



The EPOR/CD131 heteroreceptoragonist has an endothelioprotective effect against the background of pulmonary hypertension caused by monocrotalin

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Abstract

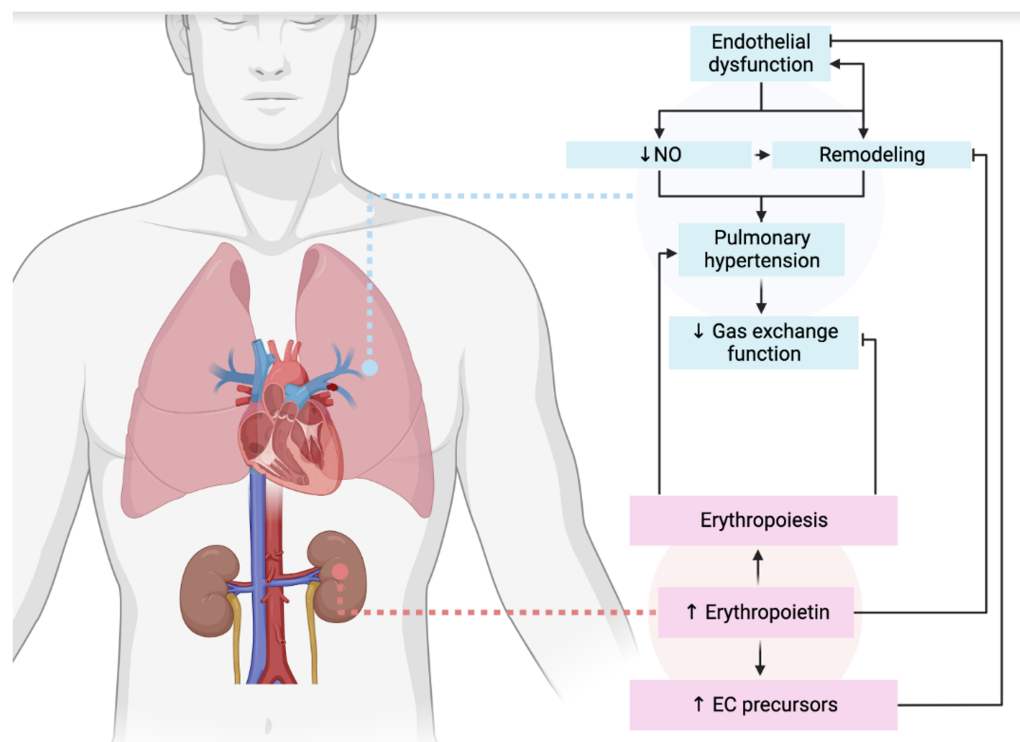
Introduction: The abnormal increase in pulmonary pressure observed in pulmonary arterial hypertension (PAH) is a consequence of increased pulmonary vascular resistance due to progressive loss and obliteration of pulmonary arteries. The initial trigger is a combination of factors that lead to endothelial damage and impaired vascular regeneration. **Aim:** research the possibilities of pharmacological correction of pulmonary arterial hypertension induced by monocrotalin using the EPOR/CD131 heteroreceptor agonist with the laboratory code EP-11-3.

Materials and Methods: The study of pharmacological activity on a model of monocrotaline induced PAH was carried out on male Sprague-Dawley rats weighing 180-220 grams. Monocrotaline (MCT) pulmonary hypertension was simulated in 30 animals using subcutaneous injection of MCT at a dose of 60 mg/kg. Seven days after the injection of MCT, the administration of the studied compounds began. The erythropoietin derivative with the laboratory code EP-11-3 and pHBSP administered subcutaneously at a dose of 25 mcg/kg once every 3 days for 21 days.

Results: On the model of monocrotalin-induced PAH, it was shown that the erythropoietin derivative with the laboratory code EP-11-3 has a pronounced endothelioprotective effect, reducing the coefficient of endothelial dysfunction, statistically significantly increasing the expression of VEGF-R2 mRNA and reducing the expression of SDF-1 mRNA, reducing the concentrations of CT-1 and PNP, and reducing the signs of remodeling of the heart and pulmonary vessels.

Conclusion: Erythropoietin derivative with laboratory code EP-11-3 has an endothelioprotective effect and reduces the manifestations of vascular remodeling in pulmonary hypertension caused by monocrotalin.

Graphical Abstract



Keywords

Monocrotaline, EPOR/CD131, progenitors of endothelial cells, endothelioprotector, pulmonary hypertension

Introduction

The relevance of the formation of holistic approaches to the search for and pharmacological evaluation of new candidate compounds for the treatment of endothelium-associated pathology is due to those nosological forms that are included in the list of endothelium-associated diseases. Thus, pulmonary arterial hypertension (PH) is a group of life-threatening progressive diseases of various genesis, characterized by a progressive increase in blood pressure (BP) in the pulmonary artery (PA), remodeling the pulmonary vessels, which leads to increased pulmonary vascular resistance and pulmonary arterial pressure and, most often, leads to right ventricular heart failure and premature death. In recent years, the role of pulmonary vascular endothelial dysfunction in PH of various genesis has been actively studied. To date, the issue of the relationship of vascular reactivity disorders associated with hypoxia in various lung pathologies, including pulmonary hypertension, and endothelial dysfunction remains relevant (Kurakula et al. 2021).

The abnormal increase in pulmonary pressure observed in pulmonary arterial hypertension is a consequence of increased pulmonary vascular resistance due to progressive loss and obliteration of small pulmonary arteries (Kurakula et al. 2021; Zagrebnaya et al. 2023). It is believed that the initial trigger mechanism

is a combination of genetic and environmental factors that lead to damage to endothelial cells (EC) and impaired vascular regeneration, which further leads to the remodeling of the pulmonary vascular bed (Rubin 2006; McLaughlin et al. 2009).

Approaches to pharmacological correction of pulmonary arterial hypertension include the use of calcium antagonists, prostacyclines, endothelin receptor antagonists and oxygen to regulate vascular tone and vascular permeability, as well as to reduce the effects of vascular remodeling.

