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RESTORATION OF THE MORPHOFUNCTIONAL STATE OF RATS LUNGS WITH EXPERIMENTAL FIBROSIS THROUGH TRANSPLANTED STEM CELLS

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Aim. To investigate the therapeutic effect of allogeneic mesenchymal stem cells (MSC) derived from bone marrow on the restoration experimentally damaged lung tissue in rats with induced pulmonary fibrosis. **Methods**. Female Wistar rats were utilized in the study. Pulmonary fibrosis was induced experimentally in the animals by administering bleomycin hydrochloride via transthoracic injection during a 45-day preparatory period. At the end of this period, all animals exhibited clinically manifested symptoms of pulmonary fibrosis. To assess the changes in the organism due to pulmonary fibrosis, blood samples, bronchoalveolar lavage samples, and lung tissue were randomly collected from five affected animals. The remaining animals were divided into four experimental groups, each consisting of five animals. In the first experimental group, allogeneic MSC were administered intrathoracically. In the second experimental group, allogeneic MSC were administered intravenously. The third experimental group received conventional treatment using medication, while the fourth experimental group (control) received placebo intrathoracically, without any active agents. Additionally, a separate fifth experimental group comprised intact (healthy) animals. The stem cells used in the experiments were obtained from the bone marrow samples of young, clinically healthy donor rats' tibia, humerus, or femur (Mazurkevych et al, 2014). Throughout the 45-day experiment, the animals in all experimental groups were monitored using clinical examination indices. At the end of the testing period, the animals were euthanized, and blood, bronchoalveolar lavage, and lung tissue samples were collected for laboratory analyses. The analysis of bronchoalveolar lavage involved microscopic examination of the collected fluid to determine cell composition, while hematological analysis encompassed the quantification of erythrocyte and leukocyte counts. Histological examination of lung tissue samples involved microscopic analysis of the lung tissue histostructure. **Results.** Following the implementation of the proposed treatment methods, it was observed on day 45 of the experiment that transplanted allogeneic MSC facilitated an increased activity in the restoration of pathologically altered lung parenchyma. The effectiveness of this process varied depending on the method of MSC application. Notably, animals in the first experimental group exhibited the absence of symptoms such as coughing and hypoxia by day 45. Furthermore, a significant decrease was observed in the indices of erythrocyte count to 7.18 ± 0.05 t/l ($p < 0.001$) and total leukocyte count to 10.6 ± 0.92 g/l ($p < 0.05$). Histological analysis revealed a complete restoration of lung tissue structure, reaching 90 % when compared to the control group. In the second experimental group, a significant decrease was noted in the total leukocyte count to 11.32 ± 0.48 g/l ($p < 0.01$) and erythrocyte count to 6.87 ± 0.18 t/l ($p < 0.001$). Histologically, the regenerative processes exhibited lower activity, reaching 70–80 % compared to the animals in the first group. Fibrous areas were observed but of smaller size than those in the conventional treatment group. In the third experimental group, despite the normalization of laboratory testing indices, namely a decrease in erythrocyte count to 7.62 ± 0.11 t/l ($p < 0.001$) and leukocyte count to 9.46 ± 0.54 g/l ($p < 0.001$), occasional fibrosis areas and thicker alveolar walls were still present in the histological structure of the lungs. The fibrosis areas decreased by only 20–30 %. **Conclusions.** Our findings demonstrate that the application of mesenchymal stem cells is a more effective approach to cell therapy for the restoration of pathologically altered lung tissue in rats with experimentally induced pulmonary fibrosis compared to conventional treatment. Traditional medication-based treatment over the 45-day experiment led to the normalization of clinical indices and laboratory tests but did not result in complete restoration of the damaged lung tis-

sue structure. These results underscore the advantages of employing allogeneic mesenchymal stem cells in the therapy of pulmonary fibrosis, indicating their potential for further investigation and clinical application.

Key words: rats, lungs, fibrosis, regenerative therapy, stem cells, erythrocytes, leukocytes, bronchoalveolar lavage.

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INTRODUCTION

Pulmonary fibrosis belongs to the group of chronic fibrous diseases, characterized by a sharp decrease in lung function and poor prognosis (Raghu et al, 2011). It develops as a progressive pathological process in the lung tissue, manifested as interstitial pneumonia with the corresponding pathohistological picture. Thoracic societies and associations in America, Europe, and Japan recommend diagnostics based on the foundations of the multidisciplinary evaluation of clinical signs, X-ray, and histological examinations (Raghu et al, 2022).

The scientists state that the triggering mechanism for this disease is constant and long-term damage to the epithelial cells in the lungs which, in its turn, induces the impairment of regeneration processes. Under these conditions, fibroblasts get activated and transit to the interstitial space, where they get differentiated into phenotypes of myofibroblasts, resistant to apoptosis processes, which start laying the extracellular matrix (Yount et al, 2016).

According to the more current data, it is possible to consider several pathogenic factors which impact the development of pulmonary fibrosis. Among these, the main factor is the imbalance of oxidative stress (Fois et al, 2018), which occurs due to excessive production of free radicals and insufficient activity of antioxidant systems and inflammation processes (Desai et al, 2018). After the damage to the lung tissue, the inflammatory process develops, during which different inflammatory cytokines (TNF-α, IL-1β, and IL-17) get activated and accumulated in its epicenter, which promotes the proliferation and transformation of alveolar fibroblasts and induces the laying of the extracellular matrix (Bolourani et al, 2021).

