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Studying the possibilities of pharmacological correction of hypoxia-induced pulmonary hypertension using a phenolic compound with a laboratory cypher KUD975

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The aim of our work was to study a pharmacological activity of a selective arginase-2 and thrombin inhibitor from a phenolic compounds group with a laboratory cypher KUD975 on a model of arterial pulmonary hypertension induced by hypoxia.

Materials and methods. To simulate pulmonary hypertension (PH), animals were placed in a normobaric hypoxic chamber and subjected to 5 weeks of hypoxia with an oxygen content of 10% in the air. After 3 weeks of hypoxia, the animals were administered with the test compound KUD975 (intragastrically, at a dose of 2 mg/kg once a day for 2 weeks). L-norvaline (intragastrically, 20 mg/kg) was used as a reference drug. To assess the development and correction of PH, measurements of cardiohemodynamics, analysis of blood gas composition, study of the number of circulating endothelial precursor cells (EPCs), quantitative PCR assessing the expression of mRNA VEGF-R2, SGF-1 (stromal growth factor-1) and MCP-1 (monocyte chemoattractant protein-1). Next, a histological examination of the lungs and heart was performed, the degree of pulmonary edema and the concentration of cardiotrophin-1 and atrial natriuretic peptide were assessed.

Results. The administration of the studied phenolic compound with laboratory cypher KUD975, as well as the reference drug L-norvaline, led to a decrease in the right ventricular systolic pressure against the background of modeling PH. The present study shows a more than twice-fold decrease in the number of circulating (EPCs) in the animals group with modeling a hypoxia-induced circulatory PH (171.3±12.1) in comparison with the group of intact animals (296.1±31.7; p=0.0018). The recovery of EPCs was noted in the animals group administered with KUD-975 and L-norvaline, up to 247.5±34.2 (p=0.0009 compared with a pulmonary arterial hypertension (PAH) and 235.6±36.4 (p=0.008 compared to PAH), respectively. The studied compounds had a protective effect by statistically significantly increasing the expression of VEGF-R2 mRNA and decreasing the expression of SGF-1 mRNA, reducing the lung moisture coefficient and the concentrations of cardiotrophin-1 and atrial natriuretic peptide and preventing vascular remodeling caused by hypoxia.

Conclusion. When studying the pharmacological activity, it was shown that the phenolic compound with the laboratory cypher KUD975 normalizes hemodynamic parameters, reduces the signs of remodeling of the heart and pulmonary vessels and has a pronounced endothelial protective effect on the model of hypoxia-induced PH, and is superior to the activity of the reference drug L-norvaline.

Keywords: pulmonary hypertension; endothelial dysfunction; nitric oxide; heterocyclic acids; endothelium; arginase-2; thrombin

Abbreviations: PH – pulmonary hypertension; PAH – pulmonary arterial hypertension; CTPH – chronic thromboembolic pulmonary hypertension; COPD – chronic obstructive pulmonary disease; RVAP – right ventricular average pressure; RVSP – right ventricular systolic pressure; HR – heart rate; EPCs – endothelial precursor cells; SDF-1 – stroma-derived growth factor; VEGF – vascular endothelial growth factor; MCP-1 – monocyte chemoattractant protein-1; RV – right ventricle; LV – left ventricle; PaO_2 – oxygen partial pressure; $PaCO_2$ – carbon dioxide partial pressure; EC – endothelial cell; MP – interventricular septum; ED – endothelial dysfunction.

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Изучение возможностей фармакологической коррекции легочной гипертензии, индуцированной гипоксией, с использованием соединения фенольной природы с лабораторным шифром КУД975

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Цель. Изучение фармакологической активности селективного ингибитора аргиназы-2 и тромбина из группы соединений фенольной природы с лабораторным шифром КУД975 на модели артериальной легочной гипертензии, индуцированной гипоксией.

Материалы и методы. Для моделирования легочной гипертензии (ЛГ) животных помещали в нормобарическую гипоксическую камеру и подвергали 5 неделям гипоксии с содержанием кислорода в воздухе 10%. После 3-х недель гипоксии животным вводили исследуемое соединение КУД975 (внутрижелудочно в дозе 2 мг/кг 1 раз в сут в течение 2 недель). В качестве препарата сравнения использовали L-норвалин (внутрижелудочно 20 мг/кг). Для оценки развития ЛГ и ее коррекции проводили измерение показателей кардиогемодинамики, анализ газового состава крови, изучение количества циркулирующих предшественников эндотелиальных клеток (ПЭК), количественную ПЦР с оценкой экспрессии мРНК VEGF-R2, SDF-1 (стромальный фактор роста-1) и MCP-1 (моноцитарный хемоаттрактантный белок-1). Далее проводили гистологическое исследование легких и сердца, оценивали степень отека легких и концентрацию кардиотрофина-1 и предсердного натрийуретического пептида.

Результаты. Введение исследуемого соединения фенольной природы с лабораторным шифром КУД975, как и препарата сравнения L-норвалина, привело к уменьшению систолического давления в полости правого желудочка сердца на фоне моделирования ЛГ. В настоящем исследовании показано снижение количества циркулирующих ПЭК более чем в 2 раза в группе животных с моделированием циркуляторной легочной гипертензии, индуцированной ЛГ (171,3±12,1), в сравнении с группой интактных животных (296,1±31,7; p=0,0018). Восстановление ПЭК было отмечено в группе животных, получавших КУД975 и L-норвалин, до 247,5±34,2 (p=0,0009 в сравнении с легочной артериальной гипертензией (ЛАГ) и 235,6±36,4 (p=0,008 в сравнении с ЛАГ) соответственно. Исследуемые соединения оказывали протективное действие, статистически значимо повышая экспрессию мРНК VEGF-R2 и снижая экспрессию мРНК SDF-1, а также снижая коэффициент влажности легких и концентрации кардиотрофина-1 и предсердного натурийуретического пептида и предотвращая сосудистое ремоделирование, вызванное гипоксией.

Заключение. При изучении фармакологической активности показано, что соединение фенольной природы с лабораторным шифром КУД975 нормализует показатели гемодинамики, уменьшает признаки ремоделирования сердца и легочных сосудов и обладает выраженным эндотелиопротективным действием на модели ЛГ, индуцированной гипоксией, и превосходит активность препарата сравнения L-норвалин.

Ключевые слова: легочная гипертензия; эндотелиальная дисфункция; оксид азота; гетероциклические кислоты; эндотелий; аргиназа-2; тромбин

Список сокращений: ЛГ – легочная гипертензия; ЛАГ – легочная артериальная гипертензия; ХТЭЛГ – хроническая тромбоэмболическая легочная гипертензия; ХОБЛ – хроническая обструктивная болезнь легких; СДПЖ – систолическое давление в полости правого желудочка; СрДПЖ – среднее давление в полости правого желудочка; ДДПЖ – диастолическое давление в полости правого желудочка; ЧСС – частота сердечных сокращений; ПЭК – предшественники эндотелиальных клеток; SDF–1 – стромальный фактор роста-1; VEGF – фактор роста эндотелия сосудов; MCP–1 – моноцитарный хемоаттрактантный белок–1; ПЖ – правый желудочек; ЛЖ – левый желудочек; PaO_2 – парциальное давление кислорода; $PaCO_2$ – парциальное давление углекислого газа; ЭК – эндотелиальная клетка; МП – межжелудочковая перегородка; ЭД – эндотелиальная дисфункция.