To date, none of the available therapeutic methods has demonstrated the potential to prevent or reduce a degree of pulmonary microvascular thrombosis in arterial pulmonary hypertension. The main obstacle to the development of effective treatment methods for PAH is an incomplete understanding of the signaling pathways leading to the development of vasoactive factors by the endothelium, providing vascular homeostasis and angiogenesis against the background of pulmonary hypertension. The initiation of angiogenesis processes is necessary for normal vascular regeneration in response to emerging pathology (Ranchoux et al. 2018).

In connection with the above, we were interested in the approach to the use of compounds that are agonists of the EPOR/CD131 heteroreceptor for the pharmacological correction of arterial pulmonary hypertension. Earlier studies have shown that the level of EPO increases in the

systemic blood flow and lungs in patients with PAH (Karamanian et al. 2014). In addition, systemic administration of EPO at a dose of 2500 ng/kg/day for 21 days to rats with monocrotalin-induced PAH improved the histological picture of the lungs, cardiac function, increased the expression of mRNA of the anti-apoptotic molecule Bcl-xL and maintained the expression of the CD31 antigen (Ikarashi et al. 2012). In addition, Satoh et al. (2006) showed that mice knocked out by the gene encoding the EPO receptor (EpoR^{-/-}) demonstrated accelerated development of PAH under chronic hypoxia in the hypobaric chamber, which was expressed in an increase in pressure in the right ventricle, hypertrophy of the right heart and the remodeling of pulmonary vessels. In this line, the mobilization of endothelial cell precursors and their recruitment into the pulmonary endothelium were significantly disrupted. This study also found that in wild-type (WT) mice, hypoxia increased EpoR expression on lung endothelial cells and activated endothelial nitric oxide synthase in the lungs (unlike EpoR^{-/-} mice).

Initially, for the purpose of cyto- and organoprotection, scientists used suberythropoietic doses of recombinant erythropoietin, and subsequently, in 2008, Michael Brines and colleagues found that signaling of non-hematopoietic effects of EPO is carried out through binding of the alpha helix B of the EPO molecule with the EPOR/CD131 heteroreceptor (Brines et al. 2008). Therefore, an urgent task is to search for erythropoietin derivatives that realize their flame retardant properties and modulate the activity of the EPOR/CD131 heteroreceptor.

The aim of this study was to research the possibilities of pharmacological correction of arterial pulmonary hypertension induced by **monocrotalin** using the EPOR/CD131 heteroreceptor agonist with the laboratory code EP-11-3.

Materials and Methods

A model of monocrotaline pulmonary hypertension

Experimental studies were approved by the Bioethical Commission of Belgorod State National Research University (Minutes No. 02/18 of 15.01.2018).

The study of pharmacological activity on a model of **monocrotaline** pulmonary hypertension was carried out on male Sprague-Dawley rats weighing 180-220 grams. **Monocrotaline (MCT)** pulmonary hypertension was simulated in 30 animals using subcutaneous injection of **MCT** (Sigma-Aldrich Corp.) at a dose of 60 mg/kg. **MCT** was dissolved in 0.3 mol/L sodium hydrochloride solution and neutralized with 0.3 mol/L sodium hydroxide solution (approximate adjusted pH 7.0) (Urakami et al. 2011). Seven days after the injection of **MCT**, the administration of the studied compounds began. The erythropoietin derivative with the laboratory code EP-11-3 and **pHBSP** were administered subcutaneously at a dose of 25 mcg/kg once every 3 days for 21 days.

Thus, the following experimental groups were formed:

- 1 – control (0.9% NaCl subcutaneously);
- 3 – **MCT** (subcutaneously once);
- 2 – **MCT** + EP-11-3 at a dose of 25 mcg/kg once every 3 days for 21 days;
- 4 – **MCT** + **pHBSP** at a dose of 25 mcg/kg once every 3 days for 21 days.

The pressure in the right ventricle of the heart and the gas composition of venous blood were measured in mice under anesthesia (2%-2.5% isoflurane in 100% oxygen) 4 weeks after the start of the experiments. To do this, the femoral vein was catheterized, then the catheter was inserted into the cavity of the right ventricle of the heart. In each animal, the pressure in the right ventricle was continuously recorded with a sampling frequency of 1 kHz for at least 30 seconds, using a piezoelectric pressure sensor and the MP-150 system (Biopac, USA). The correct anatomical position of the catheter tip was maintained by constant monitoring the pressure signal curve. Systolic pressure in the cavity of the right ventricle (SPCRV), diastolic pressure in the cavity of the right ventricle (DPCRV), heart rate (HR), dP/dt max, dP/dt min were determined. Hemodynamic parameters were determined using the Biopac MP-150 hardware complex and the AcqKnowledge 3.8.1 (USA) computer program. After measuring the hemodynamic parameters, the animal was removed from the experiment, and its blood was taken to analyze the gas composition (partial pressure of oxygen and carbon dioxide).

Methods for assessing the development of pulmonary hypertension and its correction by the studied compounds

Study of the number of circulating progenitors of endothelial cells

To measure the levels of circulating progenitors of endothelial cells (PEC), we used the method of cell culture and staining, which was described in (YanYun et al. 2015). Mononuclear cells were isolated from peripheral blood by centrifugation in Histopaque-1083 rasterizer (a solution containing polysaccharose and sodium diatrysoate, brought to a density of 1,083 g/mL) according to the manufacturer's instructions (Sigma Chemical, St. Louis, Missouri, USA). Isolated mononuclear cells were seeded in three repetitions on 96-well plates coated with 1% gelatin into a basal medium for endothelial cells (Thermo Scientific, USA) supplemented with 2% fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 mcg/mL). After 2 days of cultivation, the adhering cells were thoroughly washed with a medium and co-stained with DiI AcLDL (Thermo Scientific, USA).

Conducting a quantitative polymerase chain reaction in real time

To study the effect of the studied drugs on the molecular mechanisms of the development of pulmonary hypertension, we performed a real-time polymerase chain reaction (PCR) to determine the expression of VEGF-R2 mRNA, SDF-1 (stromal growth factor-1) and MCP-1 (monocytic chemoattractant protein-1). To perform quantitative real-time PCR, a part of the lung was homogenized and incubated for 10 minutes at 37°C in an Extract RNA solution. After lysis of the sample in the reagent, it was subjected to chloroform cleaning; the sample was collected and washed with isopropyl alcohol and 70% ethyl alcohol. The concentration of the obtained RNA was measured on an IMPLENNanoPhotometer[®] spectrophotometer and adjusted to a concentration of 300 ng/μL. Reverse transcription was performed using the MMLVRTSK021 kit in accordance with the

manufacturer's protocol (Evrogen, Russia). The level of gene expression was evaluated relative to the values of the Gapdh reference gene. The expression at a specific point was calculated using the formula: Gene expression= $[(Ct(Gapdh)/Ct(Gene\ of\ Interest))]$ (Puchenkova et al. 2020; Kuzubova et al 2022).

