УДК 616.131-008.331.1-005.755-0221.6
https://doi.org/10.20538/1682-0363-2024-4-31-37

A model of chronic thromboembolic pulmonary hypertension with the use of microencapsulated fibrin particles

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ABSTRACT

Aim. To develop a model of chronic thromboembolic pulmonary hypertension (CTEPH) in rats by embolization of the pulmonary vascular bed with microencapsulated fibrin (MF).

Materials and methods. Microencapsulated fibrin (MF) was prepared by encapsulating fibrin particles smaller than 71 μm in sodium alginate. Non-encapsulated fibrin with a particle size of 71–200 μm was used as an alternative embolic particle. Modeling was performed on male Wistar rats. The animals were divided into 4 groups. Intact (INT) animals ($n = 7$) were administered normal saline intravenously. In the NF8 group ($n = 14$), non-encapsulated fibrin was injected as embolic particles 8 times every 4 days. In the MF5 group ($n = 14$), 0.5 ml MF (9,047 ± 430 particles) was administered intravenously 5 times every 5 days. In the MF8 group ($n = 14$), MF was administered 8 times every 4 days. Six weeks after the last injection of embolic particles, cardiac catheterization with manometry and histologic examination of the lungs were performed.

Results. According to cardiac catheterization, right ventricular systolic pressure (RVSP) in the MF8 group was significantly higher compared to rats from the INT and NF8 groups ($p < 0.05$). The hypertrophy index and the percentage of collagen fibers in the structure of the vascular wall of the pulmonary artery branches were significantly higher in the MF5 and MF8 groups than in the INT and NF8 groups ($p < 0.01$). There were no significant differences between the MF5 and MF8 groups.

Conclusion. A representative CTEPH model in rats was developed, characterized by a stable increase in RVSP and pronounced structural changes in the branches of the pulmonary artery.

Keywords: chronic thromboembolic pulmonary hypertension, pulmonary embolism, experimental model, rats, microencapsulated fibrin, sodium alginate

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of financing. The research was funded by the Russian Science Foundation grant No. 23-75-10122, https://rscf.ru/project/23-75-10122/

Conformity with the principles of ethics. The study was approved by the Bioethics Committee at Saint Petersburg State Chemical and Pharmaceutical University (Protocol Rats-02.2019-5 of 12.02.2019).

For citation: Karpov A.A., Shilenko L.A., Vaulina D.D., Sidorova E.E., Akhmetova A.A., Bunenkov N.S., Vorotilov A.V., Ivkin D.Yu., Karpenko V.V., Galagudza M.M. A model of chronic thromboembolic pulmonary hypertension with the use of microencapsulated fibrin particles. *Bulletin of Siberian Medicine.* 2024;23(4):31–37. https://doi.org/10.20538/1682-0363-2024-4-31-37.

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Экспериментальная модель хронической тромбоэмболической легочной гипертензии с применением микроинкапсулированных частиц фибрина

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РЕЗЮМЕ

Цель. Разработать экспериментальную модель хронической тромбоэмболической легочной гипертензии (ХТЭЛГ) у крыс с помощью эмболизации сосудистого русла легких микроинкапсулированным фибрином (МФ).

Материалы и методы. Микроинкапсулированный фибрин изготавливался путем заключения в альгинат натрия частиц фибрина размером меньше 71 мкм. В качестве альтернативных эмболизирующих частиц использовался неинкапсулированный фибрин с размером частиц 71–200 мкм. Экспериментальное моделирование проведено на самцах крыс линии Вистар. Животные были разделены на четыре группы. Контроль (КОН) (n = 7) – внутривенно вводился физиологический раствор. НФ8 (n = 14) – в качестве эмболизирующих частиц вводился неинкапсулированный фибрин 8 раз с интервалами в 4 дня. МФ5 (n = 14) – МФ в объеме 0,5 мл (9 047 ± 430 частиц) вводился внутривенно 5 раз с интервалами в 5 дней. МФ8 (n = 14) – МФ вводился 8 раз с интервалами в 4 дня. Через 6 нед после последнего введения эмболизирующих частиц выполнялись катетеризация сердца с манометрией и гистологическое исследование легких.