Recent experimental and pre-clinical studies of the restorative ability of transplanted mesenchymal stem cells (MSC) demonstrate astonishing results of their high efficiency in restoring the cellular composition of damaged/pathologically changed tissues of different organs.

MSCs are known as a population of fibroblast-like multipotent stromal cells. They have the potential for cloning, self-restoration, and trans-differentiation *in vitro* and manifest different actions, including homing, epithelial restoration, bactericide activity, immunosimulation and secretion of growth factors, anti-inflammatory factors (Srour et al, 2015).

The most studied source of MSC is bone marrow which serves as a great place to obtain different lines of stem cells, including the ones with the ability to protect against pulmonary fibrosis.

It was experimentally proven that after the release from the bone marrow into the bloodstream, MSCs passed from the latter into the areas of experimentally damaged tissues rather easily, promoted the tissue restoration by secreting different paracrine factors and getting directly differentiated into the specialized cells of that particular place (Deng et al, 2011.) They released a wide spectrum of biologically active substances, which may regulate the local immune response for the creation of a regenerative microenvironment and inhibit inflammation, facilitating the restoration processes (Li et al, 2017).

Therefore, in modern regenerative cell therapy, transplanted stem cells are considered to be the most effective, ecological, and safe biopharmacological means to be used in human and veterinary medicine, due to their highly beneficial impact on the restoration of the structure of damaged tissues, filling this defect with full-fledged specialized cells. According to the estimates of many researchers and clinicians, in the nearest future, this will be the promising means in the treatment of chronic and acute lung diseases via decreasing the activity of pulmonary fibrosis and improving lung function due to their anti-inflammatory, antioxidant, regenerative and immunomodulating properties (Stavely and Nurgali, 2020; Xia et al, 2021).

The working idea of our experiment was to consider the main purpose of stem cells in the activity of the integral organism – to maintain the stability of the cell composition in the organism tissues (cell homeostasis) throughout its entire life.

There are no cells that live as long as the entire animal (human) organism does. The life cycle of cells of

each type (and there are more than 350 types) is different: epidermis cells live five days, erythrocytes – 120 days. A cell dies due to apoptosis (programmed death). Its residues are consumed by ubiquitous phagocytes, and its place is taken by a young specialized cell, which originated in a stem cell. Prior to becoming this specialized cell, under the effect of the local environment, a stem cell goes through several stages of division and differentiation into the corresponding mature specialized cell with the genotype of the same tissue in which it lingered (Wong et al, 2021).

It is known that the duration of restorative processes depends on the method of applying MSC and on the type of damaged tissue. For instance, regeneration is completed sooner in skin, muscles, eyes, myocardium, cartilage, and bone tissues, and longer – in the liver, kidneys, thyroid gland, and pancreas (Mazurkevych et al, 2017).

The experiments involving the transplantation of stem cells for rats with the purpose of treating pulmonary fibrosis demonstrated a considerable improvement regarding the disease symptoms via implantation to the damaged tissue and promotion of its regeneration, thus restoring the gas exchange ability of the lungs (Mitra et al, 2023).

It was determined that the therapy which involved the application of bone marrow stem cells reduced the inflammatory process and regulated the remodeling of fibrous changes in the lung tissue. For instance, stem cells administered into the blood flow immediately after the simulation of pulmonary fibrosis (after the administration of the bleomycin solution) reduced fibrous phenomena in the lung tissue even right after the administration (Ortiz et al, 2003; Zhao et al, 2008).

The antifibrous effects of stem cells were also confirmed by other researchers (Choi et al, 2014; Glassberg et al, 2017). It was proven that mesenchymal stem cells inhibit inflammatory reactions by the secretion of anti-inflammatory mediators (Prockop and Oh, 2012). It was also reported that up to 80 % of therapeutic effects of stem cells, obtained from the adult organism tissues, took place using the secretions, obtained from the stem cells, which is a promising method in the treatment of chronic diseases of the lung tissue (Hu et al, 2023).

The period of wide application of stem cells has not come yet. At present, the application of stem cells in regenerative cell therapy is used for trials, for the accumulation of objective results for further scientifically substantiated use of cells and their products in regenerative therapy.

The aim of the study was to determine the impact of transplanted mesenchymal stem cells on the activity of regenerative processes in the lung tissue of laboratory rats with experimentally induced pulmonary fibrosis.

MATERIALS AND METHODS

The experimental study was conducted at the educational and scientific laboratory, the Center for Cellular Technologies in Veterinary Medicine, at the Department of Surgery and Pathophysiology named after Acad. I. O. Povazhenko, NULES of Ukraine. Female rats of the Wistar line, 4 months old, an average bodyweight of 277.0 ± 4.6 g, were used in the experiments.

The experiment animals were kept and used in the experiments under the requirements of the effective Law of Ukraine "On Protection of Animals from Cruel Treatment" No. 3447-IV dated 21.02.2006 with recent amendments dated 08.04.2017 and other regulations, and the Directive of the European Union 2010/63/EU.

The permission for conducting the experiments according to the topic of the dissertation, which involved the use of animals, was received from the local bioethics commission of the NULES of Ukraine, minutes No. 80-1 dated 27.10.2020.