Histology

For histological examination, organs (heart and lungs) were extracted and fixed in 10% neutral formalin. Then the material was poured in standard mode into paraffin in a carousel type machine STP-120 (Microm International GmbH, Germany). Histological preparations were examined under the Axio Scope A1 microscope (Carl Zeiss Microimaging GmbH, Germany), and morphometry was performed using the Image J 1.54d program. The thickness of the pulmonary artery wall was determined; pulmonary vessels near the alveoli were evaluated, and the diameters of 20 vessels on a slide were determined. Five slices were evaluated from each animal. At the same time, the number of thrombosed vessels in the field of view was estimated (magnification of the microscope: $40 \times 10 = 400$ -fold). Approximately 20 vessels of the peribronchial pulmonary artery were evaluated on each slide stained with hematoxylin and eosin (magnification of the microscope: $\times 400$). The degree of occlusion was determined as the ratio between the outer and inner (i.e., the luminal) circumferences of each vessel.

The degree of myocardial hypertrophy of the right ventricle of the heart was determined using the image analysis software MCID 7.0 Image Research (Canada). For this purpose, a horizontal incision was made through the mouse heart at the level of the ventricles; the resulting sections were scanned, using a drawing tool; the left and right ventricles were separated manually by a thin line in the same way for all sections. Then a pixel-by-pixel analysis of the areas of the right ventricle (RV) and left ventricle (LV) with interventricular septum (IS) was performed. The results are presented in the form of a ratio of $LV/(LV+IS)$.

Determination of a degree of pulmonary edema

When removing animals from the experiment, their lungs were taken and divided into separate lobes. Then the mass of the lung fraction was determined before and after drying in a thermostat at 70°C for 72 hours. The results were expressed as the ratio of lung mass before and after drying.

Determination of concentrations of CT-1 and PNP

The concentrations of Cardiostrophin-1 (CT-1) and atrial natriuretic peptide (ANP) were measured in serum using ELISA kits (ELM-Cardiostrophin-1/EIA-ANP-1, RayBiotech, Norcross, GA, USA) in accordance with the manufacturer's instructions.

Statistics

Statistical processing was carried out using the R software computing environment. The nature of the distribution of features in the statistical sample was determined using the Shapiro-Wilk test and the Spiegelhalter test (normtest library), and the assessment of equality of variances was determined using the Levene criterion (lawstat library).

Depending on the type of distribution of characteristics and equality of variances, the significance of the results obtained was assessed using parametric (ANOVA) or nonparametric (Kruskal-Wallis test) one-way analysis of variance, and an unpaired Student t-test was used as a post-hoc analysis to identify differences in intergroup comparisons or Mann-Whitney test, respectively, with Benjamini-Hochberg correction for multiple hypothesis testing. The results were considered significant at $p \leq 0.05$.

Results

The modeling of monocrotalin-induced pulmonary hypertension resulted in a statistically significant increase in systolic pressure in the right ventricular cavity (PRVC), diastolic pressure in the right ventricular cavity (DPRVC), maximum contraction rate (dP/dt max) and minimum contraction rate (dP/dt min) and did not lead to a statistically significant change in heart rate (Table 1).

The introduction of the studied leader compound with the laboratory code EP-11-3 led to a statistically significant decrease in all the studied indicators, and the indicator dP/dt max in groups of animals with the introduction of the studied drugs was as close as possible to those values in the group of intact animals. However, the pressure values in the right ventricular cavity were far from the target values obtained in the group of intact animals (Table 1). The introduction of the initial pHBSP peptide did not lead to a statistically significant decrease in SPCR; however, it led to normalization of other indicators of cardiohemodynamics against the background of modeling pulmonary hypertension (Table 1).

Table 1. Indicators of cardiohemodynamics in groups of animals with the modeling of pulmonary hypertension and its correction using the studied compounds

Indicators of cardiohemodynamics	Intact	MCT	EP-11-3	pHBSP
SPCRV	29.3±2.6	60.3±5.92*	51.4±8.54**	53.8±9.68
DPCR	2.885±0.35	8.073±0.84*	6.967±0.72**	6.63±0.41**
dP/dt max	107.6±11.1	69.6±81.4*	81.4±10.32**	79.9±11.5**
dP/dt min	87.9±6.1	55.3±10.6*	74.2±5.7**	76.6±8.1**
HR	345.1±32.9	347.3±31.2	339.1±25.6	349.5±26.9

Note: MCT – a group of animals treated with monocrotalin to simulate pulmonary hypertension; SPCR – systolic pressure in the cavity of the right ventricle of the heart; DPCR – diastolic pressure in the cavity of the right ventricle of the heart; HR – heart rate; dP/dt max – the maximum rate of increase in intraventricular pressure; dP/dt min – the minimum rate of increase in intraventricular pressure. * – $p < 0.05$ – in comparison with the group of intact animals; ** – $p < 0.05$ – in comparison with the group of animals treated with monocrotalin (MCT).

The analysis of the results of studying the number of circulating endothelial cell precursors showed an almost 2-time decrease in the group of animals with the modeling of monocrotalin-induced pulmonary hypertension (175 ± 17.89) in comparison with the group of intact animals (296.9 ± 216.6 , $p=0.000019$). In the groups of animals that were injected with the studied compounds, the number of circulating PEC increased statistically significantly. It should be noted that in the group of animals receiving EP-11-3, the number of circulating PEC was statistically significantly higher than in the group of animals receiving pHBSP ($p=0.0022$) (Fig. 1).