Результаты. По данным катетеризации сердца, систолическое давление в правом желудочке (СДПЖ) в группе МФ8 было значимо выше по сравнению с крысами из группы КОН и НФ8 (p < 0,05). Индекс гипертрофии и процент коллагеновых волокон в структуре сосудистой стенки ветвей легочной артерии были значимо выше в группах МФ5 и МФ8, чем в группах КОН и НФ8 (p < 0,01). Значимых различий между группами МФ5 и МФ8 выявлено не было.

Заключение. Разработана репрезентативная модель ХТЭЛГ на крысах, характеризующаяся стабильным повышением СДПЖ и выраженными структурными изменениями ветвей легочной артерии.

Ключевые слова: хроническая тромбоэмболическая легочная гипертензия, тромбоэмболия легочной артерии, экспериментальная модель, крысы, микроинкапсулированный фибрин, альгинат натрия

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Исследование выполнено за счет гранта Российского научного фонда № 23-75-10122, https://rscf.ru/project/23-75-10122/

Соответствие принципам этики. Исследование одобрено биоэтической комиссией СПХФУ (протокол Rats-02.2019-5 от 12.02.2019).

Для цитирования: Карпов А.А., Шиленко Л.А., Ваулина Д.Д., Сидорова Е.Е., Ахметова А.А., Буненков Н.С., Воротилов А.В., Ивкин Д.Ю., Карпенко В.В., Галагудза М.М. Экспериментальная модель хронической тромбоэмболической легочной гипертензии с применением микроинкапсулированных частиц фибрина. *Бюллетень сибирской медицины.* 2024;23(4):31–37. https://doi.org/10.20538/1682-0363-2024-4-31-37.

INTRODUCTION

Chronic thromboembolic pulmonary hypertension (CTEPH) is a complication of pulmonary embolism (PE) [1]. This form of pulmonary hypertension is characterized by impaired thromboembolic lysis, microvascular damage, and, as a consequence, a stable increase in pulmonary artery pressure and pulmonary vascular resistance [2].

The above changes lead to right ventricular (RV) hypertrophy, which ultimately leads to RV dilatation and failure. Ten-year survival of patients with CTEPH

who do not receive specific therapy with an average pulmonary artery pressure of more than 50 mm Hg is 5%, while in patients who underwent PE without a significant increase in pulmonary artery pressure, the survival rate exceeds 50% [3]. Despite the active development of surgical and medical approaches to the treatment of CTEPH, the effect of the therapy remains insufficient [1]. At the same time, preclinical trials on new therapeutic approaches are challenging due to the lack of an experimental model that can sufficiently reflect the pathophysiological and structural changes in the vascular bed of the lungs in CTEPH.

Currently, there are two main approaches to modeling CTEPH: the use of natural thromboemboli and artificial embolic particles [4]. The use of natural thromboemboli is usually combined with the use of fibrinolysis inhibitors, such as tranexamic acid [5–8]. However, this modeling approach is labor intensive due to the need for *in vitro* production of autologous thromboemboli for each animal [9]. In addition, even with the use of tranexamic acid, an increase in the pulmonary artery pressure is unstable due to the pronounced fibrinolytic activity of blood plasma in rats [7]. On the other hand, the use of artificial particles, primarily polystyrene microspheres, in previously published works was characterized by a stable increase in the pulmonary artery pressure [10–13], which did not lead to complete reproduction of CTEPH pathogenesis, since polystyrene microspheres and similar particles were uncapable of degrading and did not contain biologically active thrombus molecules, such as fibrin degradation products (FDP) and fibrin itself.

In our previous study, we modeled CTEPH using partially biodegradable microspheres based on sodium alginate, which did not contain additional inclusions [14]. The study demonstrated a persistent increase in the pulmonary artery pressure, a decrease in exercise tolerance, and the appearance of histologic changes in the vascular bed characteristics of CTEPH. However, this model did not take into account the important role of FDPs, which have significant biological functions, including anticoagulation and proinflammatory ones [15]. Taking these data into account, it is advisable to develop a model that combines the advantages of natural thromboemboli, such as the biological effects of fibrin itself and the release of FDP, and artificial particles that have the required size and a given rate of biodegradation.

