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The effect of mesenchymal stem cells on platelet function in rats with experimental lung injury

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Abstract. Interstitial lung tissue diseases (pulmonary fibrosis) are accompanied by a decrease in thrombopoiesis. Stem cells are capable of differentiating into other cell types, making them a valuable material for veterinary cellular regenerative therapy. The purpose of the research – to explore changes in platelet counts in laboratory rats with experimental pulmonary fibrosis under the influence of stem cells. The research was performed on female *Wistar* rats, in which pulmonary fibrosis was modelled using a single transthoracic injection of bleomycin hydrochloride solution. Allogeneic mesenchymal stem cells were used to stimulate recovery processes in pathologically altered lung tissue, which were administered by different routes, and, for comparison, the conventional method of treatment. The presented results of the effect of transplanted allogeneic bone marrow mesenchymal stem cells indicate a significant change in the number and size of platelets in rats with experimental pulmonary fibrosis and an increase in the activity of regenerative processes in damaged tissues. In experimental animals, a significant increase in the number of platelets and their size was found after using allogeneic mesenchymal stem cells compared to similar data in control group animals. In addition, in the blood of animals of the experimental group, which were transplanted with mesenchymal stem cells transthoracically (directly into the lung tissue), there was a higher platelet activity than in animals with intravenous injection of mesenchymal stem cells. Platelet activation indicates an improvement in the regenerative capacity of damaged lung tissue under the influence of mesenchymal stem cells. Thus, transplanted mesenchymal stem cells stimulate platelet activity and regenerative processes in pathologically altered lung tissue in experimental fibrosis, which can be used as one of the effective methods of treating animals with this type of pathology

Keywords: allogeneic; pulmonary fibrosis; lung tissue; platelets; laboratory animals

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Introduction

Pulmonary fibrosis is a progressive chronic inflammatory lung disease with a complex pathogenesis. It is now known that wound healing mechanisms caused by repeated damage to the alveolar epithelium lead to progressive fibrosis (Pitchford *et al.,* 2019). Fibrosis is characterised by inflammatory cell infiltration, fibroblast proliferation and collagen deposition, which ultimately leads to irreversible lung function impairment (Yount *et al.,* 2016). Currently, there are no effective treatments for pulmonary fibrosis.

Mesenchymal stem cells are capable of differentiating into specialised cells of various types (more than 350) and exhibit immunomodulatory and anti-inflammatory effects. Their multipotency and migration capabilities are of interest to scientists and practitioners of human and veterinary medicine and biology, which has resulted in a large number of studies on their use for therapeutic purposes (Tzouvelekis *et al.,* 2018). In a research of bleomycin-induced pulmonary fibrosis, scientists found that using mesenchymal stem cells increased survival compared to conventional treatments (Reddy *et al.,* 2016). Under the influence of mesenchymal stem cells, there is a decrease in the activity of interleukins, tumour necrosis factor-α, and transforming growth factor-β, which decreases inflammation. In addition, there is a downregulation of matrix metalloproteinase with a decrease in collagen deposition and fibrosis (Koupenova *et al.,* 2018). Mesenchymal stem cells at the site of injury activate resident stem cells and their differentiation into local cell types (Morrison *et al.,* 2017).

Platelets and neutrophils play an important role in the progression of pneumonia (Zhan *et al.,* 2020). A series of studies have demonstrated that platelets are involved in the pathogenesis of many diseases (Levoux *et al.,* 2021). Platelet activation is triggered in the early stages after lung tissue damage, and capillary integrity is compromised when a cascade of coagulation reactions is activated. Activated platelets contribute to fibrosis through the release of active substances from their granules (Lai *et al.,* 2018; Łukasik *et al.*, 2018) or through interaction with immune and inflammatory cells (Lin *et al.,* 2017).

However, the potential role of platelets in pulmonary fibrosis has not yet been fully explored. Therefore, scientists have analysed the role of platelets in the onset of pulmonary fibrosis and changes in lung function caused by repeated exposure to low levels of bleomycin (Carrington *et al.,* 2021), as platelets accumulate and release mediators such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), which are implicated in the fibrosis process (Pitchford *et al.,* 2019). It is known that platelets are among the first cells to respond to injury and can undergo diapodosis into the extravascular space, where the release of profibrotic factors can contribute to the development of fibrosis (Eisinger *et al.,* 2018). It is now confirmed that the lungs are the main source of platelet production and their share, in particular, in mice is about 50% of the total production of these cells in the blood (Lefrançais *et al.,* 2017).