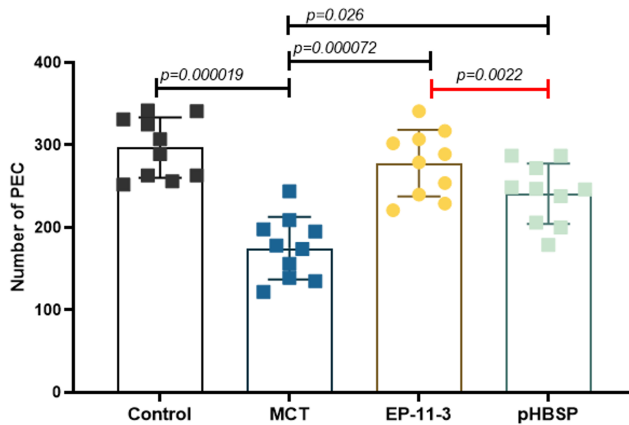


Figure 1. The effect of the studied compounds on the number of circulating endothelial progenitor cells (PEC) in the blood. **Note:** Control – a group of intact animals, MCT – a group of animals with pulmonary hypertension (PH) against the background of the administration of monocrotalin at a dose of 60 mg/kg; EP-11-3 – introduction of EP-11-3 peptide at a dose of 25 mcg/kg against the background of LH modeling; pHBSP – introduction of pHBSP peptide at a dose of 25 mcg/kg against the background of LH modeling; * – $p < 0.05$ compared with intact, ** – $p < 0.05$ compared with intact, ** – $p < 0.05$ compared with MCT.

When analyzing the results of the blood gas composition study, a similar pattern was found – a statistically significant decrease in oxygen partial pressure (PaO_2) against the background of a statistically significant increase in carbon dioxide partial pressure (PaCO_2) in the group of animals with pulmonary hypertension (Fig. 2). Compounds EP-11-3 and pHBSP statistically significantly (in comparison with the MCT group) and comparably restored the values of blood gas composition in the animals of experimental groups (Fig. 2).

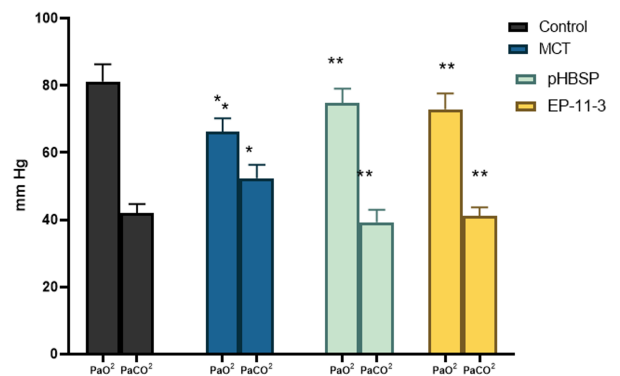


Figure 2. The effect of EP-11-3 and pHBSP on the partial pressure of oxygen and carbon dioxide in experimental groups. **Note:** Control – a group of intact animals, MCT – a group of animals with pulmonary hypertension (PH) against the background of the administration of monocrotalin at a dose of 60 mg/kg; EP-11-3 – introduction of EP-11-3 peptide at a dose of 25 mcg/kg against the background of PH modeling; pHBSP – introduction of pHBSP peptide at a dose of 25 mcg/kg against the background of PH modeling; * – $p < 0.05$ compared with intact, ** – $p < 0.05$ compared with MCT. PaO_2 – partial pressure of oxygen. PaCO_2 – partial pressure of carbon dioxide.

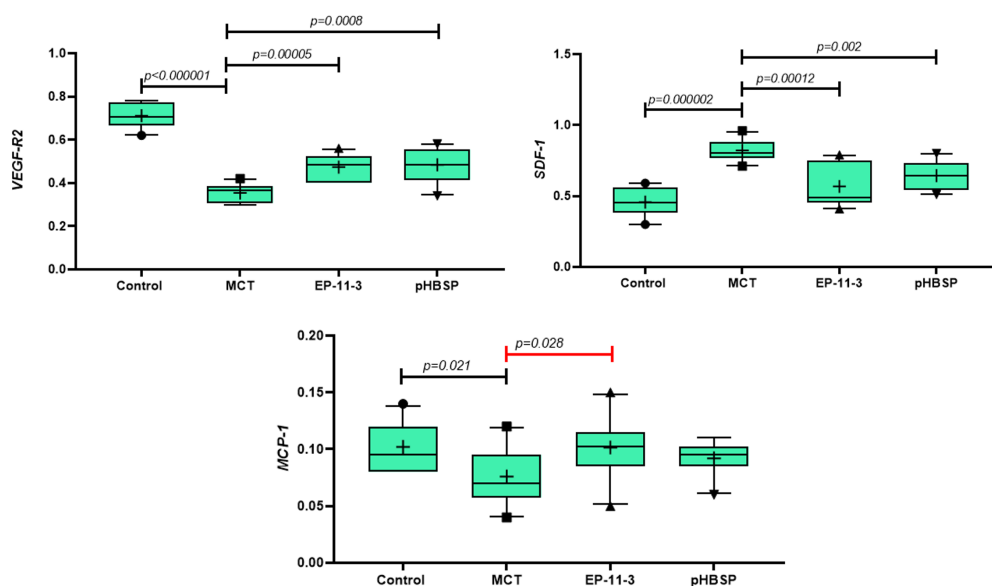


Figure 3. The effect of EP-11-3 and pHBSP on the expression of mRNA of molecular targets for the development of pulmonary hypertension. **Note:** VEGF-R2 – vascular endothelial growth factor receptor 2; SDF-1 – stromal cell factor 1; MCP-1 – monocyte chemoattractant protein-1. * – $p < 0.05$ compared to intact, ** – $p < 0.05$ compared to MCT. Control – a group of intact animals, MCT – a group of animals with pulmonary hypertension (PH) against the background of the administration of monocrotalin at a dose of 60 mg/kg; EP-11-3 – introduction of EP-11-3 peptide at a dose of 25 mcg/kg against the background of PH modeling; pHBSP – introduction of pHBSP peptide at a dose of 25 mcg/kg against the background of PH modeling.