The aim of this study was to develop an experimental model of CTEPH in rats using embolization of the pulmonary vascular bed with microencapsulated fibrin.

MATERIALS AND METHODS

We used 62 male Wistar rats in this study. The average weight was 230 ± 27 g. All animals were kept in standardized conditions and had free access to complete granulated pet food and water in agreement with the requirements according to the GOST (Russian National Standard) 33216-2014.

Production of embolic particles. At the first stage, fibrin powder from human blood plasma (Sigma-Aldrich, USA) was mechanically degraded, sifted through a sieve with a mesh size of 71 μm, and mixed with a solution of ultrapure sodium alginate (Sigma-Aldrich, USA) in a ratio of 1:7. The resulting suspension was homogenized using a submersible laboratory ultrasonic disperser with a stand (SpetsmashSonic, Russia). To obtain microencapsulated fibrin (MF), the suspension was supplied to the input of the Encapsulator B-390 system (BUCHI, Switzerland), and a 2% barium chloride solution was used as a stabilizing agent. Fibrin powder was mechanically ground, and then 71–200 μm particle fractions were separated using laboratory sieves to obtain non-encapsulated fibrin. All procedures were carried out under sterile conditions.

To model CTEPH, all animals were randomly divided into 4 groups. Intact (INT) animals (n = 8) were injected 1.5 ml of normal saline in the caudal vein 8 times every 4 days. In the NF8 group (n = 14), non-encapsulated fibrin was injected as embolic particles in a volume equivalent to that in the MF5 and MF8 groups, suspended in 1.5 ml of normal saline, 8 times every 4 days. In the MF5 group (n = 14), 0.5 ml MF (9,047 ± 430 particles) was administered in the caudal vein 5 times every 5 days. Before the injection, barium chloride solution was completely removed, and MF was suspended in 1.5 ml of normal saline. In the MF8 group (n = 14), the same volume of MF was injected in the caudal vein 8 times every 4 days. Six weeks after the last injection of embolic particles, right ventricular systolic pressure (RVSP) was measured, and a histologic examination of the lungs was performed to determine the percentage of collagen fibers in the structure of the vascular wall of the branches of the pulmonary artery and the hypertrophy index.

Study of in vivo biodegradation of embolic particles. In a separate experimental series, CTEPH was modeled in rats using the NF8 (n = 6) and MF8 (n = 6) protocols to determine the rate of embolic particle biodegradation at different time intervals.

To assess the dynamics of biodegradation of embolic particles on day 1, 2, 4, and 6 weeks after the final administration, the animals were euthanized using an isoflurane overdose. A histologic examination of the lower lobe of the right lung was performed. At each time point, embolic particles were counted in the lumen of the pulmonary artery branches along the entire cross-section of the distal third of the lung lobe using the Eclipse Ni-U light microscope (Nikon, Japan) and Nis Elements Br4 software (Nikon, Japan).

Invasive hemodynamic monitoring. The rats were anesthetized using isoflurane inhalation via the SomnoSuite Low-Flow Anesthesia System (Kent Scientific, Torrington, CT, USA). The animals were placed on a heating pad combined with the TCAT-2LV Animal Temperature Controller (Physitemp Instruments Inc., USA). Mechanical ventilation was performed using the SAR-830/AP device (CWE Inc., USA). A puncture of the heart apex was performed to measure RVSP. Pressure was recorded using the Mindray ePM 10 monitor (Mindray, China).

Histologic examination. The animals were euthanized using an isoflurane overdose. For the histologic examination, the lower lobe of the right lung was divided into 4 equal transverse levels. Micropreparations were stained according to the Picro Mallory staining method (BioVitrum, Russia) to identify collagen fibers. Quantitative analysis was carried out in two distal sections of the lung using the Eclipse Ni-U microscope (Nikon, Japan) at ×10 to ×40, as well as Nis Elements Br4 (Nikon, Japan) and ImageJ (Wayne Rasband, USA) software. For all found vessels belonging to the branches of the pulmonary artery, the following parameters were determined: the percentage of collagen fibers in the structure of the vascular wall [16], as well as the hypertrophy index, calculated as the ratio of the area of the vascular wall to the area of the entire vessel expressed as a percentage [17].