INTRODUCTION

Pulmonary hypertension (PH) is a progressive and often fatal cardiopulmonary disease characterized by an increased pulmonary artery pressure, structural changes in the pulmonary circulation, and the development of vascular complications [1].

PH, in general, consists of a mixed group of disorders, all of which ultimately lead to an increased pulmonary arterial pressure (AP). PH is clinically classified by the 6th World Symposium on Pulmonary Hypertension (WSPH, 2018) as Group 1 PH depending on the underlying etiology. Other clinical subgroups include

Group 2 PH, which develops due to the underlying heart failure (reduced or preserved ejection fraction), a valvular heart disease, or congenital heart defects [2]. Group 3 PH occurs due to lung diseases or hypoxia. Group 4 PH develops due to the pulmonary artery obstruction, which also includes chronic thromboembolic PH (CTEPH) [3]. CTEPH is characterized by a chronic organization of thrombi in the pulmonary arterioles, followed by fibrosis and vascular stenosis [4]. Group 5 PH is a complex cohort, often due to the multifactorial etiology [3].

Pulmonary arterial hypertension (PAH) is a particularly challenging form of PH because it involves a progressive hyperproliferative process that, if untreated, leads to the right ventricular failure and death [5, 6]. The pathophysiology of PAH is complex and variable, with multiple molecular mechanisms and underlying disorders involved in pathogenesis. However, the most common pathological features, regardless of the initial etiological factor, are a dysfunction of pulmonary artery endothelial cells, proliferation and migration of pulmonary artery smooth muscle cells, and a fibroblast activity dysregulation [5, 7].

Hypoxia-induced PH is a potentially severe and fatal lung disease. It is known that chronic hypoxia leads to a pulmonary vascular remodeling, PH and the right ventricular (RV) hypertrophy with a subsequent risk of developing the right ventricular failure. Chronic lung diseases such as a chronic obstructive pulmonary disease (COPD), cystic fibrosis and bronchopulmonary dysplasia can lead to diffuse chronic alveolar hypoxia [8]. The development of PH is associated with a significant morbidity and mortality in these patients [9, 10].

Despite this, there are currently a few treatments for PH, and prevention strategies remain largely unknown.

THE AIM of our work was to study a pharmacological activity of a selective arginase-2 and thrombin inhibitor from a phenolic compounds group with a laboratory cypher KUD975 on a model of arterial pulmonary hypertension induced by hypoxia.

MATERIALS AND METHODS

Experimental animals

All experimental studies were carried out in accordance with the Rules of Good Laboratory Practice, approved by Order No. 708n of the Ministry of Health of Russia dated August 23, 2010, in strict compliance with the European Convention for the Protection of Vertebrate Animals Used for Experiments or Other Scientific Purposes (Directive 2010/63/EU). Experimental studies were approved by the Bioethical Commission of Belgorod State National Research University (protocol No. 11/9 dated February 12, 2022). The vivisection was carried out in accordance with the ethical principles for the treatment of laboratory animals as set out in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (CETS No. 123).

C57Bl/6J mice (*n*=40) obtained from the Experimental Biological Clinic of Belgorod State National Research University were used as the main test system. After completing the 14-day quarantine regime, the mice were stratified by weight and placed in separate conventional cages in accordance with their belonging to the experimental group. Before and during the study, the animals were kept in rooms with artificial lighting (12 h day:12 h night) at the temperature of 21–23°C, the humidity of 38–50% and had a free access to food and water.

Study design

To simulate PH, the animals were placed in a normobaric hypoxic chamber (the authors' development) with the air gas composition control. The experimental mice were subjected to 3 weeks of hypoxia with 10% oxygen in the air. The normobaric hypoxic chamber was opened for 1 h once every 3 days to replace bedding, water bottles, and food. Wild-type (C57BL/6) mice of both sexes (10–12 weeks of age) were used, and the animals were evenly randomized by sex (females and males per group in a 50/50 ratio) and weight (weight per group in the range of 24±2 g). The animals of the control group were kept in a normobaric hypoxic chamber under the normoxia conditions. After 3 weeks of keeping the animals in a hypoxic chamber, they received various medicinal compounds for another 2 weeks under the same environmental conditions. Thus, the animals of the experimental groups were kept in a hypoxic chamber for 5 weeks [11].

Compounds under study

In this work, the pharmacological activity of a phenolic compound with laboratory code KUD975 2-((1-hydroxynaphthalene-2-yl)thio)acetyl)-*D*-proline methyl ester) was studied. The compound was synthesized by a group of scientists under the leadership of Doctor of Sciences (Chemistry) Konstantin V. Kudryavtsev (Pirogov Russian National Research Medical University). The structural formula of the compound under study shown in Figure 1.

The arginase inhibitor L-norvaline ($C_5H_{11}NO_2$, Clearsynth, India) was used as a reference drug.

KUD975 was administered intragastrically at a dose of 2 mg/kg once a day for 2 weeks. L-norvaline was used as a reference drug at a dose of 20 mg/kg intragastrically.

Thus, the following experimental groups were formed:

1. Control (1% starch solution intragastrically);
2. Hypoxia-induced PAH;

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3. PAH+KUD975 at a dose of 2 mg/kg for 14 days;

4. PAH+L-norvaline at a dose of 20 mg/kg for 14 days.

The calculation of dosage and dosing regimens of the studied compounds and reference drugs are based on their effectiveness in the experimental studies conducted previously in the field of pharmacological correction of the endothelium-associated pathology [12–15].

The doses were recalculated using interspecies coefficients, and the design of the experimental studies was carried out in accordance with the recommendations for preclinical studies[1,2].

Pressure measurement in right ventricle heart cavity, analysis of blood gas composition

A pressure measurement in the RV heart cavity and a gas composition of the venous blood were measured in mice under anesthesia (2–2.5% isoflurane in 100% oxygen) after 5 weeks from the start of the experiment. To do this, a small skin incision was made on the neck of the mice and the right external jugular vein was isolated, then catheterized with a polyethylene (PE 10) catheter. Next, the catheter was passed into the right ventricle (RV) of the heart cavity. In each animal, the BR in the pancreatic cavity was recorded continuously with a sampling frequency of 1 kHz for at least 30 sec using a piezoelectric pressure sensor and an MP-150 polygraph (BIOPAC Systems, Inc. USA). The correct anatomical position of the catheter tip was monitored by continuously monitoring the pressure signal waveform. A systolic pressure in the right ventricular cavity (RVSP), a mean pressure in the right ventricular cavity (mean RVSP), a diastolic pressure in the right ventricular cavity (RVDP), a heart rate (HR), dP/dt max, dP/dt min were determined. Hemodynamic parameters were determined using the Biopac MP-150 hardware complex (BIOPAC Systems, Inc. USA) and the AcqKnowledge 3.8.1 computer program (USA). After measuring hemodynamic parameters, the animal was removed from the experiment by the ethyl ether overdose, and the blood was drawn to analyze the gas composition (partial pressure of oxygen and carbon dioxide) [11].