Currently, it has been established that platelets can promote wound healing and play an important role in haemostasis by aggregating at sites of vascular damage with the subsequent release of growth factors, which, according to some scientists, are involved in the remodelling of certain tissues (Petito *et al.,* 2018; Pitchford *et al.,* 2019). In addition, platelets are involved in the pathogenesis of several diseases with inflammation, primarily respiratory diseases: asthma, acute lung injury, obstructive pulmonary disease, and infectious diseases with predominant lung tissue damage (Petito *et al.,* 2018; Zaid *et al.,* 2020).

Thrombocytopenia is frequently manifested in lung pathology and is associated with impaired platelet haemostasis. The association between lung damage and thrombocytopenia was further explored *in vitro* in rats with lung damage and found that the number of platelets in their peripheral blood was significantly lower than in control animals. Therefore, the lungs may play an active role in regulating platelet counts, and lung damage results in a decrease in the number of circulating platelets (Petito *et al.,* 2018).

The purpose of the study – to establish changes in the number and size of platelets in the blood of laboratory rats of the *Wistar* line with experimental pulmonary fibrosis under the influence of transplanted allogeneic mesenchymal stem cells, depending on the routes of their administration. To obtain this purpose, the following tasks were solved: to clarify the specific features of the pathogenesis of fibrotic lung diseases; to determine the clinical manifestations of pulmonary fibrosis; to determine changes in the number of platelets and their size in the presence of pathology and with using mesenchymal stem cells.

Literature Review

Platelets – small, nucleated cell fragments that play an important role in haemostasis and thrombosis. Modern research has proven that platelets are immune cells and key immune modulators, capable of expressing various receptors and molecules that allow them to respond to pathogens and interact with other immune cells. These cells have been linked to the pathogenesis of several pathological conditions, such as asthma and idiopathic pulmonary fibrosis (Chebbo *et al.,* 2021).

Platelets are established as a result of the fragmentation of a megakaryocyte, a large polyploid cell. In the bone marrow, immature megakaryocytes undergo endomitosis with the subsequent development of polyploid nuclei. Polyploid megakaryocytes extend long cytoplasmic processes known as proplatelets and release them into sinusoidal blood vessels for further division and generate mature platelets. It has been established that a single megakaryocyte can produce from 100 to 1000 platelets (Machlus & Italiano, 2011; Semple *et al.,* 2011; Hou *et al.,* 2015).

Platelets have a lifespan of 7 to 10 days, and they are constantly being established to maintain a stable blood level and volume (Holinstat, 2017; Koupenova *et al.,* 2018; Łukasik *et al.,* 2018).

Researchers have confirmed that platelets can modulate the immune response by interacting with immune cells in various pathological and physiological processes, such as inflammation, tissue remodelling, and infection (Chebbo *et al.,* 2021).

Numerous observations have identified the potential role of platelets in the pathophysiology of pulmonary diseases (Zhan *et al.,* 2020). *In vivo* microscopy studies in laboratory animals, megakaryocytes have been identified as platelet-producing cells in the lung vasculature. Moreover, the lungs are responsible for almost 50% of the total platelet production (Lefrançais *et al.,* 2017). In addition, researchers have identified extravascular pulmonary megakaryocytes that are not involved in platelet production. RNA sequencing analysis has established that pulmonary megakaryocytes have an innate immunity phenotype compared to bone marrow megakaryocytes and may be involved in the regulation of the pulmonary immune response (Lefrançais *et al.,* 2017). These scientific results suggest that platelets may be involved in pulmonary immune haemostasis.