Upon further analysis of the data obtained, it was found that the levels of VEGFR2 mRNA expression in the lungs were statistically significantly reduced, and the levels of SDF-1 were statistically significantly increased with MCT. When using EP-11-3 and pHBSP compounds, a comparable and statistically significant increase in VEGF-R2 mRNA expression and a decrease in SDF-1 mRNA expression were found. The initial peptide did not affect the expression of MCP-1 mRNA, which decreased as a result of modeling monocrotalin-induced pulmonary hypertension (Fig. 3, MCP-1). At the same time, a statistically significant increase in the expression of monocyte chemoattractant protein-1 (MCP-1) mRNA was found in the group of animals treated with erythropoietin derivatives with the laboratory code EP-11-3 (Fig. 3, MCP-1).

The degree of pulmonary edema in the experimental groups was assessed by the ratio of the mass of wet and dry lungs. The modeling of pulmonary hypertension using monocrotalin increased the ratio of the weight of a wet lung to a dry one by 25%. The degree of pulmonary edema was statistically significantly reduced with the use of EP-11-3 and pHBSP compounds, the values of this indicator in the experimental groups were lower than in the group of animals with pulmonary hypertension, and the values of the humidity coefficient in the groups using the studied compounds did not differ from the target values set in the group of intact animals (Fig. 4).

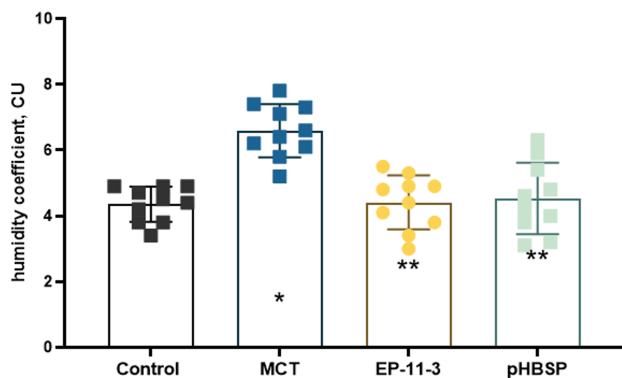


Figure 4. Quantitative assessment of the formation of pulmonary edema by assessing the ratio of the mass of wet and dry lungs in the groups of ruminants receiving EP-11-3 and pHBSP against the background of modeling pulmonary hypertension. * – $p < 0.05$ compared to intact, ** – $p < 0.05$ compared to MCT. **Note:** Control – a group of intact animals, MCT – a group of animals with pulmonary hypertension (PH) against the background of the administration of monocrotalin at a dose of 60 mg/kg; EP-11-3 – introduction of EP-11-3 peptide at a dose of 25 mcg/kg against the background of PH modeling; pHBSP – introduction of pHBSP peptide at a dose of 25 mcg/kg against the background of PH modeling; * – $p < 0.05$ compared with intact, ** – $p < 0.05$ compared with MCT.

When studying the plasma concentrations of cytokines cardiotrophin-1 (CT-1) and atrial natriuretic peptide (ANP), it was found that the levels of both factors increased statistically significantly when modeling monocrotalin-induced pulmonary hypertension. Thus, in the group of animals with untreated pulmonary hypertension (MCT),

the concentration of CT-1 increased by more than 6 times and the concentration of ANP – by more than 5 times. The use of EP-11-3 and pHBSP compounds led to a comparable and statistically significant decrease in the concentrations of CT-1 and ANP in blood plasma (Fig. 5).

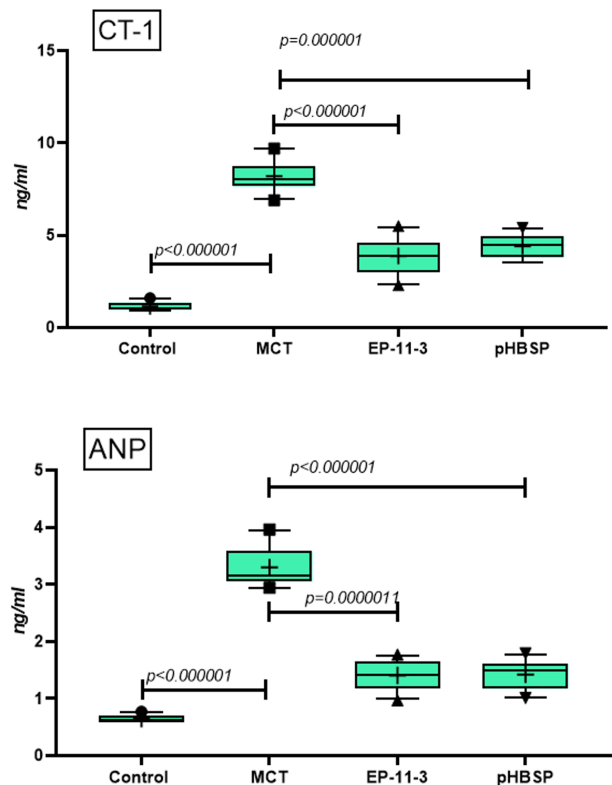


Figure 5. Effect of EP-11-3 and pHBSP on plasma concentrations of cytokines cardiotrophin-1 (CT-1) and atrial natriuretic peptide (ANP). **Note:** Control – a group of intact animals, MCT – a group of animals with pulmonary hypertension (PH) against the background of the administration of monocrotalin at a dose of 60 mg/kg; EP-11-3 – introduction of EP-11-3 peptide at a dose of 25 mcg/kg against the background of PH modeling; pHBSP – introduction of pHBSP peptide at a dose of 25 mcg/kg against the background of PH modeling; * – $p < 0.05$ compared with intact, ** – $p < 0.05$ compared with MCT.

When analyzing the wall thickness of the pulmonary artery, it was found that against the background of modeling pulmonary hypertension with the introduction of monocrotaline, the studied indicator increases by more than 2 times from 7.06 ± 0.76 microns to 16.25 ± 1.99 microns. The introduction of EP-11-3 and pHBSP leads to a statistically significant (in comparison with the MCT group) decrease in the wall thickness of the pulmonary artery. There were no statistically significant differences in the effectiveness of reducing this indicator between the groups receiving EP-11-3 and pHBSP (Fig. 6, LA wall thickness).

When assessing the number of thrombosed vessels in the field of vision, it was found that the percentage of thrombosed vessels in the lungs of animals receiving the studied peptides decreased statistically significantly, more effectively in the group of animals receiving EP-11-3. The percentage of thrombosed vessels in the lungs of animals of this group was statistically significantly lower ($p = 0.0033$) than in the group of animals receiving the pHBSP comparison drug (Fig. 6, vascular occlusion).