Thirty rats of the Wistar line were used in the study. During the preparatory period of 45 days, pulmonary fibrosis was experimentally induced in 25 rats using a single instillation of 0.3 ml of bleomycin hydrochloride solution (Bleomycin, Nippon Kayak Co., Ltd., Takasaki Plant, Japan) into the lungs with the estimation of 1.0 mg/100 g of bodyweight of the animal in 0.3 ml sterile physiological solution of sodium chloride 0.9 % of room temperature. The solution was administered once into the thoracic cavity (Boiko et al, 2013).

In the initial state (day 45 of pulmonary fibrosis simulation), five animals were selected from the total number of sick animals and removed from the experiment. The samples of blood, bronchoalveolar lavage, and the pieces of lung tissue were taken for laboratory testing to determine the nature of changes induced by pulmonary fibrosis. Other 20 animals with expressed symptoms of pulmonary fibrosis were divided into four experimental groups, five animals in each. There was a separate fifth experimental group – five intact animals.

The animals of the first experimental group had a single transthoracic administration of the investigated preparation (mesenchymal stem cells) in the dose of 2 mln/animal into the right side.

The animals of the second experimental group had an intravenous administration of the investigated preparation (mesenchymal stem cells) in the same dose. The animals of the third experimental group were prescribed the traditional method of treatment – the solution of dexamethasone (4 mg/ml, KRKA, Slovenia) in the dose of 0.08 mg/kg of the bodyweight, intramuscular administration for three weeks with the interval of two days and gradual decrease in the dose, the solution of hyaluronidase, 64 units (Lidasa-Biofarma, PP BIOFARMA, Ukraine) in the dose of 0.85 units/kg, intramuscular administration for three weeks with the interval of two days (Boiko et al, 2013).

The control group animals were subjected to the transthoracic administration of 0.3 ml phosphate buffer solution (Sigma, USA) into the thoracic cavity, into the right side.

The fifth experimental group – intact (healthy) animals.

The allogeneic mesenchymal stem cells to be used in this study were obtained from the bone marrow of the tibia, humerus, or femur of clinically healthy four-day-old rats-donors (10 animals), after washing using the methods, developed by the Department specialists (Mazurkevych et al, 2014). The cells were cultivated in the disposable Petri dishes, containing the following media: DMEM – Dulbecco's Modified Eagle Medium (Sigma, USA) – 80 %; FBS – Fetal Bovine Serum (Sigma, USA) – 20 % and 10 mcl/cc − antibiotic-antimycotic (Sigma, USA). The cultivation was performed in CO_2 incubator at 37 °C and 5 % concentration of CO_2 until the 90–100 % formation of the monolayer. The attached cells were removed using the 0.25 % solution of trypsin/ethylenediaminetetraacetic acid (EDTA) (Sigma, USA) (Mazurkevych et al, 2014). During the cell cultivation, the requirements of ensuring optimal conditions of temperature, humidity, pH, and nutrients in the media were met. The passaging and reproduction of cells with the purpose of preserving their stability and viability were conducted according to the relevant protocol (Kovpak et al, 2020).

Taking into consideration the specificities of changes in the organism of animals under pulmonary fibrosis which did not allow for setting an accurate diagnosis by the indices of clinical observations and laboratory analyses, we used the method of histological examination of structural changes in the lung tissue to evaluate the nature of the disease.

The objects of the study were the samples of blood and lung tissue, randomly taken in the initial state from five animals with experimentally induced fibrosis, and the ones, taken on days 7, 14, 30, and 45 of the experiment from five animals in each group, after the suggested treatment methods. This article presents the study results, obtained in the initial state and on day 45 after the application of the experimental and conventional methods of treatment.

Then the animals were removed from the experiment by euthanasia via the intracranial administration (Shoyaib et al, 2020) of the lethal dose of thiopental sodium (Tiopenat, Brovafarma, Ukraine) in the dose of 40 mg/kg of the bodyweight (Plumb, 2008).

The obtained blood samples were transferred into special tubes with K3 EDTA (AQUISEL, Italy) to prevent the formation of the blood clot and further used for the morphological study.

The pieces of lungs for histological studies were taken at the end of the experiment (on day 45 of the study). The obtained pieces were fixed in the 10 % neutral aqueous solution of formalin, dehydrated in ethanols of increasing concentration, and poured into paraffin through chloroform. The cuts of 8–10 mcm were obtained by the sledge microtome and dyed with Carazzi's hematoxylin and eosin (Horalskyi, 2011).

The statistical processing of the obtained results was conducted in Statistica 8.0 (StatSoft Inc., USA, 2012). The experimental data are presented as M ± m (M – mean arithmetic value; ± – mean error of average value). The differences between the parameters of the control and experimental groups were determined by the dispersion analysis (ANOVA), where the differences were deemed reliable at $P < 0.05$.

RESULTS OF INVESTIGATIONS

In the initial state (prior to the prescription of the treatment), pulmonary fibrosis with its characteristic symptoms was confirmed in the animals of all four experimental groups (Tanabe et al, 2020): shortness of breath, hypoxia, tachycardia. The corresponding changes in hematologic indices are presented in **Table 1.**

The presented results demonstrated that the groups with experimentally induced pulmonary fibrosis had a reliably increased number of erythrocytes up to 8.61 ± 0.20 t/l ($P < 0.01$) and the total number of leukocytes of 12.70 ± 0.15 g/l ($P < 0.01$). Obviously, the development of erythrocytosis in the experimental animals was a result of breathing hypoxia, which developed under the pathological process in the lung tissue; moderate leukocytosis demonstrated the pathological process in the lungs (Surtaieva and Mazurkevych, 2022).