Study of the number of circulating endothelial progenitor cells

To measure the level of circulating endothelial progenitor cells (EPCs), the cell culture and staining method described in the study by Pan Y et al, was used [16]. Mononuclear cells were isolated from peripheral blood by centrifugation (Eppendorf 5430R centrifuge, Germany) in a Histopaque-1083 solution (solution containing polysucrose and sodium diatrizoate adjusted to the density of 1.083 g/ml) according to the manufacturer's instructions (Sigma Chemical, USA). The isolated mononuclear cells were seeded in a triplicate on 96-well plates coated with 1% gelatin in the endothelial cell basal medium (Thermo Scientific, USA) supplemented with 2% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 µg/ml). After 2 days of culture, the adherent cells were thoroughly washed with the medium and co-stained with DiI AcLDL (Thermo Scientific, USA).

Quantitative PCR

To investigate the studied drugs effect on the molecular mechanisms of the PH development, a real-time polymerase chain reaction to determine the expression of mRNA VEGF-R2, SDF-1 (stromal growth factor-1) and MCP-1 (monocyte chemoattractant protein-1) was performed. To carry out a quantitative real-time PCR, a part of the lung was homogenized and incubated for 10 min at 37°C in the "Extract RNA" solution. After lysing the sample in the reagent, it was subjected to the chloroform purification, the supernatant sample was collected and washed with isopropyl alcohol and 70% ethyl alcohol. The concentration of the resulting RNA was measured on an IMPLEN NanoPhotometer® NP80 Spectrophotometer (IMPLEN, Germany) and adjusted to the concentration of 300 ng/µl by adding deionized water (CJSC Evrogen, Russia). A reverse transcription was performed using the MMLVRTSK021 kit in accordance with the protocol of the manufacturer (CJSC Evrogen, Russia). The gene expression level was assessed relative to the values of the reference gene Gapdh. The expression at a specific point was calculated using the formula [8]:

Gene Expression=[(Ct(Gapdh)/Ct (Gene of Interest)]

Methods of histological examination

For a histological examination, organs (the heart and lungs) were removed and fixed in 10% neutral formalin. Then the material was poured into paraffin in a standard mode in a carousel-type machine "STP-120" (Microm International GMbH, Germany). The examination of histological preparations was performed under an Axio Scope A1 microscope (Carl Zeiss Microimaging GMbH, Germany); morphometry was performed using the Image J 1.54d program.

The thickness of the pulmonary artery wall was determined, the pulmonary vessels near the alveoli were assessed, and the diameters of 20 vessels were determined on a slide. Five sections from each animal were evaluated. At the same time, the number of

[1] Guidelines to experimental (preclinical) study of new pharmacological substances. Khabrieva RU, editor. Scientific Center for Examination of Medical Products Applications; 2nd ed., revised. Moscow: Shiko Publishing House, 2005. 826 p. Russian

[2] Guidelines for conducting preclinical studies of drugs: in 2 parts. Scientific Center for Examination of Medical Products Applications. Mironov AN et al, editors. Moscow: "Grif and K", 2012. Part 1. 940 p. Russian

thrombosed vessels in the field of view was assessed (microscope magnification ×400). Approximately 20 peribronchial pulmonary artery vessels were assessed on each hematoxylin and eosin-stained slide (microscope magnification ×400). The degree of occlusion was determined as the ratio between the outer and inner (i.e., luminal) circumferences of each vessel. The degree of cardiac RV myocardial hypertrophy was determined using the image analysis Software MCID 7.0 Image Research. For this purpose, a horizontal section 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 manually divided with a thin line in the same way for all sections. Then, a pixel-by-pixel analysis of the RV and LV areas with the interventricular septum (IVS) was carried out. The results are presented as the ratio of RV/(LV+IVS).

Determination of pulmonary edema degree

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

Biochemical markers

Cardiotrophin-1 (CT-1) and atrial natriuretic peptide (ANP) were measured in serum using ELISA kits (ELM-Cardiotrophin-1/EIA-ANP-1, RayBiotech, Norcross, USA) according to the manufacturer's instructions.

Statistical analysis

The data were tested for a normal distribution using the Shapiro–Wilk test. The normally distributed data were compared using an ordinary one-way analysis of variance (ANOVA) with Tukey post hoc test. The non-normally distributed data were compared with the Kruskal–Wallis test and Dunn's post hoc test. The differences were determined at a significance level of p <0,05. The experimental data are presented as M±SD values. The statistical analysis was performed using GraphPad Prism 9.2.0 Software.

RESULTS

Modeling PH in a hypoxic chamber led to a statistically significant increase in RVSP by almost twice, RVDP – by more than twice, a maximum contraction speed (dP/dt max) and a minimum contraction speed (dP/dt min) and did not lead to a statistically significant heart rate change (Table 1). The administration of the test compound with a laboratory code KUD975, as well as the reference drug L-norvaline, led to a statistically significant decrease in all studied parameters, and the indicator dP/dt max in the groups of the animals with the administration of the study drugs was as close to those values in the group of the intact animals as possible (Table 1).

To characterize the state of the vascular endothelium, the number of circulating EPCs which statistically significantly decreased in the animals with circulatory PH induced by hypoxia, was assessed (Fig. 1).

This study shows a more than 2-fold decrease in the number of circulating EPCs in the group of animals with modeling of circulatory PH induced by hypoxia (171.3±12.1) in comparison with the group of intact animals (296.1±31.7; p=0.0018). In the groups of animals that had been administered the test compounds, the number of circulating EPCs increased statistically significantly (Fig. 2).

When analyzing the results of the blood gas composition study, a similar picture was found – a statistically significant decrease in the partial oxygen pressure (PaO^2) against the background of a statistically significant increase in the partial carbon dioxide pressure ($PaCO^2$) in the group of animals with PH. The compounds KUD975 and L-norvaline statistically significantly (in comparison with the PAH group) and comparable restored the values of the blood gas composition in the animals of the experimental groups (Fig. 3).

It was established 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 the compound KUD975 and L-norvaline, a statistically significant increase in the expression of VEGF-R2 mRNA and a decrease in the expression of SDF-1 mRNA were established.

Herewith, the degree of increase in the VEGF-R2 mRNA expression in the group of animals receiving KUD975 was statistically significantly higher than that in the group of the animals receiving L-norvaline. At the same time, the studied compounds did not affect the expression of MCP-1 mRNA when modeling hypoxia-induced PH (Fig. 4).

The degree of pulmonary edema in the experimental groups was assessed by the ratio of the weight of fluid and dry lungs. Simulating pulmonary hypertension with hypoxia increased the fluid-to-dry lung weight ratio by 33%. The degree of pulmonary edema was statistically significantly reduced with the use of the compound KUD975 and L-norvaline. The value of this indicator in the experimental groups was lower than in the group of animals with PH. The value of the lungs humidity coefficient in the group of animals using KUD975 was as close as possible to the target values set in the group of intact animals (Fig. 5).

