunogenic staphylokinase in patients with intermediate-high risk PE has begun (permission of the Ministry of Health of Russia No. 106 от 21.03.2024 г., clinicaltrials.gov No. NCT06362746) [24].

A clinical study of recombinant non-immunogenic staphylokinase is being conducted with its intra-arterial intrathrombal administration in patients with thrombosis of the arteries of the lower extremities in comparison with surgical methods of treatment FORAT (researcher-coordinator — Academician I.I. Zatevakhin, permission of the Ministry of Health of Russia No. 184 dated March 18, 2022, clinicaltrials.gov No. NCT05372718) [25].

Along with the Russian Federation, the recombinant non-immunogenic staphylokinase drug is registered in a number of CIS countries (Tajikistan, Turkmenistan) and is currently undergoing registration in the EAEU countries, Azerbaijan, Georgia, and Uzbekistan.

Study limitations

In the presented clinical study, the recombinant non-immunogenic staphylokinase drug was administered in accordance with the instructions for medical use as a single bolus. Studies after bolus-infusion administration of the drug were not conducted. Studies of the neutralizing activity of blood plasma and titers of specific antibodies after repeated use of the drug were also not conducted. Clinical experience with its double use indicates the preservation of efficacy and the absence of neutralizing activity of blood plasma, which was shown in a patient with massive pulmonary embolism against the background of a shrapnel wound [26].

CONCLUSION

Given the annual increase in the number of thrombolysis procedures, the risks of adverse events should be taken into account. In this regard, a comprehensive study of the allergenic and immunotoxic properties of the recombinant non-immunogenic staphylokinase molecule in preclinical and clinical studies is extremely relevant. Based on the results of a full range of tests, it was convincingly proven that recombinant non-immunogenic staphylokinase does not have allergenic, immunogenic, and immunotoxic properties. The data obtained during these studies will undoubtedly contribute to a more active spread of thrombolytic therapy using non-immunogenic staphylokinase, which in the future will improve the quality of medical care for the population.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Sergey S. Markin — formulation of design, editing and final approval of the article; Sergei V. Ivanov — writing the article; Igor P. Beletsky — formulation of design, administration; Marina V. Zakharova — conducting a research, statistical data processing; Eduard A. Ponomarev — conducting a research; Evgenii V. Arzamascev — conducting a research, statistical data processing. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made significant contributions to the development of the concept, research and preparation of the article, read and approved the final version before publication).

REFERENCES

1. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020;396(10258):1204–22. DOI: 10.1016/S0140-6736(20)30925-9. Erratum in: Lancet. 2020;396(10262):1562. DOI: 10.1016/S0140-6736(20)32226-1
2. Collen D. Staphylokinase: a potent, uniquely fibrin-selective thrombolytic agent. Nature Medicine. 1998;4(3):279–84. DOI: 10.1038/nm0398-279
3. Christener RB, Boyle MD. Role of Staphylokinase in the acquisition of plasmin-(ogen) dependent enzymatic activity by Staphylococci. J Infect Dis. 1986;173(1):104–12. DOI: 10.1093/infdis/173.1.104
4. Verstraete M. Third-Generation Thrombolytic Drugs. Am J Med. 2000;109(1):52–8. DOI: 10.1016/s0002-9343(00)00380-6
5. Toul M, Nikitin D, Marek M, Damborsky J, Prokop Z. Extended mechanism of the plasminogen activator staphylokinase revealed by global kinetic analysis: 1000-fold higher catalytic activity than that of clinically used alteplase. ACS Catalysis. 2022;12:3807–14. DOI: 10.1021/acscatal.1c05042
6. Markin SS, Semenov AM, Markov VA, Nizov AA, Ponomarev EA, Lebedev PA. Clinical trial of fibrinselective thrombolytic pharmaceutical agent "Fortelyzin®" (III phase). RUDN Journal of Medicine. 2012;(1):105–10. EDN: OPPLCH
7. Weigle WO, Cochrane CG, Dixon FJ. Anaphylactogenic properties of soluble antigen-antibody complexes in guinea pig and rabbit. Journal of Immunology. 1960;85:469–77.
8. Descotes J, Verdier F. Popliteal lymph node assay. In: Methods in Immunotoxicology; Volume 1. Wiley-Liss, Inc.; 1995.
9. Jerne NK, Nordin AA. Plaque formation in agar by single antibody-producing cells. Science. 1963;140(3565):405.
10. Markov VA, Duplyakov DV, Konstantinov SL, Klein GV, Aksentev SB, Platonov DYu, Vyshlov EV, Ponomarev EA, Rabinovich RM, Makarov EL, Kulibaba EV, Yunevich DS, Kritskaia OV, Baranov EA, Talibov OB, Gerasimets EA. Fortelyzin® in comparison with Metalyse® for ST-elevated myocardial infarction: one-year results and clinical outcomes of a multicenter randomized study FRIDOM1. Russian Journal of Cardiology. 2018;(11):110–16. DOI: 10.15829/1560-4071-2018-11-110-116
11. Markov VA, Duplyakov DV, Konstantinov SL, Klein GV, Aksentiev SB, Platonov DYu, Vyshlov EV, Ponomarev EA, Rabinovich RM, Makarov EL, Kulibaba EV, Yunevich DS, Kritskaya OV, Baranov EA, Talibov OB, Kutsenko VA, Orlovsky AA, Vyazova NL, Koledinsky AG, Semenov AM, Semenov MP, Yarovaya EB, Uskach TM, Shakhnovich RM, Tereshchenko SN, Markin SS. Advanced results of Fortelyzin® use in the FRIDOM1 study and real clinical practice. Russian Journal of Cardiology. 2022;27(8):5178. DOI: 10.15829/1560-4071-2022-5178
12. Lequin RM. Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). Clinical Chemistry. 2005;51(12):2415–8. DOI: 10.1373/clinchem.2005.051532
13. Collen D, Moreau H, Stockx L, Vanderschueren S. Recombinant staphylokinase variants with altered immunoreactivity. II. Thrombolytic properties and antibody induction. Circulation. 1996;94:207–16.
14. Markin SS, Semenov AM, Arzamascev EV, Markov VA, Vyshlov EV, Nizov AA, Aksent'ev SB6, Ponomarev EA, Gridasov GN, Lebedev PA, Barbarash OL, Kashtalap VV. Fortelyzin in patients with acute myocardial infarction. Medical Academic Journal. 2012;12(1):80–6. EDN: QBXHQB
15. Collen D, Stockx L, Lacroix H, Suy R. Vanderschueren S. Recombinant Staphylokinase Variants With Altered Immunoreactivity IV. Identification of variants with reduced antibody induction but intact potency. Circulation. 1997;95(2):463–72.
16. Laroche Y, Heymans S, Capaert S, De Cock F, Demarsin E, Collen D. Recombinant staphylokinase