Researchers have identified that during the progression of pulmonary fibrosis, a variety of immune cells and inflammatory cells, including macrophages and neutrophils (Gregory *et al.,* 2015; Huang *et al.,* 2017) are involved in processes at different stages of the disease

(Keane *et al.,* 2005). Activated platelets contribute to neutrophil activation by releasing CD40 L, which binds to CD40 receptors on neutrophils (Storey & Sinha, 2016) or by secretion of P-selectin/CD62 P, which binds to the neutrophil-expressed glycoprotein P-selectin-1 (PSGL-1) ligand (Rahman *et al.,* 2014).

Platelets are involved both in coagulation processes under physiological conditions and contribute to the development of inflammation and wound healing (Stavenuiter *et al.,* 2013; Webster *et al.,* 2013). Platelet activation occurs in many types of diseases with inflammation and fibrosis (Pitchford *et al.,* 2019) and in the very early stages of lung damage. In this case, the inflammatory response may be secondary to platelet activation (Pitchford *et al.,* 2019).

Peraçoli *et al.* (2008) note that platelets can have both pro-inflammatory and anti-inflammatory effects. Alpha granules contain the anti-inflammatory factor transforming growth factor-β. They release interleukin-10, which inhibits the secretion of tumour necrosis factor-α by monocytes (Gudbrandsdottir *et al.,* 2013). It has been concluded that platelets inhibit the secretion of inflammatory mediators by macrophages through mechanisms involving cyclooxygenase during sterile or bacterial systemic inflammation (Xiang *et al.,* 2013).

Materials and Methods

The research was conducted in 2021. Experimental animals were kept and used for experimental studies according to the requirements of the current Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty Treatment" (2006) and other bylaws, and Directives of the European Union 2010/63/EU (2010). In particular, the laboratory animals were kept in a separate room of the Povazhenko Department of Surgery and Pathophysiology in cages with 12-hour daylight hours at an air temperature of 20 to 23°C and with free access to water and food.

The object of the study was blood samples obtained from rats (with experimental pulmonary fibrosis), female *Wistar* rats, aged 4 months, with an average body weight of 277.0±4.26 g. During the preparatory period, lasting 45 days, pulmonary fibrosis was modelled in animals by a single instillation of 0.3 mL of bleomycin hydrochloride solution (Bleocin, Nippon Kayak Co., Ltd., Takasaki Plant, Japan) into the lungs at the rate of 1.0 mg/100 g of animal body weight in 0.3 mL of sterile physiological sodium chloride solution 0.9% at room temperature. The solution was injected once, transthoracically, straight into the lung tissue (Boiko *et al.,* 2013). The bleomycin solution was administered under light anaesthesia using telazol 100 mg/mL (Zoetis, Spain) at a dose of 30 mg/kg body weight, intramuscularly (Plumb, 2008) and in combination with Madison 0.1% (Brovapharma, Ukraine) at a dose of 0.25 mg/kg, intramuscularly (Plumb, 2008).

All animals with pronounced symptoms of pulmonary fibrosis on day 45 (baseline) were divided into 4 experimental groups of 20 animals each. Animals of the first experimental group were injected with mesenchymal stem cells transthoracically, directly into the lung tissue at a dose of 3 million/animal once, on the right side; animals of the second experimental group were injected with allogeneic mesenchymal stem cells intravenously at a similar dose. The animals of the third experimental group were treated with the conventional method of treatment – administration of dexamethasone solution 4 mg/mL (KRKA, Slovenia), at a dose of 0.08 mg/kg body weight, intramuscularly, for 3 weeks with an interval of 2 days with a gradual dose reduction, hyaluronidase solution 64 U (Lidaza-Biopharma, FZ "BIOPHARMA", Ukraine) at a dose of 0.85 U/kg, intramuscularly, for 3 weeks with an interval of 2 days (Boiko *et al.,* 2013); control group animals were injected with 0.3 mL of phosphate-buffered saline (Sigma,

USA) transthoracically, into the lung tissue directly, on the right side; the fifth experimental group was intact (healthy) animals.

Stem cells for use in the experiments were obtained from the bone marrow of young clinically healthy donor animals (rats) according to the methods developed by researchers of the Povazhenko Department of Surgery and Pathophysiology (Mazurkevych *et al.,* 2014).