When studying the concentration of cytokines

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CT-1 and ANP in the blood plasma, it was found out that the level of both factors increased statistically significantly when PH was modeled using hypoxia. Thus, in the group of PH animals without treatment (PAH), the concentrations of CT-1 and ANP increased by more than 5 times (Fig. 6). The use of the compounds KUD975 and L-norvaline led to a statistically significant decrease in the concentrations of CT-1 and ANP in the blood plasma.

A histological picture of the lungs in the group of animals with circulatory PH induced by hypoxia is presented in Figure 7.

When analyzing the thickness of the pulmonary artery (PA) wall, it was found out that against the background of modeling circulatory PH using hypoxia, the studied indicator increased more than twice from 0.742±0.049 to 1.728±0.24 μm.

The administration of KUD975 and L-norvaline led to a statistically significant (compared to the PAH group) decrease in the thickness of the PA wall. There were no statistically significant differences in the effectiveness of reducing this indicator between the groups receiving KUD975 and L-norvaline. When assessing the number of thrombosed vessels in the field of view, it was found out that this indicator in the lungs of animals receiving KUD975 and L-norvaline was statistically significantly lower than in the group of animals with PH without treatment (Fig. 7).

When assessing the effectiveness of correcting the morphological manifestations of PH in the heart, it was shown that KUD975 and L-norvaline in the studied doses had a pronounced pharmacological activity, statistically significantly reducing the cross-sectional area of cardiomyocytes. The reduction in RV hypertrophy was also confirmed by a histological examination, demonstrating a decrease in the hypoxia-induced increase in the RV/(LV+S) ratio with both KUD975 and L-norvaline treatment (Fig. 8).

DISCUSSION

It is now clear that the development of new arginase inhibitors represents a promising strategy for the treatment of diseases associated with the nitroxidergic system. Given the different expression of arginase-1 and arginase-2 in tissues and their different physiological actions, a large number of specific and selective inhibitors of these two isoforms of the enzyme are available today. For example, endothelial cells express both isoforms of arginases, but it is not known exactly what the role of each of these isoforms is in the development of endothelial dysfunction (ED). A considerable controversy remains regarding the role of arginase expression in various conditions such as atherosclerosis and other forms of vascular inflammation. For example, hyperglycemia in diabetes causes ED through the activation of p38 mitogen-activated protein kinase (MAPK). That causes Arg1 upregulation in coronary arteries and increased Arg2 expression in mesenteric arteries [17, 18].

The studies have shown that arginase blockade can prevent the reduction of angiogenesis by increasing the NO-induced VEGF expression, initiate a vascular repair in the experimental ischemic retinopathy (a NOS function normalization and a reduction of a superoxide production) [19], promote wound healing in mice, and prevent morphofunctional changes in the cardiovascular system against the background of preeclampsia [20].

Thus, there is a substantial evidence for the therapeutic potential of arginase inhibition against endothelium-associated pathology interrelated with a low bioavailability of NO. Therefore, this enzyme is very attractive from the point of view of research and development of new compounds – drug candidates for the treatment of endothelium-associated pathology. On the other hand, we know the effectiveness of a multidirectional approach to the pharmacological correction of ED, when two or more compounds, different in their mechanism of action and point of application, are used for therapeutic effects [21, 22].

The combination effect of one compound on 2 different targets, representing two different parts of the ED pathogenesis, seems promising for the development of new drug candidates. Thrombin was chosen as a second target, which, in addition to arginase-2, is interesting for the inhibition in the conditions accompanied by ED. The interaction of platelets with vessel walls plays an important role in acute cardiovascular diseases [23].

Thrombin is a powerful platelet activator, having a pronounced effect on the endothelium. Endothelial cells (ECs) have an antithrombotic activity by releasing nitric oxide (NO) and prostacyclin, which are potent vasodilators and inhibitors of the platelet activity. The blood clotting enzyme thrombin, produced on the surface of damaged endothelium, induces blood clotting and has many functional effects on the endothelium itself. Thrombin acts on ECs by stimulating the synthesis and release of various agents, such as inflammatory mediators, vasoactive substances and growth factors. It causes adhesion of leukocytes to the endothelium, triggering the expression of adhesion molecules on the cell surface, and causes a disruption of the endothelial permeability. It is known that the effect of thrombin on EC is mediated by its receptor. To date, different responses of EC to thrombin have been shown. In general, capillary endothelial cells appear to be particularly sensitive to this enzyme. Thrombin-induced ED in the microvasculature can have pathological consequences and contribute to a target organ damage in the endothelium-associated pathology [24, 25].

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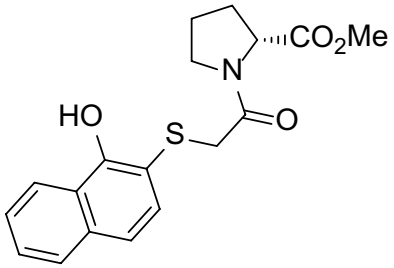

Figure 1 – Structural formula of compound under study with laboratory cypher KUD975 – 2-((1-hydroxynaphthalene-2-yl)thio)acetyl)-*D*-proline methyl ester

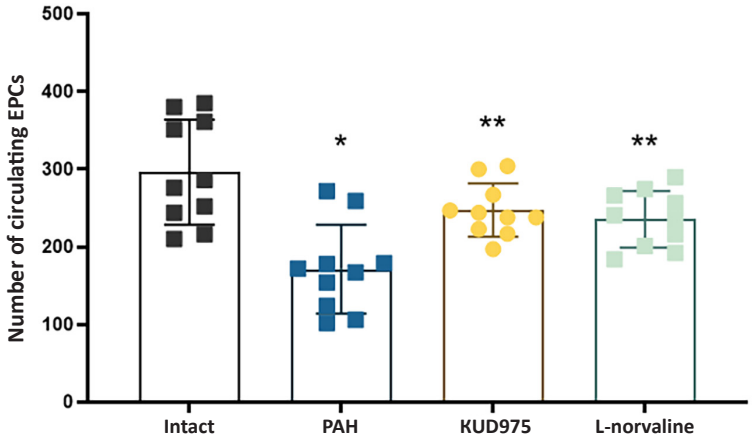

Figure 2 – Effect of compounds under study on the number of circulating endothelial cell precursors (EPCs) in animals' blood in experimental groups with pulmonary hypertension against the background of hypoxia

Note (here and in Fig. 3–6): intact – group of intact animals; PAH – hypoxia-induced pulmonary arterial hypertension; KUD975 – administration of the compound KUD975 at a dose of 2 mg/kg against the background of PH simulation; L-norvaline – administration of L-norvaline at a dose of 20 mg/kg against the background of PH modeling; * – p <0.05 compared to intact, ** – p <0.05 compared to PAH.