Table 1. Hematologic indices of rats in the initial period, (M ± m, n = 5)

Indices	Intact animals	Animals with experimentally induced pulmonary fibrosis
Number of erythrocytes, t/l	7.84 ± 0.08	8.61 ± 0.20 **
Total number of leukocytes, g/l	10.0 ± 0.75	12.7 ± 0.15 **

Note: * – p<0.05; ** – p<0.01; *** – p<0.001; reliable data as compared to the same values for the intact animals.

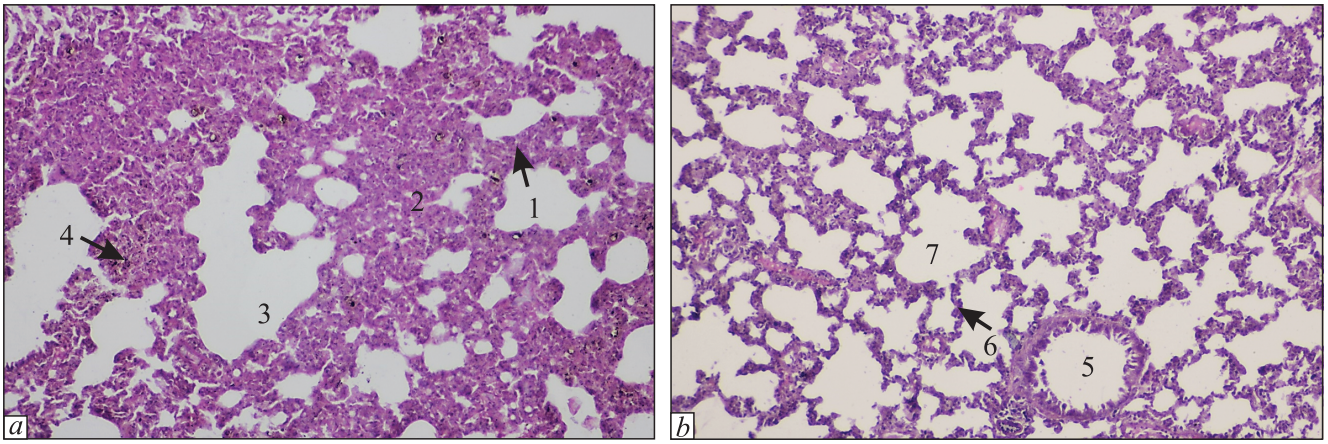

Fig. 1. The histostructure of the lung of the rat with experimentally induced fibrosis (*a*); of the intact animal (*b*): 1 – thickened alveolar wall; 2 – fibrous area; 3 – large cavity of an irregular form; 4 – hemosiderin; 5 – bronchus; 6 – a wall of alveolus; 7 – an alveolar lumen. Carazzi's hematoxylin and eosin, ×50

Acute lung inflammation, induced by the injection of bleomycin, is known to gradually pass into the chronic form. With the increasing level of breathing hypoxemia, there is a higher activity of erythropoiesis which is seen in a gradual increase in the number of erythrocytes in the blood even regardless of higher activity of the processes of erythrocyte destruction. In its turn, higher activity of red bone marrow functioning causes the occurrence of erythrocytes with smaller and larger average volume of erythrocytes in blood (anisocytosis) when erythrocytes of different sizes are observed in the field of vision of the blood smear under the microscope (Surtaieva and Mazurkevych, 2022).

The animals with experimentally induced pulmonary fibrosis had leukocytosis which demonstrated the inflammatory process in the chronic form.

Histological studies revealed considerable structural changes in the lung tissue **(Fig. 1).**

As seen in Figure 1, in the histostructure of lung parenchyma under experimentally induced pulmonary fibrosis (*a*) there was a considerable thickening of alveolar walls (1) and considerable areas of fibrotically changed lung tissue (2) as compared to the intact animals (*b*). Large cavities were formed in some places, which corresponded to several alveoli, located nearby. In the fibrosis areas, there was also either complete closure or absence of blood vessels and some accumulations of hemosiderin (4) which, in our opinion, occurred due to the destruction of erythrocytes remaining in the lung tissue. On day 45 of the study, the intact rats had a typical microscopic structure for this species of animals. Each part consisted of bronchs of different caliber (5) and alveoli (6, 7). In the plane of the histologic preparation, alveoli had different sizes and forms, as the three-dimensional form of these bags was irregular and different parts from each alveolar bag got into the plane of the cut.

On day 45 after the prescription of the corresponding treatment, the animals of the first, second, and third experimental groups had positive changes in the indices of their clinical and laboratory testing: the animals did not have shortness of breath and hypoxia symptoms anymore, there was an improvement in hematologic indices, and the results of the analysis of bronchoalveolar lavage. At the same time, the histostructure of the experimentally damaged lung tissue of the animals from each experimental group had its specificities. The results of hematologic examinations are presented in **Table 2**.

Table 2. The dynamics of hematologic indices in the rats on day 45 after the application of different treatment methods, (M ± m, n = 5)

Treatment method	Number of erythrocytes, t/l	Total number of leukocytes, g/l
1. Administration of MSC into the thoracic cavity	7.18 ± 0.05***	10.60 ± 0.92*
2. Intravenous administration of MSC	6.87 ± 0.18***	11.32 ± 0.48**
3. Conventional treatment	7.62 ± 0.11***	9.46 ± 0.54***
4. Control:	9.32 ± 0.26	13.1 ± 0.10
5. Intact animals	8.10 ± 0.11	9.7 ± 0.40

Note: * – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$; reliable data as compared to the same values for the control group animals; MSC – mesenchymal stem cells.