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2025-13-1-31-44

variants with reduced antigenicity due to elimination of B-lymphocyte epitopes. Blood. 2000;96(4):1425–32.

17. Markin SS, Lapshin RD, Baskina OS, Korotchenko SA, Mukhina IV, Ivanov SV, Semenov MP, Beregovykh VV, Semenov AM. Nebulized Non-Immunogenic Staphylokinase in the Mice Acute Lung Injury Model. Int J Mol Sci. 2022;23(16):9307. DOI: 10.3390/ijms23169307
18. Tereshchenko SN, Bagnenko SF, Markov VA, Miroshnichenko AG, Serebrennikov II, Krylov SO, Lishchenko AN, Gorbacheva SM, Kuznetsov VV, Ostroumova LA, Ihaev AB, Duplyakov DV, Chefranova ZhYu, Konstantinov SL, Vyshlov EV, Ponomarev EA, Rabinovich RM, Petrushin MA, Kutsenko VA, Koledinsky AG, Vyazova NL, Stryabkova GI, Uskach TM, Minnullin IP, Gaponova NI, Trukhanova IG, Prokhasko LV, Mukhin SI, Kostylev VV, Krause OV, Belova LP, Lesnikov EV, Zhukov GP, Pribylov SA, Farsiyants AV, Zhirov AV, Shtegman OA, Ivanov VB, Timoschenko ES, Makarov EL, Tolstoy OA, Sachkov DYu, Karamova IM, Rakhmatullin AR, Kostogryz VB, Volkov ES, Rukosuev EV, Yurkin EP, Shakhnovich RM, Yavelov IS, Erlikh AD, Ivanov SV, Semenov AM, Semenov MP, Yarovaya EB, Markin SS. Safety of prehospital thrombolysis with the non-immunogenic staphylokinase in 51,021 patients with ST-segment elevation myocardial infarction: data from the FRIDOM-registry. Russian Journal of Cardiology. 2025;30(6):6355. DOI: 10.15829/1560-4071-2025-6355
19. Boytsov SA, Shakhnovich RM, Tereschenko SN, Erlikh AD, Pevsner DV, Gulyan RG, Rytova YuK, Dmitrieva NYu, Voznyuk YaM, Musikhina NA, Nazarova OA, Pogorelova NA, Sanabasova GK, Sviridova AV, Sukhareva IV, Filinova AS, Shylko YuV, Shirikova GA. Features of the Reperfusion Therapy for ST-Segment Elevation Myocardial Infarction According to the Russian Registry of Acute Myocardial Infarction – REGION-IM. Kardiologiia. 2024;64(2):3–17. DOI: 10.18087/cardio.2024.2.n2601
20. Gusev EI, Martynov MYu, Nikonov AA, Shamalov NA, Semenov MP, Gerasimets EA, Yarovaya EB, Semenov AM, Archakov AI, Markin SS; FRIDA Study Group. Non-immunogenic recombinant staphylokinase versus alteplase for patients with acute ischaemic stroke 4.5 h after symptom onset in Russia (FRIDA): a randomised, open label, multicentre, parallel-group, non-inferiority trial. Lancet Neurology. 2021;20(9):721–728. DOI: 10.1016/S1474-4422(21)00210-6
21. Gusev EI, Martynov MYu, Shamalov NA, Yarovaya EB, Semenov MP, Semenov AM, Orlovsky AA, Kutsenko VA, Nikonov AA, Aksentiev SB, Yunevich DS, Alasheev AM, Androfagina OV, Bobkov VV, Choroshavina KV, Gorbachev VI, Korobeynikov IV, Greshnova IV, Dobrovolskiy AV, Elemanov UA, Zhukovskaya NV, Zakharov SA, Chirkov AN, Korsunskaya LL, Nesterova VN, Nikonova AA, Nizov AA, Girivenko AI, Ponomarev EA, Popov DV, Pribylov SA, Semikhin AS, Timchenko LV, Jadan ON, Fedyanin SA, Chefranova ZhYu, Lykov YuA, Chuprina SE, Vorobev AA, Archakov AI, Markin SS. Nonimmunogenic staphylokinase in the treatment of acute ischemic stroke (FRIDA trial results). S.S. Korsakov Journal of Neurology and Psychiatry. 2022;122(7):56–65. DOI: 10.17116/jnevro202212207156