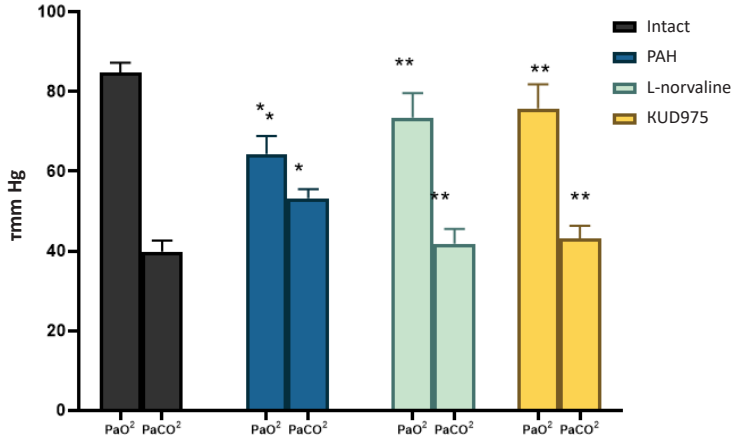

Figure 3 – Effect of KUD975 and L-norvaline on partial pressure of oxygen and carbon dioxide in experimental groups with pulmonary hypertension against the background of hypoxia

Note: PaO^2 – partial pressure of oxygen; $PaCO^2$ – partial pressure of carbon dioxide; PAH – hypoxia-induced pulmonary arterial hypertension.

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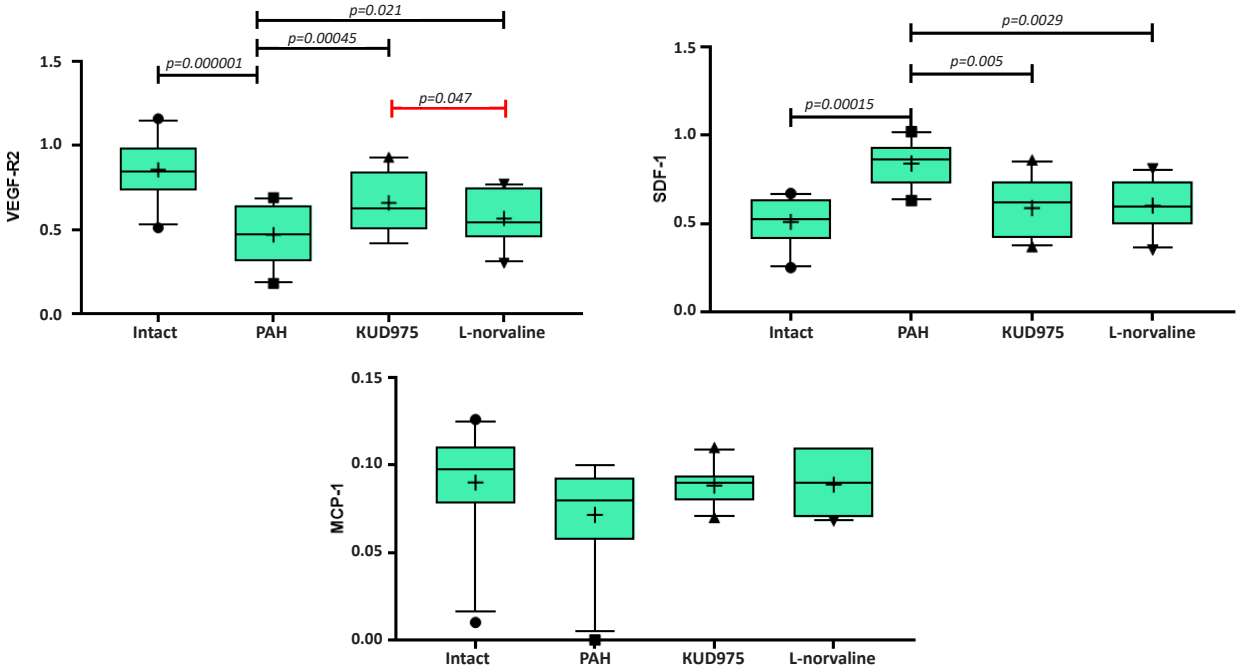

Figure 4 – Effect of KUD975 and L-norvaline on the expression of mRNA molecular targets for the development of pulmonary hypertension against the background of hypoxia

Note: VEGF-R2 – vascular endothelial growth factor receptor 2; SDF-1 – stromal cell factor 1; MCP-1 – monocyte chemoattractant protein-1.

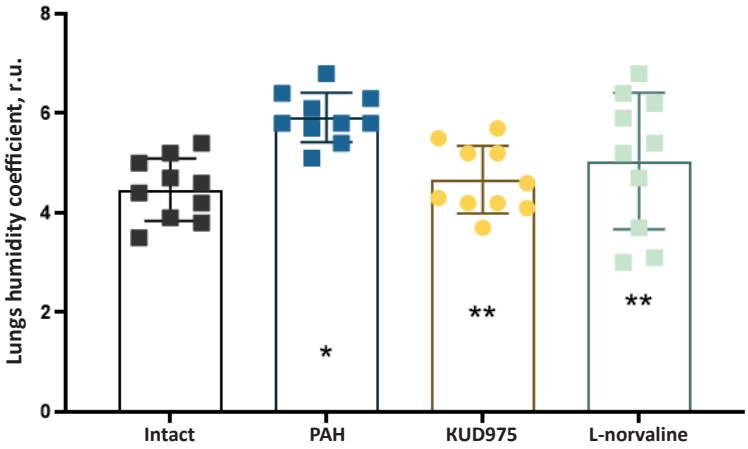

Figure 5 – Quantitative assessment of pulmonary edema formation by assessing ratio of humidity and dried lungs mass in groups of animals receiving KUD975 and L-norvaline against the background of modeling pulmonary hypertension with hypoxia

Note: * – p <0.05 compared to intact; ** – p <0.05 compared to PAH.

Table 1 – Indicators of cardiohemodynamics in animals' groups with pulmonary hypertension modeling and its correction with the help of studied compounds

Indicators	Control	PAH	PAH+KUD975	PAH+L-norvaline
RVSP	28.8±4.84	51.8±19.23*	32.5±7.51**	33.1±4.9**
RVDP	2.527±0.32	5.706±0.78*	3.918±0.5**	4.019±0.59**
dP/dt max	104.5±17.2	68.8±15.5*	88.4±10.9**	87.6±11.2**
dP/dt min	99.4±11.5	74.9±9.25*	86±7.53**	82.5±7.81**
HR	365.6±19.4	336.8±24.37	344.2±31.6	350.7±26.5

Note: PAH is a group of animals with hypoxia-induced pulmonary hypertension; RVSP – systolic pressure in the cavity of the right ventricle of the heart; RVDP – diastolic pressure in the cavity of the right ventricle of the heart; HR – heart rate; dP/dt max – maximum rate of increase in intraventricular pressure; dP/dt min – minimum rate of increase in intraventricular pressure. * – p <0.05 compared with the group of intact animals; ** – p <0.05 compared with the group of animals with experimental pulmonary arterial hypertension without treatment (PAH group).