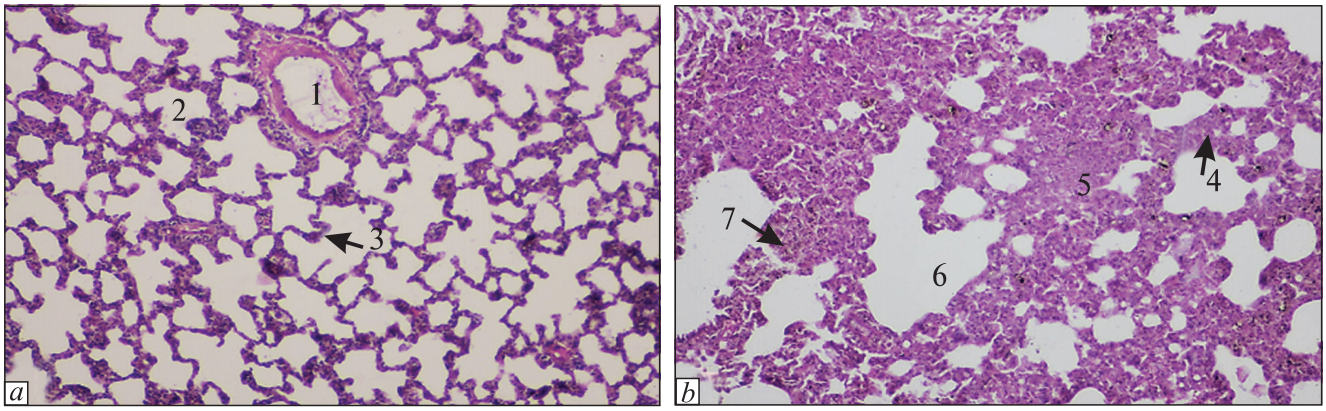

Fig. 2. The histostructure of the lung tissue of the rat on day 45 after the administration of MSC into the thoracic cavity (*a*); control group (*b*): 1 – bronchiole; 2 – a lumen of alveolus; 3 – an alveolar wall; 4 – a thickened alveolar wall; 5 – fibrous area; 6 – a large cavity of an irregular form; 7 – hemosiderin. Carazzi's hematoxylin and eosin, ×50

As seen in Table 2, in animals of the first experimental group, the administration of MSC directly into the thoracic cavity promoted a reliable decrease in the number of erythrocytes down to 7.18 ± 0.05 t/l ($P < 0.001$) and the decrease in the total number of leukocytes down to 10.6 ± 0.92 g/l ($P < 0.05$) as compared to the indices of the control group animals.

In the bronchoalveolar lavage, the number of macrophages reliably increased 1.5 times ($P < 0.001$), and the number of lymphocytes decreased 2.8 times ($P < 0.001$).

The activity of erythropoiesis in the animals of the second experimental group, which were administered MSC directly in blood, decreased even more, which must be the consequence of the direct impact of transplanted MSC on the activity of the bone marrow.

There was a reliable decrease in the total number of leukocytes down to 11.32 ± 0.48 g/l ($P < 0.01$) and the number of erythrocytes – down to 6.87 ± 0.18 t/l ($P < 0.001$) as compared to the control group, which was within the physiological parameters for this species of animals.

In the bronchoalveolar lavage, the number of macrophages reliably increased 1.5 times ($P < 0.001$), and simultaneously the number of lymphocytes decreased 2.7 times ($P < 0.001$).

In the animals of the third experimental group, the changes in indices towards normalization of both erythropoiesis and leukopoiesis may be conditioned by the use of glucocorticoid hormone in the treatment scheme, which is known for its considerable impact on the hemopoiesis activity.

An increase in the number of macrophages ($P < 0.05$) and a decrease in the number of lymphocytes were found in the cytological smears from the bronchoalveolar lavage. There was also a reliable decrease in the number of erythrocytes down to 7.62 ± 0.11 t/l ($P < 0.001$) and in the total number of leukocytes down to 9.46 ± 0.54 g/l ($P < 0.001$) as compared to the control group.

The results of histologic changes in the regenerating lung tissue are presented in **Fig. 2**.