Data analysis was performed using the R 4.2.2 software. The Newman – Keuls test was used to assess the statistically significant difference between the groups. Results were presented as the median and the interquartile range Me [Q_1; Q_3]. The differences were considered significant at $p < 0.05$.

RESULTS

Characteristics of embolic particles. The size of MF particles was 205 ± 38 μm (Fig. 1, *a*), particles of non-encapsulated fibrin were 155 ± 60 μm in diameter after suspension in normal saline and ultrasonic treatment.

The study of embolic particle biodegradation revealed a consistent decrease in the number of detected MF particles. By the time CTEPH simulation was completed, the number of these particles was 6% of the baseline. When assessing the biodegradation of non-encapsulated fibrin after 2 weeks, only 2% of particles were detected, and no embolic particles were detected further on (Fig. 1, *b*).

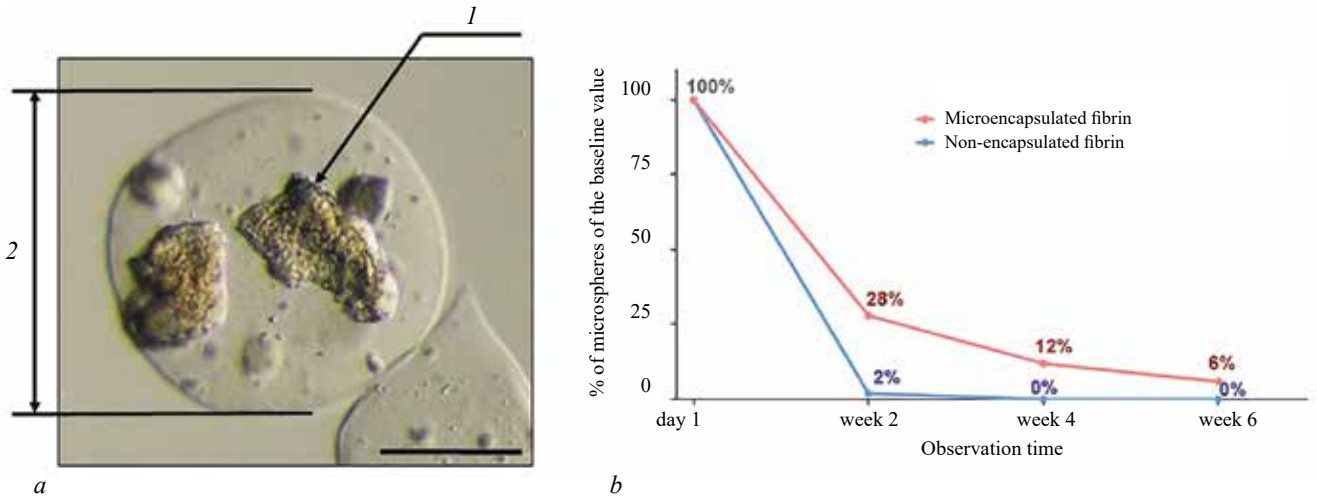

Fig. 1. Embolic particles: *a* – representative fibrin micrograph (bar = 100 μm), *1* – fibrin particles, *2* – microcapsule with fibrin; *b* – embolic particle biodegradation in the vascular bed

During embolic particle administration, mortality in the main series of experiments was 4 animals in the NF8 group, and 2 and 4 animals in the MF5 and MF8 groups, respectively. The cause of death was the development of acute right heart failure or paradoxical embolism with stroke.

According to cardiac catheterization data, RVSP in the MF8 group was significantly higher than in the INT and NF8 groups ($p < 0.05$). There were no significant differences in the RVSP levels between the INT, NF8, and MF5 groups (Fig. 2).