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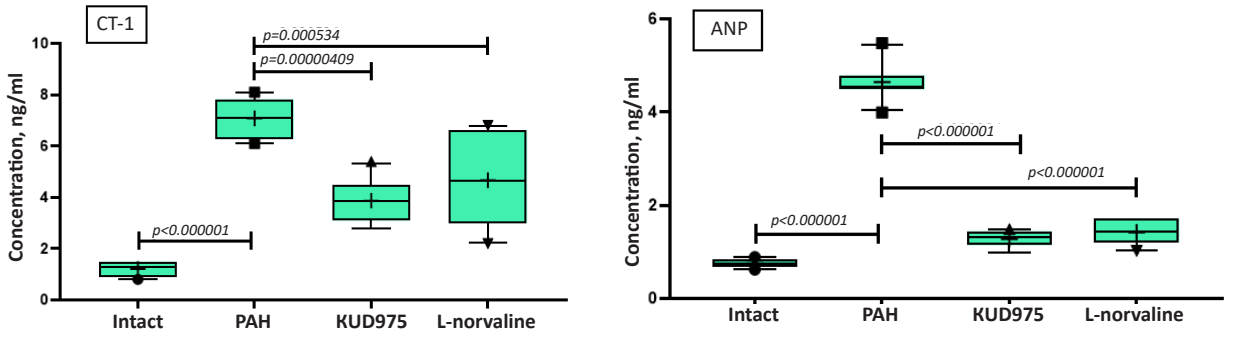

Figure 6 – Effect of KUD975 and L-norvaline on plasma concentrations of cytokines cardiotrophin-1 and atrial natriuretic peptide

Note: CT-1 – cardiotrophin-1; ANP – atrial natriuretic peptide.

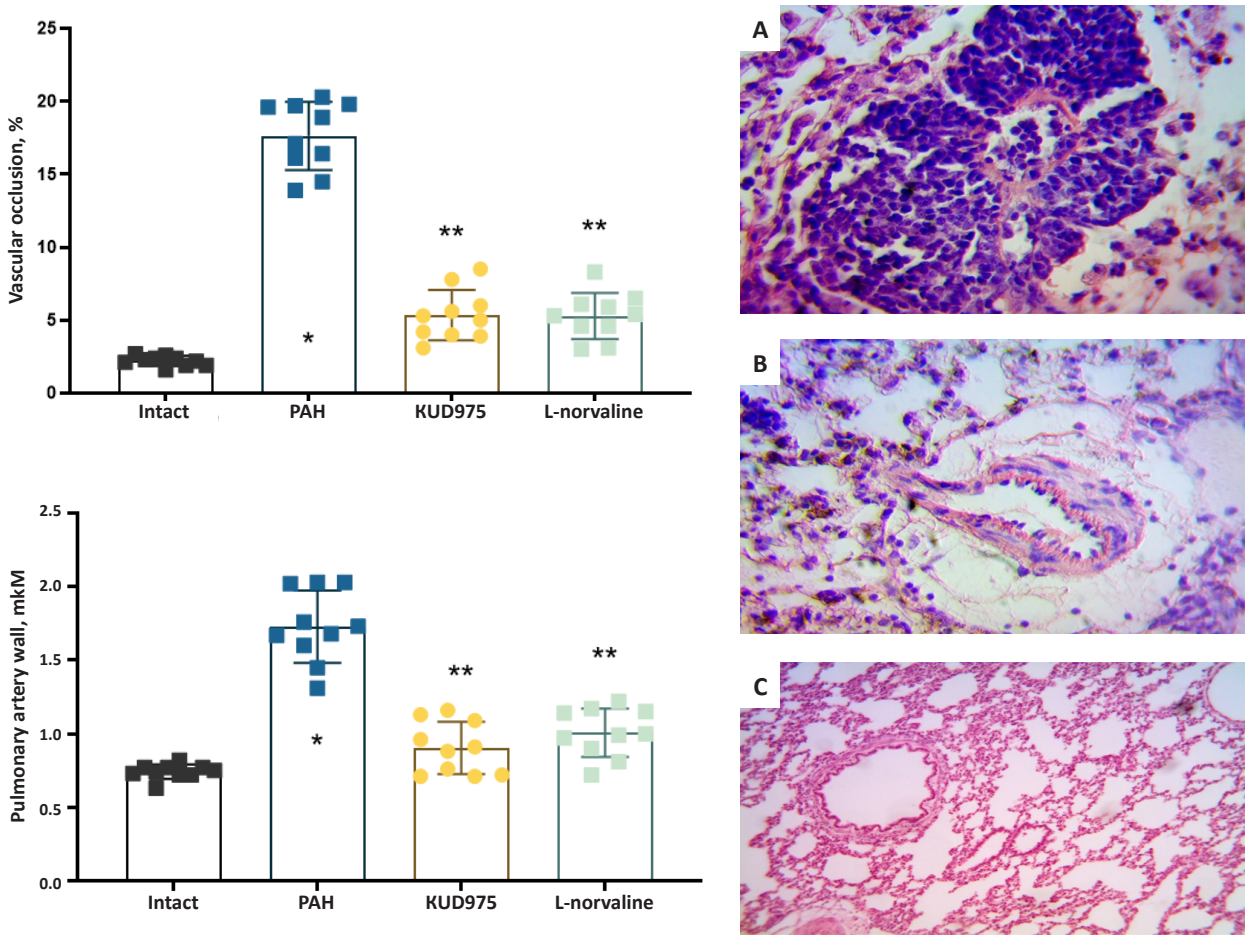

Figure 7 – Effect of KUD975 and L-norvaline on histological lungs structure when modeling circulatory pulmonary hypertension with hypoxia

Note: PA wall thickness – pulmonary artery wall thickness; A – micrograph of pulmonary artery wall (×400); B – lung microphotograph, perivascular fibrosis, stained with hematoxylin+eosin (×400); * – p <0.05 compared to intact; ** – p <0.05 compared to PAH.

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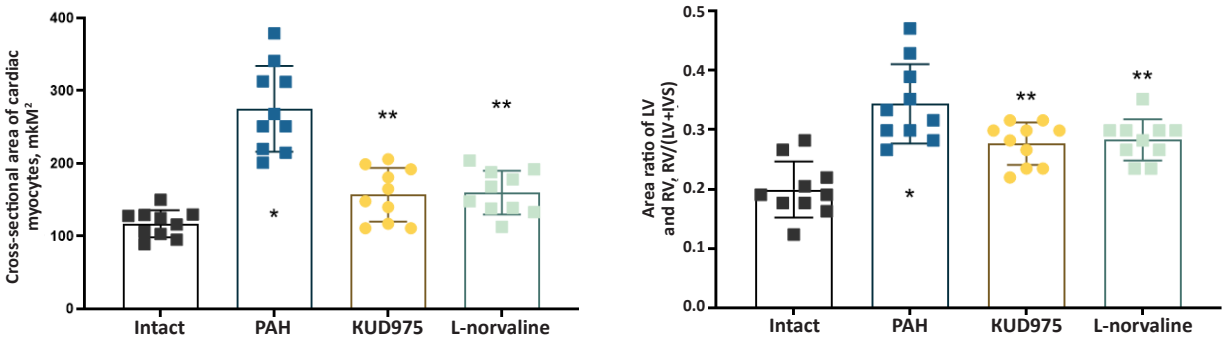

Figure 8 – Effect of KUD975 and L-norvaline on histological structure of right ventricle of animals' hearts in experimental groups

In this study, the concept of privileged structures was used to search for the compounds of a phenolic nature that have an endothelial protective effect. To conduct a virtual search for targets, several online services available on a non-commercial basis, were used. The physicochemical properties of the studied low molecular weight organic compounds were calculated using the following computational algorithms: OpenBabel, Molinspiration online service, online service of the "virtual laboratory of computational chemistry" VCCL. The phenolic compound with the laboratory code KUD975 investigated in this study is an inhibitor of arginase-2 and thrombin – the participation of these enzymes in the pathogenesis of the vascular endothelium dysfunction is currently beyond doubt. The inhibition of arginase-2, first of all, makes it possible to switch parts of the L-arginine-eNOS-NO metabolic pathway to the effective generation of NO and normalization of endothelium-dependent reactions in response to acetylcholine and vascular homeostasis in general [26, 27]. At the same time, the inhibition of thrombin leads to a slowdown in the release of thromboxane A2 by platelets, causing a powerful vasoconstriction, which is prevented by the simultaneous thrombin-induced release of prostacyclin and NO from ECs. Therefore, the inhibition of the thrombin production is an effective therapeutic strategy to correct a thrombin-induced activation of platelet-vascular wall interactions in ED [24].