The first day of mesenchymal stem cell injection was considered day zero (baseline). Blood samples for laboratory tests were taken randomly from 5 animals at baseline (before the experiment) and on days 7, 14, 30 and 45 of the experiment. For this purpose, the animals were euthanised by intraperitoneal injection (Shoyaib *et al.,* 2019) of a lethal dose of sodium thiopental (Thiopenate, Brovapharma, Ukraine) at a dose of 40 mg/kg body weight (Plumb, 2008). After the solution was injected into the abdominal cavity, euthanasia occurred due to the cessation of vital functions, namely breathing and heartbeat. Immediately after euthanasia, blood was immediately taken from the heart cavity for further haematological examination. The body of the euthanised animal was fixed in a dorsal position on a flat surface, perpendicular to the thorax. A puncture was performed near the sternum using a 2 mL syringe with a G 29 needle. The obtained blood samples were placed in special tubes with K3 EDTA (AQUISEL, Italy) to prevent the development of a blood clot and for further morphological examination on an automatic haematological analyser (Exigo BM800, USA) according to the manufacturer's instructions.

No animal deaths were recorded in the experimental groups during the experiment.

Statistical processing of the results was performed using the Microsoft Excel 2010 software package. The experimental data are presented as M±m (M – arithmetic mean; ± – standard error of the mean). The significance between the values of the control and experimental groups was determined by Student's t-test, and the values were considered significant at $P<0.05$.

Results and Discussion

Changes in the number of platelets in the blood of laboratory rats at different stages of the experiment have certain features depending on the method of application of stimulants for the restoration of damaged lung tissue in fibrosis and the period after the application of these methods (Table 1, Fig. 2).

Table 1. Platelet count in the blood of rats with experimental pulmonary fibrosis under the influence of mesenchymal stem cells and conventional treatment, 10^9/L, M±m, n=5

Stages of the experiment	Group of animals				
	1 experimental	2 experimental	3 experimental	4 (control)	5 experimental
Baseline state	650.20±35.93	650.20±35.93	650.20±35.93	650.20±35.93	668.30±28.30
7th day	895.80±5.96***	867.20±44.20***	420.00±46.36*	566.80±38.49	688.30±11.27
14th day	954.00±34.58***	940.00±27.56***	571.40±26.51	665.20±46.50	676.50±18.04
30th day	842.00±32.30***	893.00±6.21***	559.40±28.26	531.00±28.56	700.20±6.21
45th day	884.20±15.80***	879.60±20.44***	611.70±20.60	597.00±14.03	718.90±19.08

Note: **P<0.05; *P<0.01; ***P<0.001, significantly compared with control group animals*
Source: *author's development*

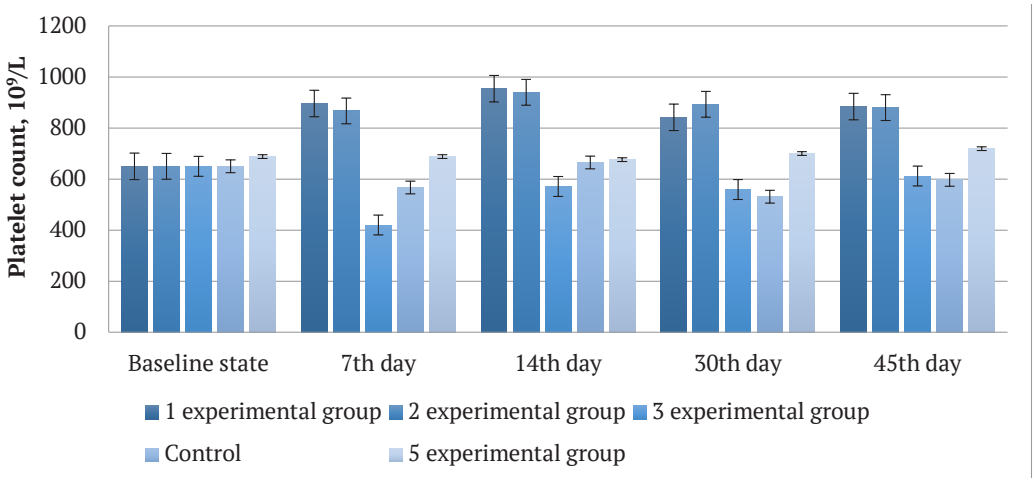

Figure 1. Dynamics of platelet count in the blood of rats with experimental pulmonary fibrosis under the influence of mesenchymal stem cells and conventional treatment

Thus, in the baseline state (before using the drugs), the platelet count in the blood of animals with experimental pulmonary fibrosis was lower than that of intact animals. Evidently, this is a consequence of the development of a pathological process in the lungs due to the modelling of experimental fibrosis in animals.