According to the results of the histologic examination, the hypertrophy index and the percentage of collagen fibers in the vascular wall structure in the MF5 and MF8 groups were significantly higher than in the INT and NF8 groups ($p < 0.01$). There were no significant differences between the MF5 and MF8 groups (Fig.3).

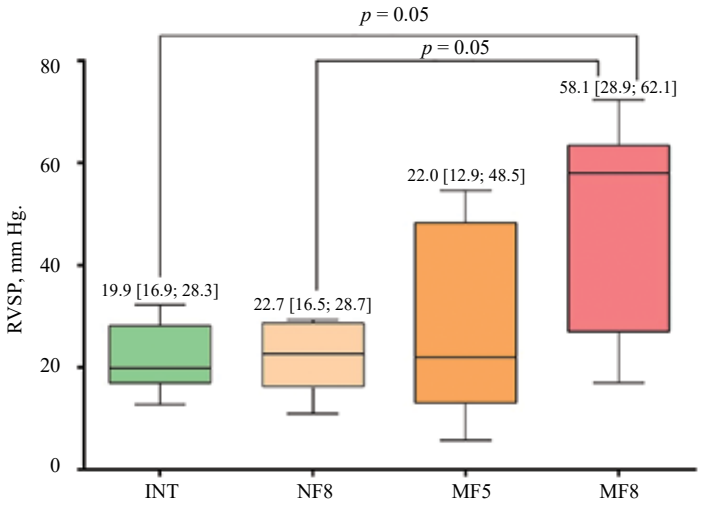

Fig. 2. Right ventricular systolic pressure 6 weeks after the last embolic particle administration according to cardiac catheterization data