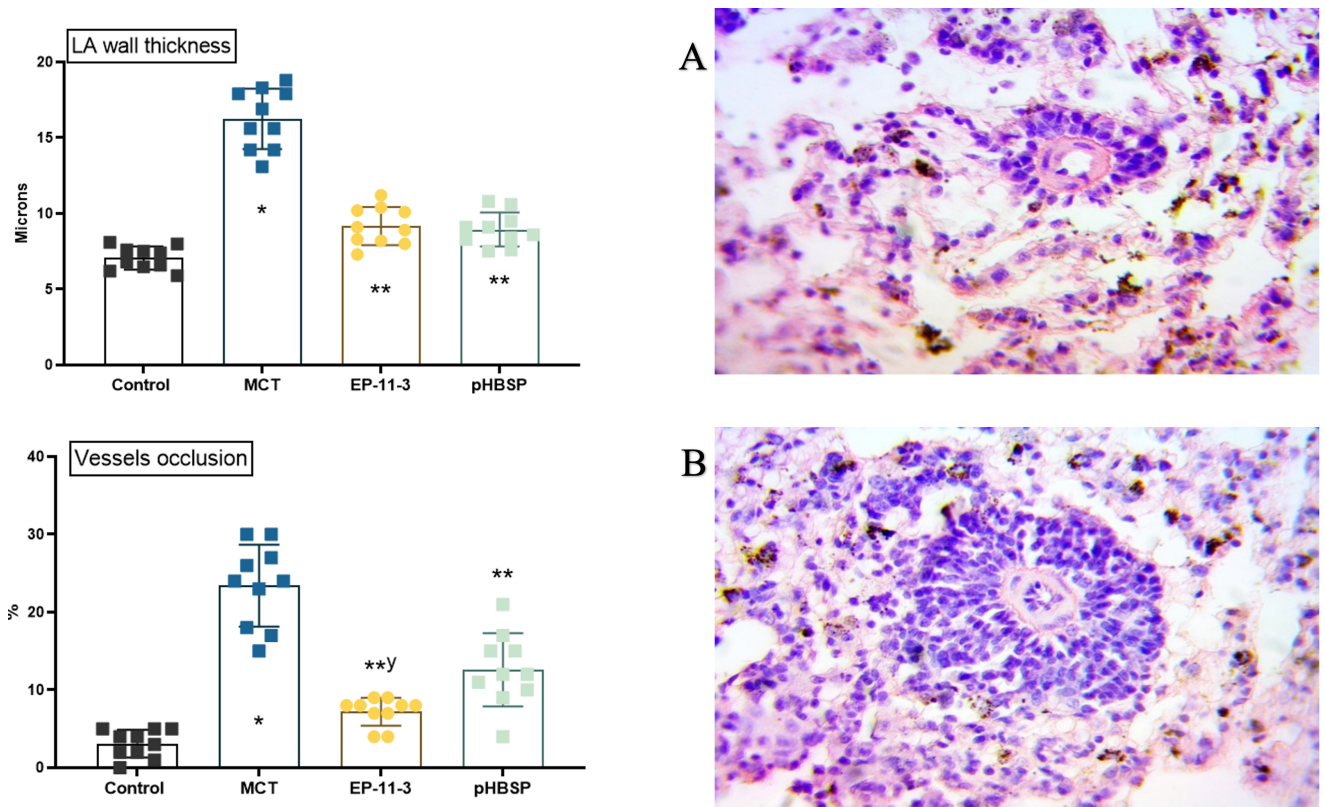


Figure 6. The effect of EP-11-3 and pHBSP on the histological structure of the lungs. *Note:* The thickness of the LA wall is the thickness of the pulmonary artery wall; A – representative microphotography of the lung section of animals treated with EP-11-3 compound, x400; B – representative microphotography of the lung of animals from the MCT group – extensive perivascular lymphohistiocytic infiltration. hematoxylin+eosin; Control – a group of intact animals, MCT – a group of animals with pulmonary hypertension (PH) against the background of the administration of **monocrotalin** at a dose of 60 mg/ kg; EP-11-3 – introduction of EP-11-3 peptide at a dose of 25 mcg/kg against the background of PH modeling; pHBSP - introduction of pHBSP peptide at a dose of 25 mcg/kg against the background of PH modelingx400. * – at $p < 0.05$ compared to intact, ** – at $p < 0.05$ compared to MCT, γ – at $p < 0.05$ compared to the pHBSP group.

Microscopic examination of histological preparations of the heart revealed that the cross-sectional area of cardiomyocytes increased statistically significantly in the group of animals with pulmonary hypertension modeling using **monocrotalin** (Figure 7). When evaluating the effectiveness of correction of morphological manifestations of pulmonary hypertension, it was shown that EP-11-3 and pHBSP in the studied doses have

pronounced pharmacological activity, statistically significantly reducing the cross-sectional area of cardiomyocytes (Figure 7). Also, the decrease in pancreatic hypertrophy was confirmed by histological examination, which demonstrated a decrease in the increase in the ratio of pancreas/(LV+S) caused by **monocrotalin**, both with the introduction of the EP-11-3 peptide and pHBSP.

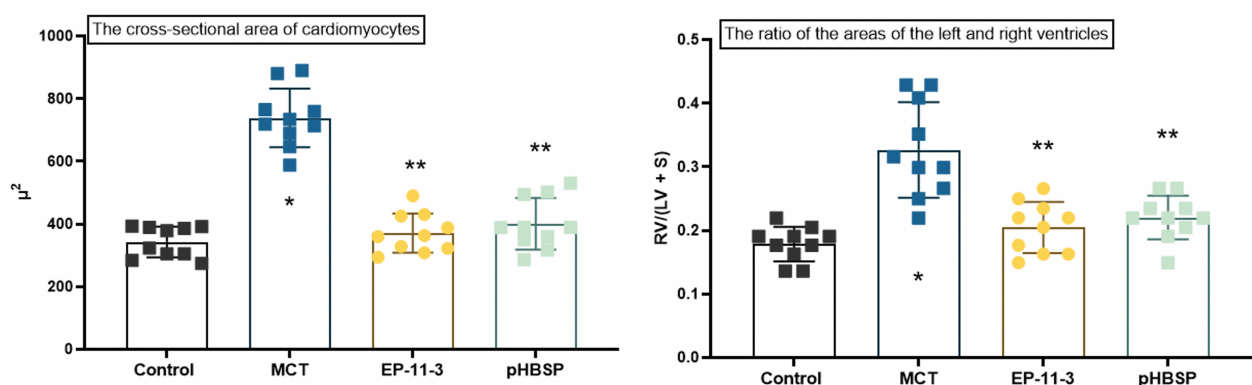


Figure 7. The effect of EP-11-3 and pHBSP on the histological structure of the right ventricle of the heart of animals in experimental groups. Stained with hematoxylin and eosin. Magnification x 400. *Note:* * – at $p < 0.05$ compared to intact, ** – at $p < 0.05$ compared to MCT.