On day 7 of the experiment, after using allogeneic mesenchymal stem cells, a significant increase in the examined indicators was found in the blood of animals of groups 1 and 2 compared to those of the control group (group 4). In particular, a significant increase in the number of platelets by 37% ($P<0.001$) and 35% ($P<0.001$), respectively, was found in the blood of animals of groups 1 and 2 compared to the control group. A significant decrease in the number of platelets by 26% ($P<0.05$) was found in the blood of animals of group 3 compared to the baseline.

In the blood of animals of the control group, the number of platelets remained lower than in intact animals. The lowest number of platelets was recorded in the blood of animals of experimental group 3 (conventional treatment), and it was significantly lower than in the control group by 26% ($P<0.05$).

On the 14th day of the experiment, the number of platelets in the blood of animals of experimental groups 1 and 2 significantly increased by 30% ($P<0.001$) and 29% ($P<0.001$), respectively, compared to the control group. On the contrary, the number of platelets in the blood of animals of experimental group 3, which were treated with the conventional method, decreased by 14%.

On day 30 of the study, an increase in the examined indicators in the blood of animals of experimental groups 1 and 2 was found compared to the control group, but they were lower than the same indicators on day 14 of the experimental study. Thus, the number of platelets in the blood of animals of experimental group 1 significantly increased by 37% ($P<0.001$) and of experimental group 2 – by 40% ($P<0.001$) compared to the control group. A tendency to a slight increase in the number of platelets (by 5%) was observed in the blood of animals of experimental group 3.

On day 45 of the experimental study, an increase in quantitative indicators was noted in the groups of animals treated with allogeneic mesenchymal stem cells, in particular: in experimental group 1, the total number of platelets significantly increased by 32% ($P<0.001$), and in the blood of animals of experimental group 2, this indicator significantly increased by 30% ($P<0.001$) compared to the control group; in the blood of animals of experimental group 3, only a tendency to increase the number of these cells (by 2.4%) was recorded.

Thus, the results of the study indicate that transplanted allogeneic mesenchymal stem cells stimulate thrombocytosis in animals with pulmonary fibrosis. Therewith, the highest activity of platelet count increase was recorded in the first 14 days of the experiment when the regenerative processes in the damaged lungs were the highest. Subsequently, by day 45 of the experiment, the intensity of platelet growth decreased. The methods of conventional treatment (experimental group 3) do not contribute to a significant increase in the number of platelets, as evidenced by the comparison of the corresponding indicators in animals of experimental group 3 with those in control and intact rats.

Forty years ago, researchers were convinced that platelets were just "non-living" cell fragments that were only involved in the development of a platelet clot to stop bleeding (Leslie *et al.,* 2010; Garraud *et al.,* 2015). Only in the last decade has the number of discoveries about platelet biology and function increased, demonstrating (Andrade *et al.,* 2020; Levoux *et al.,* 2021) that these cells are among the most biotechnologically advanced. In addition, it has been reported in the literature that platelets enhance the wound-healing activity of mesenchymal stem cells, which is consistent with this research. However, the mechanisms by which they improve the therapeutic potential of stem cells remain unclear (Levoux *et al.,* 2021). There is evidence that after platelets are activated, they transfer respiratory-competent mitochondria to mesenchymal stem cells through dynamin- and clathrin-dependent endocytosis, which contributes to the therapeutic efficacy of these cells after engraftment and has been demonstrated in relevant tissue injury models in mice (Levoux *et al.,* 2021).