22. Kirienko AI, Leontyev SG, Tereschenko SH, Yavelov IS, Shakhnovich RM, Erlikh AD, Talibov OB, Yarovaya EB, Semenov AM, Semenov MP, Ivanov SV, Beregovykh VV, Archakov AI, Markin SS; FORPE study group. Non-immunogenic recombinant staphylokinase versus alteplase for patients with massive pulmonary embolism: a randomised open-label, multicenter, parallel-group, non-inferiority trial FORPE. Journal of Thrombosis and Haemostasis. 2025;23(2):657–67. DOI: 10.1016/j.jtha.2024.09.035
23. Kirienko AI, Leontyev SG, Yarovaya EB, Konstantinov SI, Orlov BB, Meray I, Duplyakov DV, Oleynikov VE, Vasilyeva EYu, Ponomarev EA, Pribylov SA, Komarova AG, Bobkov VV, Rabinovich RM, Klein GV, Shogenov ZS, Karabach YuV, Zolkin VN, Kulabukhov VV, Kashtalap VV, Linev KA, Stryabkova GI, Yasnopolskaya NV, Tolmacheva VYu, Chefranova ZhYu, Lykov YuA, Panina ES, Solovieva NV, Rybin EV, Furman NV, Kulibaba EV, Makukhin VV, Koledinsky AG, Mullova IS, Cherepanova NA, Pavlova TV, Savvinova PP, Libov IA, Igoshin AS, Bogomazov IYu, Pecherina TB, Lyudnev LO, Vlasov PN, Avdeeva IV, Maksimov DB, Komissarova EV, Ivanov VS, Vyazova NL, Vyshlov EV, Kurtasov DS, Kutsenko VA, Ivlev OE, Soplenkova AG, Tereshchenko SN, Yavelov IS, Shakhnovich RM, Erlikh AD, Talibov OB, Semenov AM, Semenov MP, Ivanov SV, Romashova YuA, Beregovykh VV, Archakov AI, Markin SS. Non-immunogenic staphylokinase — a thrombolytic agent in the treatment of massive pulmonary embolism: results of the FORPE clinical trial. Russian Journal of Cardiology. 2024;29(11):6157. DOI: 10.15829/1560-4071-2024-6157
24. Tereshchenko SN, Yarovaya EB, Leontiev SG, Yavelov IS, Shakhnovich RM, Erlikh AD, Uskach TM, Duplyakov DV, Kutsenko VA, Ivlev OE, Soplenkova AG, Semenov AM, Semenov MP, Ivanov SV, Markin SS. Non-immunogenic staphylokinase in patients with massive intermediate-high risk pulmonary embolism: protocol of the FORPE-2 multicenter, double-blind, randomized, placebo-controlled trial. Russian Journal of Cardiology. 2025;30(2):6291. DOI: 10.15829/1560-4071-2025-6291
25. Zatevaxin II, Chupin AV, Karpenko AA. Intraarterial thrombolysis with non-immunogenic staphylokinase compared with surgical methods of treatment in patients with arterial thrombosis of the lower extremities of varying degrees of ischemia: Protocol of a multicenter open randomized clinical trial of FORAT. Journal of Angiology and Vascular Surgery. 2025;31(1):142–51. Russian
26. Konstantinov SL, Stryabkova GI, Basarab AI, Kravchuk VN, Kazhanov IV, Ivanov SV, Chefranova ZhYu, Markin SS. Non-immunogenic staphylokinase in the treatment of high-risk massive pulmonary embolism in the early postoperative period due to femoral fragment wound. Rational Pharmacotherapy in Cardiology. 2023;19(5):495–501. DOI: 10.20996/1819-6446-2023-2957

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