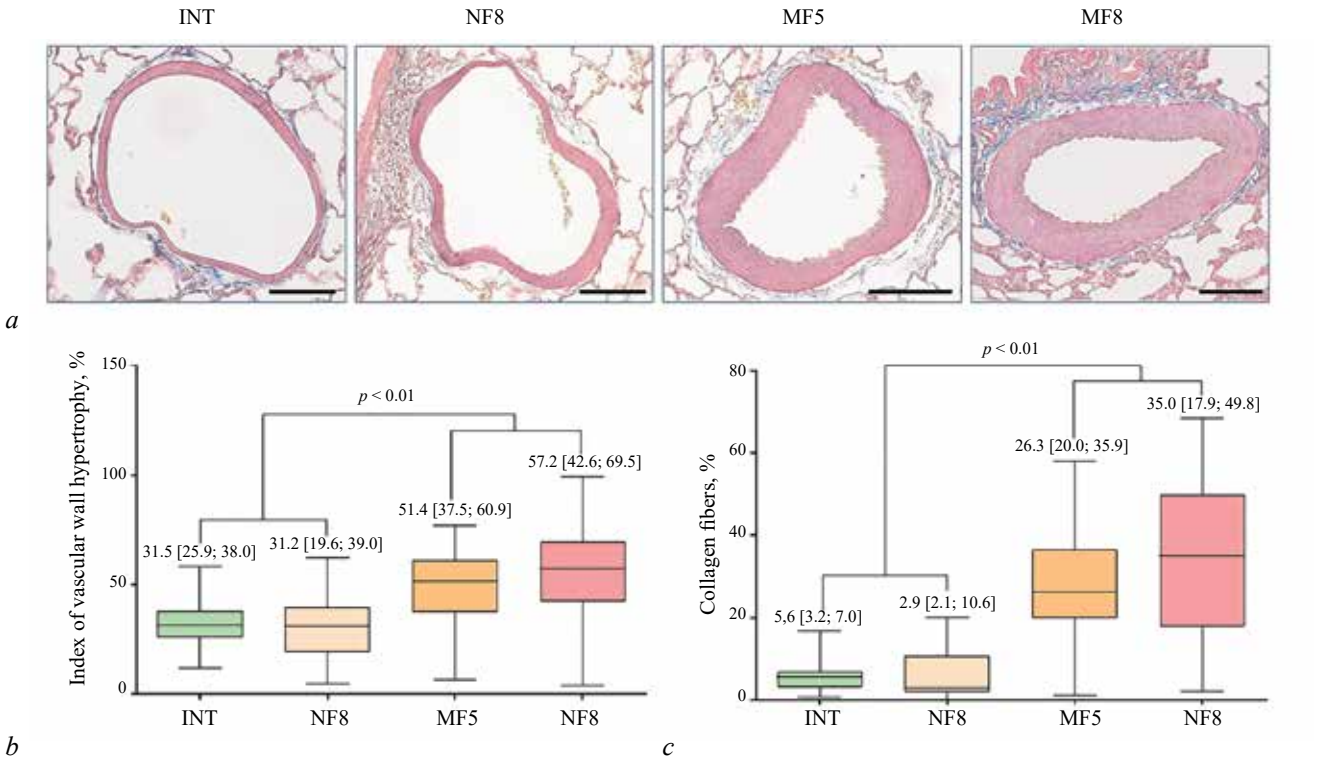

Fig. 3. Results of the histologic examination of pulmonary artery branches: *a* – representative micrograph (bar = 100 μm); *b* – index of vascular wall hypertrophy; *c* – collagen fibers in vascular wall structure, %

DISCUSSION

As a result of the study, a new experimental CTEPH model was developed, characterized by a stable increase in RV pressure and significant remodeling of the pulmonary circulation vessels. This was achieved by repeated intravenous administration of MF, which, as shown in [18, 19], is capable of stimulating the migration of leukocytes directly, being a chemoattractant, and through enhancing the secretion of cytokines by leukocytes and endothelial cells. The use of this type of embolic particles made it possible to combine the advantages of both natural thromboemboli – partial biodegradation and release of biologically active substances (fibrin and FDP), and artificial ones – a controlled rate of particle biodegradation and convenient dosing. In addition, in contrast to autologous thrombi, the use of fibrin has significantly reduced efforts to produce thromboemboli.

When modeling CTEPH in this study, the MF8 group was characterized by a persistent increase in RVSP. In the MF5 group, the dose of embolic particles administered was insufficient to reproduce stable pulmonary hypertension. According to epy histologic examination, a significant increase in the index of vascular wall hypertrophy and the percentage of fibrosis in its structure was noted in both groups where MF was used and did not differ significantly between them. The use of non-encapsulated fibrin did not lead to significant changes in either the RVSP level or the remodeling of the pulmonary artery branches.

Similar results were achieved in our previous study, where microencapsulated autologous thrombi were used as embolic particles [9]. However, this model was characterized by significant efforts, which made its practical application difficult. In addition, in contrast to previously published articles on artificial particles based on polystyrene [10–13], the developed embolic particles were capable of partial and controlled biodegradation and release of biologically active substances. These properties contributed to greater pathophysiological accuracy of the presented model.

CONCLUSION

The developed model could be used both to study the CTEPH pathogenesis and to test new therapeutic approaches to the treatment of this disease. In the future, it is planned to conduct a comparative study of empty alginate microspheres with microencapsulated fibrin to most clearly demonstrate the role of biologically active substances released during the biodegradation of thromboemboli during the CTEPH development.

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Authors' contribution

Karpov A.A. – conception and design, analysis and interpretation of the data, critical revision of the manuscript for important intellectual content, drafting of the manuscript. Shilenko L.A. – conception and design, production of embolic particles, invasive hemodynamic monitoring, drafting of the manuscript. Vaulina D.D. – production of embolic particles, analysis and interpretation of the data, drafting of the manuscript. Sidorova E.E., Akhmetova A.A., Karpenko V.V. – intravenous administration of embolic particles, histologic examination, analysis and interpretation of the data. Bunenkov N.S. – analysis and interpretation of the data, drafting of the manuscript. Vorotilov A.V. – production of embolic particles, invasive hemodynamic monitoring. Ivkin D.Yu. – critical revision of the manuscript for important intellectual content, drafting of the manuscript. Galagudza M.M. – project leader; conception and design, critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication.

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Received 11.05.2024;
approved after peer review 01.08.2024;
accepted 12.09.2024