Therewith, in recent years, researchers have begun to explore platelet-rich plasma together with stem cells to stimulate the regenerative potential of mesenchymal stem cells (Andia *et al.,* 2018; Henschler *et al.,* 2019). Several studies have demonstrated that using platelet-rich plasma (PRP) increases the therapeutic efficacy of stem cells (Qian *et al.,* 2017; Mahmoudian-Sani *et al.,* 2018; Hersant *et al.,* 2019) and improves their survival after exposure to oxidative stress (Hersant *et al.,* 2019). For example, Qian *et al.* (2017) found that PRP (a natural product) isolated from whole blood is capable of producing numerous growth factors to regulate physiological activities. These factors have a stimulatory effect on the proliferation and differentiation of various stem cells in injury models.

Nowadays, Etulain (2018) has proven the stimulating role of growth factors released by activated platelets in wound healing. They operate as a result of their paracrine secretion, focusing on the participation of respiratory-competent mitochondria, both in membrane-encapsulated microparticles and as free organelles, during stem cell-based therapies (Marcoux *et al.,* 2017). Two-photon microscopy in live animals transfused with blood demonstrated that extracellular mitochondria interact with neutrophils *in vivo*, causing neutrophil adhesion to the endothelial wall (Marcoux *et al.,* 2017). In addition, these scientists noted that PRP treatment stimulates the proangiogenic potential of mesenchymal stem cells in mice *in vitro*, depending on the dose, through increased secretion of soluble factors such as VEGF and

SDF-1. Therewith, the therapeutic use of PRP in laboratory animals improves survival and activates the proliferation of *in vitro* cultured mesenchymal stem cells. These effects are accompanied by changes in energy metabolism in these cells, including the rate of oxygen consumption and production of adenosine triphosphoric acid in the mitochondria. However, the molecular mechanism and consequences of this phenomenon have not been fully understood. Only two studies have described that mitochondria released by activated platelets can be absorbed by platelets, which triggers an immune or regenerative response (Boudreau *et al.,* 2014; Zhao *et al.,* 2017).

During the experiment, the average volume of platelets changed according to the indicators of their quantitative composition (Table 2, Fig. 2).

Table 2. Changes in the mean platelet volume in the blood of rats with experimental pulmonary fibrosis and under the influence of mesenchymal stem cells and conventional treatment, fl, M±m, n=5

Stages of the experiment	Group of animals				
	1 experimental	2 experimental	3 experimental	4 (control)	5 experimental
Baseline state	5.36±0.05	5.36±0.05	5.36±0.05	5.36±0.05	6.06±0.10
7th day	7.18±0.23**	7.54±0.25***	5.26±0.05*	5.88±0.21	6.10±0.05
14th day	9.54±0.26***	9.52±0.28***	5.38±0.04	5.54±0.08	6.17±0.06
30th day	6.62±0.06***	6.18±0.29*	5.88±0.21*	5.38 0.04	6.10±0.12
45th day	6.34±0.18**	6.15±0.05***	6.02±0.06**	5.54±0.10	6.14±0.13

Note: **P<0.05; **P<0.01; ***P<0.001, significantly compared with control group animals*
Source: *author's development*

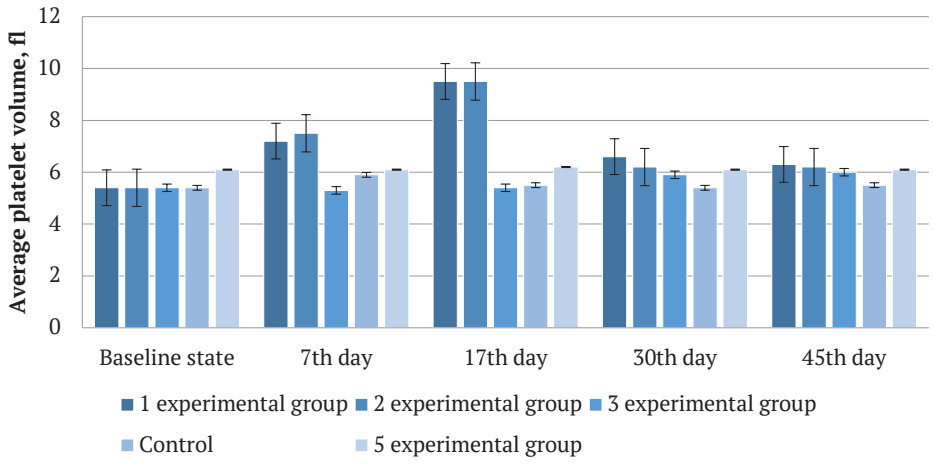

Figure 2. Changes in the average platelet volume in the blood of rats with experimental pulmonary fibrosis under the influence of MSCs and conventional treatment