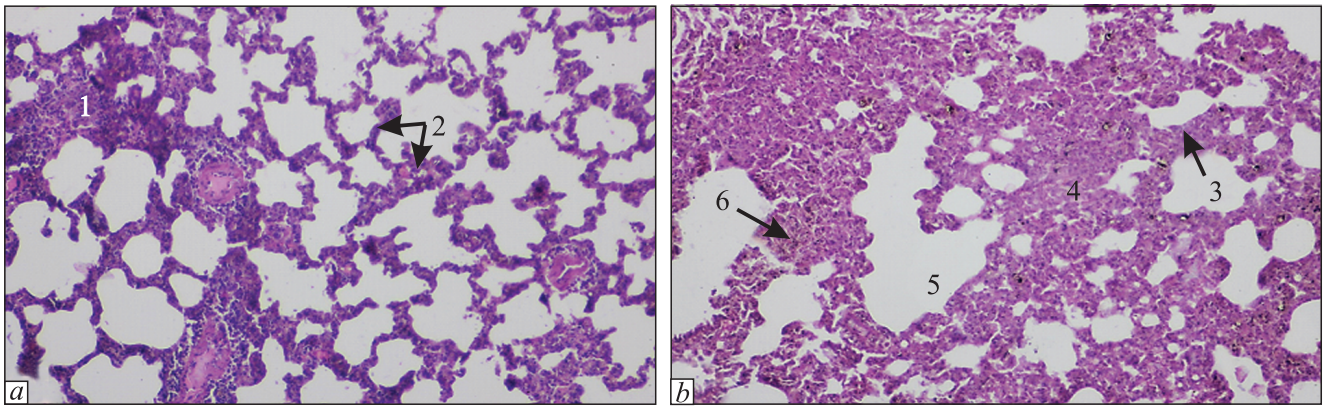

Fig. 3. The histostructure of the lung tissue of the rat on day 45 after the intravenous administration of MSC (*a*); control group (*b*): 1 – residues of the fibrous area; 2 – different thickness of alveolar walls; 3 – a thickened alveolar wall; 4 – fibrous area; 5 – a large cavity of an irregular form; 6 – hemosiderin. Carazzi's hematoxylin and eosin, ×50

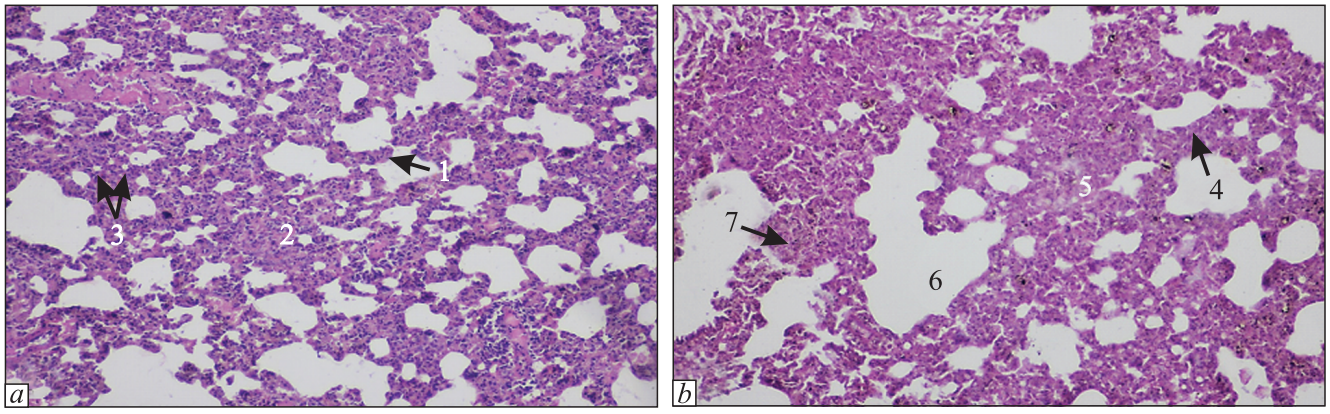

Fig. 4. The histostructure of the lung tissue of the rat on day 45 after the conventional treatment (*a*); control group (*b*): 1 – a thickened wall of the alveolus; 2 – a small fibrous area; 3 – newly formed alveoli; 4 – a thickened alveolar wall; 5 – a fibrous area; 6 – a large cavity of an irregular form; 7 – hemosiderin. Carazzi's hematoxylin and eosin, ×50

As seen in Figure 2, on day 45 after the transplantation of the allogeneic MSC into the thoracic cavity, the lung histostructure of the animals of the first experimental group acquired the microscopic composition, typical for this species of animals, and was within the physiological norm. Each part consisted of bronchi of different caliber (1) and alveoli (2), the thickness of alveolar walls did not differ from the one under normal conditions (3) as compared to the control group (*b*).

Therefore, 45 days after the transplantation of allogeneic MSC by the method of intrathoracic injection, there was a complete restoration of the lung tissue structure (up to 90 %) and these tissues had the histostructure of intact animals (Fig. 1, *b*).

On day 45 after the transplantation of the allogeneic MSC via their intravenous administration, the lungs of the rats of the second experimental group still had rare fibrous areas (1), but they were three times smaller (which was 70–80 % of the restored structure of the lung tissue) than in the animals from the group under the conventional treatment. The alveolar walls were not visibly thickened (2), but different alveoli had walls of different thickness (3) **(Fig. 3).**

On day 45, after the application of conventional methods of treating experimental fibrosis, the fibrous areas (2) were still visible in the lungs of the rats from the third experimental group. The alveoli had different sizes, and their walls were visibly thicker (1). A considerable number of newly formed alveoli were found (3). Under the conventional treatment, the fibrous areas decreased only by 20–30 % **(Fig. 4).**

DISCUSSION

The application of stem cell transplantation by different administration methods for the purpose of treating pulmonary fibrosis in rats, a practically incurable disease, may be used as a promising method of cell

therapy, as it allows for restoring the structure of the damaged areas, and thus the function of the pathologically changed lung tissues almost to the physiological parameters.

The application of stem cells is currently widely used as an experimental method of regenerative medicine, which is confirmed by the literature data regarding various pathologies (Lee et al, 2009; Wang et al, 2013). Therefore, we decided to use stem cells for the experimental pathology of the lung tissue, namely, experimentally induced fibrous lung disease in rats, to study the regenerative possibilities of MSC as an efficient method of treatment. The promising results of pre-clinical trials involving stem cells demonstrate that they can be a potential therapeutic variant for the treatment of chronic lung diseases, including pulmonary fibrosis (Tzouvelekis et al, 2018; Zhao et al, 2021).