Discussion

When forming a strategy for searching for new erythropoietin derivatives with tissue-protective properties without hematopoietic activity, the Research Institute of Pharmacology of Living Systems of the Belgorod State National Research University upgraded the original HBSP peptide by adding tripeptide motifs RGD, KGD and PGP (Golubev et al., 2020). The obtained compounds combined cytoprotective and antiplatelet effects, had endothelioprotective activity and were able to protect the vascular wall from atherosclerotic damage. This study presents the development and study of a second pool of compounds – derivatives of the HBSP peptide with the laboratory code EP-11-3 (UEQLERALNTS) obtained by searching for groups of related peptides to the pHBSP molecule using the BLAST program.

Thus, the results of a study of the pharmacological activity of erythropoietin derivatives on a model of pulmonary hypertension caused by monocrotalin showed that the compound with the laboratory code EP-11-3 has a pronounced protective effect comparable or superior to that of the original peptide pHBSP.

The endothelioprotective effect was expressed in a statistically significant decrease in systolic pressure in the cavity of the right ventricle, while the comparison drug, the initial peptide pHBSP, did not lead to a statistically significant decrease in this indicator. In this study, against the background of modeling pulmonary hypertension, the number of circulating endothelial cell precursors (CECP) in experimental groups was studied. More and more studies demonstrate that circulating endothelial progenitor cells are involved in vascular homeostasis (Pompilio et al. 2009). It was shown that the number of circulating CECP decreased by more than 2 times in groups of animals with modelled monocrotalin-induced pulmonary hypertension. In the groups of animals that were injected with the studied compounds, the number of circulating PEC increased statistically significantly – in the group of animals receiving EP-11-3, the number of circulating PEC was statistically significantly higher than in the group of animals receiving pHBSP.

To study the effect of the studied compounds on factors involved in the delivery of circulating endothelial cell precursors to the endothelium of the affected vessels, the expression of mRNA factors necessary for the delivery of PEC to the affected vessel walls was studied: vascular endothelial growth factor (VEGF), the first subtype of its receptors (VEGF-R1) and stromal cell factor -1 (SDF-1). Since inflammatory processes are involved in the pathophysiology of PAH, we also measured the levels of monocytic chemoattractant protein-1 (MCP-1), the main marker of inflammation in inflammatory processes against the background of PAH (Peng et al. 2020).

It was found that the levels of VEGF-R2 mRNA expression in the lungs were statistically significantly reduced, and the levels of SDF-1 were statistically significantly increased in PAH. When using EP-11-3 and rNBSP compounds on models of monocrotalin-induced pulmonary hypertension, a statistically significant increase in VEGF-R2 mRNA expression and a decrease in SDF-1 mRNA expression were found. At the same time, the initial pHBSP peptide did not affect the

expression of MCP-1 mRNA in the simulation of pulmonary hypertension induced by monocrotalin.

To further assess the state of the cardiovascular system against the background of modeling pulmonary hypertension, we measured the content of cardiotrophin-1 (CT-1) and atrial natriuretic peptide (ANP) in blood plasma. The first cytokine is associated with myocardial hypertrophy and cardiovascular pathology, and the second is a hormone secreted by the atria in response to high blood pressure – its effect is to reduce preload on the heart, thereby reducing blood pressure. When studying the plasma concentrations of cytokines cardiotrophin-1 and atrial natriuretic peptide (ANP), it was found that the levels of both factors increased statistically significantly when modeling monocrotalin-induced pulmonary hypertension. Thus, in the group of animals with untreated pulmonary hypertension (MCT), the concentration of CT-1 increased by more than 6 times and the concentration of ANP – by more than 4 times. The use of erythropoietin derivatives EP-11-3 and pHBSP led to a comparable and statistically significant decrease in the concentrations of CT-1 and ANP in blood plasma.

The degree of pulmonary edema in the experimental groups was assessed by the ratio of the mass of wet and dry lungs. Modeling of pulmonary hypertension using monocrotalin increased the ratio of wet to dry lung weight by 25-33%. The degree of pulmonary edema was statistically significantly reduced with the use of EP-11-3 and pHBSP compounds; the values of this indicator in the experimental groups were lower than in the group of animals with pulmonary hypertension, and the values of the humidity coefficient in the groups using the studied peptides did not differ from the target values set in the group of intact animals.

Previously, scientists had shown that when modeling pulmonary hypertension with a combination of monocrotalin and abdominal aortocaval bypass, recombinant erythropoietin reduced vascular occlusion of intraacinar pulmonary vessels and the thickness of the medial wall of preacinar ones, and also increased the density of capillaries of the right ventricle. Nevertheless, functional tests revealed a more modest effect: for example, increased mean pressure in the pulmonary artery and decreased contractility of the right ventricle in the model did not change under the influence of EPO therapy. The same scientific group, using a similar model in rats with pulmonary hypotransea under the influence of EPO, along with a decrease in lung remodeling, found an increase in the number of circulating precursors and a restriction of LH-induced activation of the enzyme hemoxygenase-1 compared with the control (van Loon et al. 2015).