Thus, on day 7 of the experiment, the average platelet volume in the blood of animals of experimental groups 1 and 2 significantly increased by 18% (P<0.01) and 22% (P<0.001), respectively, compared to animals of the control group. In animals of the experimental group 3, on the contrary, this index significantly decreased by 10% (P<0.05) compared to the control group and intact rats.

On day 14 of the experiment, the average platelet volume in the blood of rats of experimental groups 1 and 2 significantly increased by 42% (P<0.001) and 41.8% (P<0.001), respectively. In animals of experimental group 3, which were treated with the conventional method of treatment, the examined index decreased by 3% compared to the control group, but it was already higher than on day 7 of the study.

On day 30 of the experiment, the average platelet volume in the blood of animals of experimental group 1 significantly increased by 19% (P<0.001), and in rats of experimental group 2 – 13% (P<0.05). A significant increase in the average platelet volume by 8.5% (P<0.05) was observed in animals of experimental group 3. On day 45 of the experiment, the average platelet volume significantly increased in animals of all experimental groups: in experimental group 1 – by 13% (P<0.01), experimental group 2 – by 10% (P<0.001), and experimental group 3 – by 8% (P<0.01).

Thus, compared to intact animals, both the number of platelets and their average volume in animals of experimental groups 1 and 2 significantly increased in the first 14 days of the experiment after using mesenchymal stem cells. However, in animals of experimental group 3, which received conventional treatment, these indicators were lower, which is obviously due to the delay or absence of a stimulating effect on thrombocytosis and, accordingly, on the anti-inflammatory activity of platelets.

Thus, platelets are circulatory components derived from megakaryocytes present in the bone marrow that play an important role in physiological and pathophysiological processes such as thrombosis, haemostasis, inflammation and wound healing (Holinstat, 2017). Platelets contain cytokines or growth factors that initiate the regenerative process of ageing. They begin to be activated by biologically active growth factors in the target area through a paracrine effect. At the same time, platelets release growth factors that affect the activation of macrophages and stem cells at the site of damaged tissue. In addition, Du Toit *et al*. (2007) emphasise that tissue regeneration is facilitated by the proliferation and differentiation of stem cells. These nucleus-free cells contain secretory organelles, including alpha granules, containing various proteins and growth factors. As described by Gremmel *et al.* (2016) platelets contain functional mitochondria that provide energy for their metabolism.

Gomperts & Strieter (2007) note that in pathologically altered lungs, regenerative processes can be impaired by the depletion of specific chemical factors, which we observed in the control group. It is noted that the supplementation of these factors in combination with platelets can more effectively stimulate the regeneration of functional blood vessels and alveolar structures in pathologically altered lung tissue (Mammoto *et al.,* 2016).

Mammoto *et al.* (2016) demonstrated that platelets stimulate vascular and alveolar regeneration of the adult mouse lung via Ang1-LRP5-Tie2 signalling. These results may improve the efficiency of lung regeneration and lung organ engineering, as unregulated angiogenesis contributes to the pathogenesis of various chronic lung diseases.

Hersant *et al.* (2019) note that activated platelets potentiates several important properties of mesenchymal stem cells, including their proliferation, survival and angiogenic potential, which is why an increase in platelet count

was observed in animals treated with stem cells. Rodrigues *et al.* (2010) and Lai *et al.* (2018) confirmed that platelets exert their cytoprotective and mitogenic effects on mesenchymal stem cells through the production of mitogenic growth factors. Therewith, as noted by Gurtner *et al.* (2008) and Sadtler *et al.* (2016), platelet factors play an important role throughout the healing process of damaged tissues.

Since interstitial fibrotic diseases of the lung tissue disrupt the mechanisms of tissue repair, understanding how bone marrow mesenchymal stem cells regulate platelet activation may prove to be a strategy for regenerating damaged tissues.