It was previously shown that phenolic compounds prevent morphological changes in the cardiovascular system when modeling preeclampsia [28]. In the present study, it was shown that in a model of circulatory PH caused by hypoxia, the administration of the lead compound under study with the laboratory cypher KUD975, as well as the reference drug L-norvaline, led to a statistically significant decrease in RVSP and speed parameters of cardiohemodynamics. Against the background of PH modeling, the number of circulating EPCs in the experimental groups were studied. More and more studies demonstrate that circulating EPCs are involved in vascular homeostasis [29]. This study shows a more than 2-fold decrease in the number of circulating EPCs in the groups of animals with modeling circulatory PH and a statistically significant increase in the number of EPCs in the groups of animals that had been administered with the test compounds, which indicates the endothelial protective effect of KUD975.

To study the effect of the investigated compounds on the factors involved in the delivery of circulating EPCs to the endothelium of the affected vessels, the mRNA expression of factors necessary for the delivery of EPCs to the affected vascular walls, were studied: a vascular endothelial growth factor (VEGF), the first subtype of its receptors (VEGF-R1) and stromal cell factor-1 (SDF-1). As inflammatory processes are involved in the pathophysiology of PAH, the levels of monocyte chemoattractant protein-1 (MCP-1), a major marker of inflammation in inflammatory processes associated with PAH, were also measured [30]. It was found out 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 the compounds KUD975 and L-norvaline in the model of hypoxia-induced PH, a statistically significant increase in the expression of VEGF-R2 mRNA and a decrease in the expression of SDF-1 mRNA were found out. Moreover, the degree of increase in VEGF-R2 mRNA expression in the group of animals receiving KUD975 was statistically significantly higher than that in the group of animals receiving L-norvaline.

To further assess the state of the cardiovascular system against the background of PH modeling, the content of CT-1 and ANP in the blood plasma was measured. The first cytokine is associated with myocardial hypertrophy and cardiovascular pathology, and the second is a hormone secreted by the atria in response to a high BP – its effect is to reduce preload on the heart, thereby lowering BP [31]. The decrease in the concentrations of CT-1 and ANP under the influence of the studied compounds indicates a decrease in the manifestations of vascular remodeling caused by PH and is consistent with the data obtained from the histological examination.

The development of PAH and its correction by the studied compounds was confirmed by histological studies. Thus, in the animals with a PH, a progressive pulmonary vascular remodeling, including a significant increase in wall thickness, occlusion and muscularization of intraacinar vessels, as well as an increase in wall thickness and wall / lumen ratio of preacinar pulmonary vessels compared with controls was observed. In the animals' hearts with PH, RV hypertrophy, including an increase in the cross-sectional area of cardiomyocytes and the ratio of the RV and LV areas of the heart was found out. The administration of KUD975 and L-norvaline made it possible to reduce signs of pulmonary vascular remodeling by reducing the thickness of the PA wall and the occlusion degree of intraacinar pulmonary vessels compared to the animals with PH induced and hypoxia.

CONCLUSION

Thus, when studying the pharmacological activity, it was shown that a compound of the phenolic nature with the laboratory cypher KUD975 normalizes hemodynamic parameters, reduces the signs of remodeling of the heart and pulmonary vessels and has a pronounced endothelial protective effect on the model of PH induced by hypoxia, and surpasses the activity of the reference drug L-norvaline in terms of the effectiveness of increasing the number of circulating EPCs, increasing the expression of VEGF-R2 mRNA and reducing the concentration of CT-1.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHOR'S CONTRIBUTION

The author confirms that her authorship meets the international criteria of the ICMJE.
Liliya V. Korokina – development of research design, planning and implementation of the experimental part of the study, evaluation and interpretation of results, literature analysis, preparation of graphic material, writing the text of the article.

REFERENCES

1. Bousseau S, Sobrano Fais R, Gu S, Frump A, Lahm T. Pathophysiology and new advances in pulmonary hypertension. BMJ Med. 2023;2(1):e000137. DOI: 10.1136/bmjmed-2022-000137
2. Shah AJ, Beckmann T, Vorla M, Kalra DK. New drugs and therapies in pulmonary arterial hypertension. Int J Mol Sci. 2023;24(6):5850. DOI: 10.3390/ijms24065850
3. Humbert M, Kovacs G, Hoeper MM, Badagliacca R, Berger RMF, Brida M, Carlsen J, Coats AJS, Escribano-Subias P, Ferrari P, Ferreira DS, Ghofrani HA, Giannakoulas G, Kiely DG, Mayer E, Meszaros G, Nagavci B, Olsson KM, Pepke-Zaba J, Quint JK, Rådegran G, Simonneau G, Sitbon O, Tonia T, Toshner M, Vachiery JL, Vonk Noordegraaf A, Delcroix M, Rosenkranz S; ESC/ERS Scientific Document Group. 2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Heart J. 2022;43(38):3618–731. DOI: 10.1093/eurheartj/ehac237. Erratum in: Eur Heart J. 2023;44(15):1312.
4. Hoeper MM, Mayer E, Simonneau G, Rubin LJ. Chronic thromboembolic pulmonary hypertension. Circulation. 2006;113(16):2011–20. DOI: 10.1161/CIRCULATIONAHA.105.602565
5. Swisher JW, Weaver E. The evolving management and treatment options for patients with pulmonary hypertension: current evidence and challenges. Vasc Health Risk Manag. 2023;19:103–26. DOI: 10.2147/VHRM.S321025
6. Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, Williams PG, Souza R. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. Eur Respir J. 2019;53(1):1801913. DOI: 10.1183/13993003.01913-2018
7. Zolty R. Novel experimental therapies for treatment of pulmonary arterial hypertension. J Exp Pharmacol. 2021;13:817–57. DOI: 10.2147/JEP.S236743
8. Ball MK, Waypa GB, Mungai PT, Nielsen JM, Czech L, Dudley VJ, Beussink L, Dettman RW, Berkelhamer SK, Steinhorn RH, Shah SJ, Schumacker PT. Regulation of hypoxia-induced pulmonary hypertension by vascular smooth muscle hypoxia-inducible factor-1α. Am J Respir Crit Care Med. 2014;189(3):314–24. DOI: 10.1164/rccm.201302-0302OC
9. Khemani E, McElhinney DB, Rhein L, Andrade O, Lacro RV, Thomas KC, Mullen MP. Pulmonary artery hypertension in formerly premature infants with bronchopulmonary dysplasia: clinical features and outcomes in the surfactant era. Pediatrics. 2007;120(6):1260–9. DOI: 10.1542/peds.2007-0971
10. Kim GB. Pulmonary hypertension in infants with bronchopulmonary dysplasia. Korean J Pediatr. 2010;53(6):688–93. DOI: 10.3345/kjp.2010.53.6.688
11. Samillan V, Haider T, Vogel J, Leuenberger C, Brock M, Schwarzwald C, Gassmann M, Ostergaard L. Combination of erythropoietin and sildenafil can effectively attenuate hypoxia-induced pulmonary hypertension in mice. Pulm Circ. 2013;3(4):898–907. DOI: 10.1086/674758
12. Gilinsky MA, Polityko YK, Markel AL, Latysheva TV, Samson AO, Polis B, Naumenko SE. Norvaline reduces blood pressure and induces diuresis in rats with inherited stress-induced arterial hypertension. Biomed Res Int. 2020;2020:4935386. DOI: 10.1155/2020/4935386
13. Pokrovskiy MV, Korokin MV, Tsepeleva SA, Pokrovskaya TG, Gureev VV, Konovalova EA, Gudyrev OS, Kochkarov VI, Korokina LV, Dudina EN, Babko AV, Terehova EG. Arginase inhibitor in the pharmacological correction of endothelial