In this study, the development of pulmonary arterial hypertension and its correction by the studied compounds was confirmed by histological studies. Thus, in animals with pulmonary hypertension, the progressive remodeling of pulmonary vessels was observed, including a significant increase in wall thickness, occlusion and muscularization of intraacinar vessels, as well as an increase in wall thickness and the wall/lumen ratio of preacinar pulmonary vessels compared with the control. Hypertrophy of the right ventricle was found in the heart of animals with pulmonary hypertension, including an increase in the cross-sectional area of cardiomyocytes and the ratio of the areas of the right and left ventricles of the heart. In the study of erythropoietin derivatives, it was shown that in groups of animals treated with EP-11-3 and pHBSP on the model of monocrotalin-induced pulmonary hypertension, positive dynamics was

also observed, which is expressed in a statistically significant decrease in the wall thickness of the pulmonary artery and a decrease in the cross-sectional area of cardiomyocytes. Against the background of monocrotalin-induced pulmonary hypertension in the group of animals treated with EP-11-3, the percentage of thrombosed vessels in the lungs was statistically significantly lower ($p=0.0033$) than in the group of animals treated with the comparison drug **pHBSP**.

Based on the experimental data obtained, special attention should be paid to the effectiveness of the 11-amino acid derivatives of erythropoietin studied in this work. The integral function of erythropoietin and its derivatives in systemic and local shifts during adaptation to hypoxia suggests its participation in the systemic response in pulmonary hypertension. Since pulmonary vascular dysfunction leads to impaired gas exchange function and impaired hemodynamics, it is logical to assume that the resulting systemic hypoxia leads to the activation of feedback, through the secretion of erythropoietin by the kidneys and other EPO-producing organs. The proposed mechanism of implementation of erythropoietin and its derivatives modeling the activity of the EPOR/CD131 heteroreceptor of endothelioprotective action in pulmonary hypertension is shown in Figure 8.

To date, the use of erythropoietin in endothelium-associated pathology has not been sufficiently studied. In a line of mice that constitutively overexpress human erythropoietin, a decrease in vascular remodeling processes, a decrease in vascular resistance and vasoconstrictor response were demonstrated both under normal conditions and during the modeling of chronic normobaric hypoxia. These facts were confirmed by the results of our studies with the introduction of erythropoietin derivatives devoid of hematopoietic activity – the introduction of **pHBSP** and EP-11-3 peptides leads to

normalization of vascular homeostasis in animals against the background of modeling pulmonary hypertension with the introduction of **monocrotalin** and hypoxia.

A number of studies have confirmed the fabric-protective effects of HBSP on various models. Despite the fact that HBSP exhibits protective effects similar to EPO, its short half-life in blood plasma, which is approximately 2 minutes, limits its use in the clinic (Brines et al. 2008).

Proteomic analysis has shown that mediated tissue protection is associated with the regulation of energy metabolism and reduction of oxidative stress (Peng et al. 2020).

Conclusion

On the model of monocrotalin-induced pulmonary hypertension, it was shown that the erythropoietin derivative with the laboratory code EP-11-3 has a pronounced endothelioprotective effect, reducing the coefficient of endothelial dysfunction, statistically significantly increasing the expression of VEGF-R2 mRNA and reducing the expression of SDF-1 mRNA, reducing the concentrations of CT-1 and PNP, and reducing the signs of remodeling of the heart and pulmonary vessels. At the same time, EP-11-3, unlike the original **pHBSP** peptide, which mimics the spatial structure of the erythropoietin b chain, statistically significantly reduces systolic pressure in the cavity of the right ventricle of the heart and increases the number of circulating endothelial cell precursors.

Conflict of Interests

The authors declare the absence of a conflict of interests.

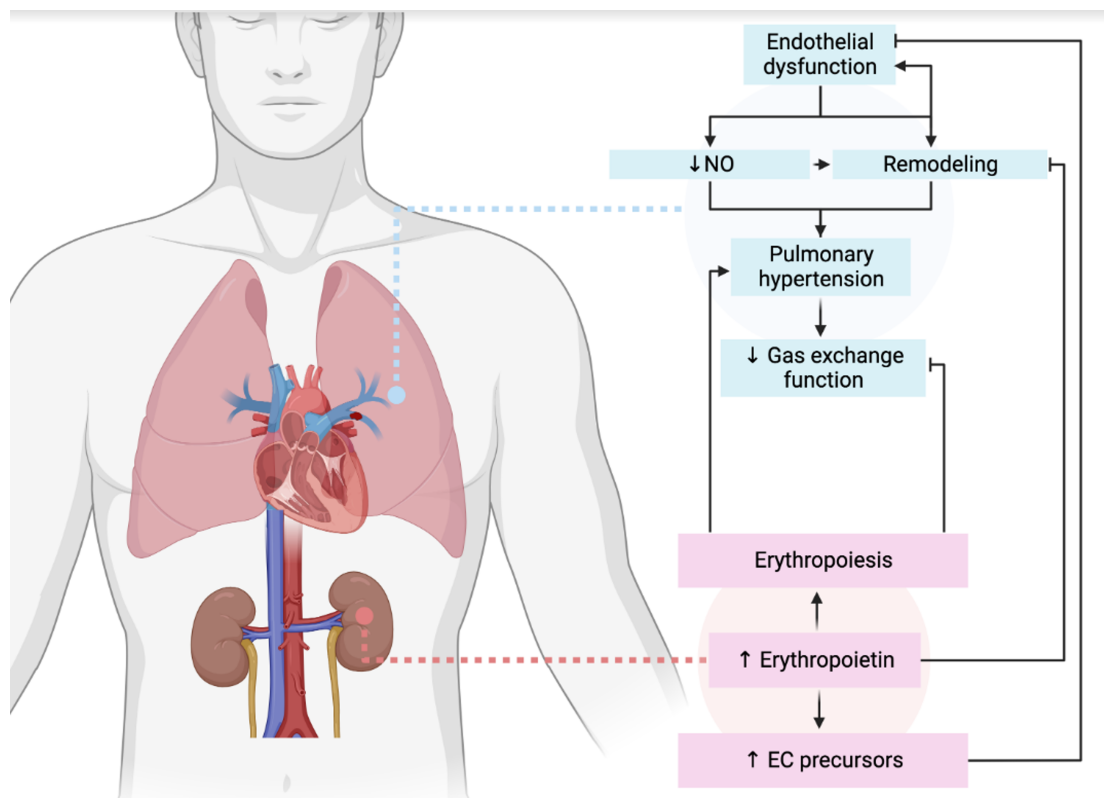


Figure 8. Compensatory role of erythropoietin in the pathogenesis of pulmonary hypertension (PH). *Note:* EC – endothelial cells.

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