Conclusions

As a result of using allogeneic bone marrow mesenchymal stem cells, their positive effect on thrombocytosis activity in an experimental study of lung injury leading to fibrotic changes in laboratory rats was established.

An increase in the number of platelets and their average volume was established in animals of experimental groups 1 and 2, which were transplanted with allogeneic mesenchymal stem cells. Thus, after transthoracic injection, there was an increase in platelets on days 7 ($P<0.001$), 14 ($P<0.001$), 30 ($P<0.001$) and 45 ($P<0.001$) compared to the control group. An increase in the average volume of erythrocytes was observed on days 7 ($P<0.01$), 14 ($P<0.001$), 30 ($P<0.001$) and 45 ($P<0.01$) of the experimental study. In the group of animals with intravenous stem tissue management, an increase in platelets was observed on days 14 ($P<0.001$), 30 ($P<0.001$) and 45 ($P<0.001$) and an increase in the average volume of erythrocytes on days 14 ($P<0.001$), 30 ($P<0.05$) and 45 ($P<0.001$).

During the application of the conventional method of treatment, an increase in the average platelet volume was found in the blood of animals on days 30 and 45, and in other studied parameters – on day 7. The transplanted mesenchymal stem cells stimulate the establishment of megakaryocytes, which are the precursors of platelets. As a result, the therapeutic efficacy of mesenchymal stem cells after their engraftment increases, since platelets can enhance the reparative properties of mesenchymal stem cells. Therefore, it is assumed that platelets may play an important role in the anti-fibrotic effect of stem cells.

The processes of platelet activation in fibrotic lung diseases and their molecular mechanisms under the influence of stem cells require further detailed research to determine the prospects of their use as an alternative to classical methods of regeneration of damaged tissues.

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Conflict of Interest

None.

Reference

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Вплив мезенхімальних стовбурових клітин на тромбоцитарну ланку в щурів за експериментального ушкодження легень

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Анотація. Інтерстиціальні захворювання легеневої тканини (фіброз легень) супроводжуються зниженням процесів тромбопоезу. Стовбурові клітини здатні до диференціювання в інші типи клітин, завдяки чому вони стають цінним матеріалом для ветеринарної клітинної регенеративної терапії. Мета роботи – дослідити зміни показників тромбоцитарної ланки у лабораторних щурів з експериментальним фіброзом легень за впливу стовбурових клітин. Дослідження проведені на щурах-самицях лінії *Wistar*, в яких моделювали фіброз легень із застосуванням однократного трансторакального введення розчину гідрохлориду блеоміцину. З метою стимуляції відновлювальних процесів у патологічно зміненій легеневій тканині використовували алогенні мезенхімальні стовбурові клітини, які вводили різними шляхами, та, для порівняння, – традиційний метод лікування. Наведені результати впливу трансплантованих алогенних мезенхімальних стовбурових клітин кісткового мозку свідчать про вірогідні зміни кількості та розміру тромбоцитів у щурів з експериментальним фіброзом легень, а також про підвищення активності регенеративних процесів в ушкоджених тканинах. У дослідних тварин встановлено вірогідне збільшення кількості тромбоцитів та їх розмірів після застосування алогенних мезенхімальних стовбурових клітин порівняно з аналогічними даними у тварин контрольної групи. Крім того, в крові тварин дослідної групи, яким трансплантували мезенхімальні стовбурові клітини трансторакально (безпосередньо в легеневу тканину), відмічали більшу активність тромбоцитів, ніж у тварин з внутрішньовенним уведенням мезенхімальних стовбурових клітин. Активація тромбоцитів вказує на покращення регенеративної здатності ушкоджених тканин легень за впливу мезенхімальних стовбурових клітин. Таким чином, трансплантовані мезенхімальні стовбурові клітини стимулюють активність тромбоцитів та регенеративні процеси в патологічно зміненій легеневій тканині за експериментального фіброзу, що може бути використано як один із ефективних методів лікування тварин за цього виду патології

Ключові слова: алогенні; легеневий фіброз; легенева тканина; тромбоцити; лабораторні тварини