While administering stem cells into the thoracic cavity under experimentally induced pulmonary fibrosis in rats, we noted histologic restoration of the lung tissue structure, and it was similar to the structure of intact group animals. After the transthoracic administration of stem cells into the thoracic cavity, the cells penetrate the lung tissue directly, because a large area of the lungs is intensely vascularized and has high tissue permeability, which, in its turn, facilitates fast and targeted penetration of the cells into the pathological process zone, getting accumulated in the tissue in a much larger amount than after the intravenous administration (Agu et al, 2001).

The researchers have also demonstrated that after the intramuscular administration, most stem cells penetrate the lung tissue, which is an efficient method of cell transplantation in the lungs due to passing through the lung tissue (Fischer et al, 2019; Harting et al, 2019). After the intravenous administration of stem cells, we detected rare fibrous areas, which allows for assumptions that some systemically administered stem cells first penetrate the lungs and then can migrate into the liver and spleen, which was confirmed by the scientists (Rustad and Gurtner, 2012; Mezey, 2022).

A relevant criterion in the evaluation of the changes in the animal organism after the application of different methods of restoring the pathologically changed lung tissue after the experimentally induced pulmonary fibrosis was the study of histologic changes in the lungs, which clearly demonstrated the specificities of restoring the lung tissue structure in the animals of each experimental group. Tanabe et al (2020) recommend conducting the histologic examination of the lung tissue as one of the main methods of detecting pulmonary fibrosis.

Our results coincide with the results of other researchers (Hu et al, 2023) and confirm the decrease in the number of fibrous areas in the lung tissue due to the ability of stem cells to migrate to the damaged tissues, to get proliferated and differentiated into target tissues, replacing and regenerating the damaged tissues (Qin et al, 2023)

Thus, the application of the histologic examination method in the differential diagnostics of pulmonary fibrosis in animals should be considered the definitive method which allows for determining the specificities of structural changes in the lung parenchyma on the cellular and subcellular level to single out the pulmonary fibrosis proper out of dozens of other kinds of lung damage, known as interstitial lung diseases (ILDs), which currently cover several dozens of nosological forms (pulmonary fibrosis, interstitial pneumonias, pneumoconiosis, pulmonary hyalinosis, and histiocytosis, etc. (Reinero, 2019)) that differ in their etiology, pathogenetic specificities, histologic picture and have different clinical courses and prognoses (Serrano et al, 2022; Morrow et al, 2022).

During the examination of histologic material of pulmonary fibrosis, a remarkable weakening of alveolar epithelium was detected, which then causes the abnormal activation of fibroblasts and collagen deposition (Adamson et al, 1988; Bagnato and Harari, 2015) that was not observed by us in the groups of animals with the application of mesenchymal stem cells. Our results of histologic examination demonstrated positive outcomes of treatment after the application of mesenchymal stem cells, which was characterized by the decrease of fibrous areas in the lung tissue down to 70–80 % after intravenous administration of stem cells. While transthoracically administering mesenchymal stem cells into the thoracic cavity, we noted histologic restoration of the lung structure (Fig. 2), and it was similar to the structure of intact group animals.

Therefore, the application of stem cells promotes the reliable elimination of fibrous changes in the lung tissue under pulmonary fibrosis, experimentally induced by bleomycin. It is known that under the effect of bleomycin, oxidative stress develops in the lungs, which plays a relevant role in damaging the lung tissue due to its toxic impact and activation of NADP-oxidase (Jun et al, 2011). In its turn, it causes inflammatory reactions and the development of pulmonary fibrosis (Bale et al, 2016).

The application of stem cells may decrease the oxidative stress related to the inflammation, and thus diminish the development of pulmonary fibrosis (Hecker et al, 2009; He et al, 2011; Murthy et al, 2009; Hecker et al, 2012; Osborn-Heaford et al, 2012). These arguments may have decisive consequences for further development of regenerative treatment methods with the application of stem cells as an alternative to the conventional protocols of treatment in order to reduce inflammatory and fibrous processes in the lungs.

CONCLUSIONS

The application of mesenchymal stem cells to eliminate structural changes in the lungs under the experimentally induced fibrosis in laboratory animals (rats) resulted in effective restoration of the morphofunctional state of the pathologically changed lung tissue: on day 45 after the transplantation of allogeneic MSC into the thoracic cavity, the animals of the first experimental group had almost complete (up to 90 %) restoration of the lung tissue structure, and thus the normalization of laboratory testing indices to the physiological parameters for this species.

After the intravenous administration of stem cells, the animals had 70–80 % restored structure of the lung tissue; there were rare fibrous areas of insignificant sizes which was accompanied by the normalization of hematologic indices.

In the animals of the third experimental group, against the background of the normalization of laboratory testing indices, namely, a decrease in the number of erythrocytes down to 7.62 ± 0.11 t/l ($P < 0.001$) and a decrease in the number of leukocytes – to 9.46 ± ± 0.54 g/l ($P < 0.001$), the histological structure of the lungs still had the occasional fibrosis areas and the alveolar walls were much thicker, the fibrosis areas decreased only by 20–30 %.

Further pre-clinical and clinical trials should give answers to the questions about the similarity between the pathogenesis of experimentally induced pulmonary fibrosis and its spontaneous form for the application of transplanted MSC in the treatment of spontaneous forms of pulmonary fibrosis and to find out the difference in the treatment impact of pharmacological preparations and transplanted stem cells.