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2023-11-4-312-323

dysfunction. Int J Hypertens. 2011;2011:515047. DOI: 10.4061/2011/515047
14. Gudyrev OS, Faitelson AV, Sobolev MS, Pokrovskiy MV, Pokrovskaya TG, Korokin MV, Povetka EE, Miller ES, Soldatov VO. A study of osteoprotective effect of l-arginine, l-norvaline and rosuvastatin on a model of hypoestrogen-induced osteoporosis in rats. I.P. Pavlov Russian Medical Biological Herald. 2019;27(3):325–32. DOI: 10.23888/PAVLOVJ2019273325-332
15. Kudryavtsev KV, Korokin MV, Yakushev VI. Study the effect of compound of phenolic nature on the formation of blood clots using intravital microscopy and fluorescent labels. Research result: pharmacology and clinical pharmacology. 2015;1(4(6)):4–8. DOI: 10.18413/2313-8971-2015-1-4-4-8
16. YanYun P, Wang S, Yang J, Chen B, Sun Z, Ye L, Zhu J, Wang X. Interruption of CD40 pathway improves efficacy of transplanted endothelial progenitor cells in monocrotaline induced pulmonary arterial hypertension. Cell Physiol Biochem. 2015;36(2):683–96. DOI: 10.1159/000430130
17. Caldwell RB, Toque HA, Narayanan SP, Caldwell RW. Arginase: an old enzyme with new tricks. Trends Pharmacol Sci. 2015;36(6):395–405. DOI: 10.1016/j.tips.2015.03.006
18. Kossmann S, Schwenk M, Hausding M, Karbach SH, Schmidgen MI, Brandt M, Knorr M, Hu H, Kröller-Schön S, Schönfelder T, Grabbe S, Oelze M, Daiber A, Münzel T, Becker C, Wenzel P. Angiotensin II-induced vascular dysfunction depends on interferon-γ-driven immune cell recruitment and mutual activation of monocytes and NK-cells. Arterioscler Thromb Vasc Biol. 2013;33(6):1313–9. DOI: 10.1161/ATVBAHA.113.301437. Erratum in: Arterioscler Thromb Vasc Biol. 2013;33(8):e126.
19. Wang L, Bhatta A, Toque HA, Rojas M, Yao L, Xu Z, Patel C, Caldwell RB, Caldwell RW. Arginase inhibition enhances angiogenesis in endothelial cells exposed to hypoxia. Microvasc Res. 2015;98:1–8. DOI: 10.1016/j.mvr.2014.11.002
20. Kavalukas SL, Uzgare AR, Bivalacqua TJ, Barbul A. Arginase inhibition promotes wound healing in mice. Surgery. 2012;151(2):287–95. DOI: 10.1016/j.surg.2011.07.012
21. Statsenko ME, Derevianchenko MV. Correction of endothelial dysfunction in hypertensive patients with type 2 diabetes mellitus during combined antihypertensive therapy. Terapevticheskii Arkhiv. 2014;86(8):90–3. Russian
22. Shcheblykin DV, Bolgov AA, Pokrovskii MV, Stepenko JV, Tsuverkalova JM, Shcheblykina OV, Golubinskaya PA, Korokina LV. Endothelial dysfunction: developmental mechanisms and therapeutic strategies. Research Results in Pharmacology. 2022;8(4):115–39. DOI: 10.3897/rrpharmacology.8.80376
23. Dehghani T, Panitch A. Endothelial cells, neutrophils and platelets: getting to the bottom of an inflammatory triangle. Open Biol. 2020;10(10):200161. DOI: 10.1098/rsob.200161
24. De Pablo-Moreno JA, Serrano LJ, Revuelta L, Sánchez MJ, Liras A. The vascular endothelium and coagulation: homeostasis, disease, and treatment, with a focus on the von willebrand factor and factors VIII and V. Int J Mol Sci. 2022;23(15):8283. DOI: 10.3390/ijms23158283
25. Rabiet MJ, Plantier JL, Dejana E. Thrombin-induced endothelial cell dysfunction. Br Med Bull. 1994;50(4): 936–45. DOI: 10.1093/oxfordjournals.bmb.a072935
26. Lundberg JO, Weitzberg E. Nitric oxide signaling in health and disease. Cell. 2022;185(16):2853–78. DOI: 10.1016/j.cell.2022.06.010
27. Mahdi A, Kövamees O, Pernow J. Improvement in endothelial function in cardiovascular disease – Is arginase the target? Int J Cardiol. 2020;301:207–14. DOI: 10.1016/j.ijcard.2019.11.004
28. Lokteva TI, Rozhkov IS, Gureev VV, Gureeva AV, Zatolokina MA, Avdeeva EV, Zhilinkova LA, Prohoda EE, Yarceva EO. Correction of morphofunctional disorders of the cardiovascular system with asialized erythropoietin and arginase II selective inhibitors KUD 974 and KUD 259 in experimental preeclampsia. Research Results in Pharmacology. 2020;6(1):29–40. DOI: 10.3897/rrpharmacology.6.50851
29. Naito H, Iba T, Takakura N. Mechanisms of new blood-vessel formation and proliferative heterogeneity of endothelial cells. Int Immunol. 2020;32(5):295–305. DOI: 10.1093/intimm/dxaa008
30. Peng B, Kong G, Yang C, Ming Y. Erythropoietin and its derivatives: from tissue protection to immune regulation. Cell Death Dis. 2020;11(2):79. DOI: 10.1038/s41419-020-2276-8
31. Guo HJ, Jiang F, Chen C, Shi JY, Zhao YW. Plasma brain natriuretic peptide, platelet parameters, and cardiopulmonary function in chronic obstructive pulmonary disease. World J Clin Cases. 2021;9(36): 11165–72. DOI: 10.12998/wjcc.v9.i36.11165

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