Adherence to ethical standards. The study was conducted with adherence to the standards of working with animals according to the Procedure of conducting experiments involving animals in scientific institutions (a regulation of the Ministry of Education and Science, Youth, and Sport of Ukraine, Order No. 249, dated March 01, 2012). The permission for conducting the experiments according to the topic of the dissertation, which involved the use of animals was received from the local bioethics commission of the NULES of Ukraine, minutes No. 80-1 dated 27.10.2020.

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Відновлення морфофункціонального стану легень у щурів із експериментальним фіброзом трансплантованими стовбуровими клітинами

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Мета. З'ясувати вплив трансплантованих алогенних мезенхімальних стовбурових клітин (МСК) кісткового мозку на активність відновлення експериментально ушкодженої легеневої тканини у щурів із експериментальним фіброзом (легеневий фіброзу. **Методи**. В дослідженні використовували щурів-самиць породи Wistar. У підготовчий період тривалістю 45 діб у тварин моделювали легеневий фіброз шляхом одноразового трансторакального введення гідрохлориду блеоміцину. В кінці підготовчого періоду у всіх тварин клінічно виявлені виражені симптоми легеневого фіброзу. Рандомізовано у 5 хворих тварин були відібрані зразки крові, бронхоальвеолярного лаважу та легеневої тканини для оцінки змін в організмі за легеневого фіброзу. Інших тварин розділяли на 4 дослідні групи по 5 голів у кожній. У вихідному стані тваринам першої дослідної групи вводили алогенні МСК у грудну порожнину, тваринам другої дослідної групи – алогенні МСК внутрішньовенно; тваринам третьої дослідної групи застосували медикаментозне лікування за традиційним методом, тваринам четвертої дослідної групи (контроль) уводили внутріторакально плацебо без активних засобів. Окремо була п'ята дослідна група – інтактні (здорові) тварини. Стовбурові клітини для використанні в дослідах отримували із зразків кісткового мозку великогомілкової, плечової або стегнової кістки від мо-

лодих клінічно здорових щурів-донорів (Mazurkevych та ін, 2014). Впродовж дослідного періоду, який тривав також 45 діб, контролювали зміни в організмі тварин всіх дослідних груп за показниками клінічного огляду. В кінці дослідного періоду тварин виводили із досліду, відбирали зразки крові, бронхоальвеолярний лаваж та легеневої тканини для лабораторних аналізів. Аналіз бронхоальвеолярного лаважу включав мікроскопічне дослідження отриманої рідини для визначення клітинного складу. Гематологічний аналіз включав встановлення кількості еритроцитів і загальної кількості лейкоцитів. Гістологічними дослідженнями зразків легеневої тканини передбачалось мікроскопічне дослідження гістоструктури легеневої тканини. **Результати**. Після застосування запропонованих методів лікування на 45 добу експерименту було встановлено, що трансплантовані алогенні МСК сприяють підвищенню активності відновлення патологічно зміненої паренхіми легень. Ефективність цього процесу залежить від способу застосування МСК. Так, у тварин першої дослідної групи на 45-й день експерименту зникли симптоми, такі як кашель і гіпоксія. Достовірно знизилися показники кількості еритроцитів до 7,18 ± 0,05 Т/л ($P < 0,001$) та загальної кількості лейкоцитів 10,6 ± 0,92 Г/л ($P < 0,05$). Гістологічна структура легеневої тканини повністю відновилась, до 90 % порівняно контрольної групи. У тварин другої дослідної групи відмічали достовірне зменшення загальної кількості лейкоцитів до 11,32 ± ± 0,48 Г/л ($P < 0,01$) та кількості еритроцитів до 6,87 ± ± 0,18 Т/л ($P < 0,001$). Гістологічно підтверджено, що активність регенеративних процесів була меншою до 70–80 %, ніж у тварин першої групи; виявлялись поодинокі фіброзні поля, але вони були набагато менших розмірів, ніж у тварин у групі за медикаментозного лікування. У тварин третьої дослідної групи, незважаючи на нормалізацію показників лабораторних досліджень, а саме зниження кількості еритроцитів до 7,62 ± 0,11 Т/л ($P < < 0,001$) та зменшення кількості лейкоцитів 9,46 ± ± 0,54 Г/л ($P < 0,001$), гістологічна структура легень залишалась із острівками фіброзу та стінки альвеол були виразно потовщені, фіброзні ділянки зменшилися лише на 20–30 %. **Висновки**. Отримані нами результати демонструють, що застосування мезенхімальних стовбурових клітин виявилося кращим методом клітинної терапії для відновлення патологічно зміненої легеневої тканини у щурів з експериментальним легеневим фіброзом порівняно з медикаментозним лікуванням. Лікування тварин медикаментозним методом упродовж 45 днів експерименту призвело до нормалізації клінічних показників і лабораторних досліджень, але не призвело до повного відновлення структури пошкодженої легеневої тканини. Ці результати свідчать про переваги використання алогенних мезенхімальних стовбурових клітин у терапії легеневого фіброзу та можуть вказувати на їхній потенціал для подальшого дослідження та використання у клінічній практиці.

Ключові слова: щурі, легені, фіброз, регенеративна терапія, стовбурові клітини, еритроцити, лейкоцити, бронхоальвеолярний лаваж.

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