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Research Article

Combined use of arginase II inhibitors and tadalafil for the correction of monocrotaline pulmonary hypertension

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Abstract

Introduction: The concept of the regulatory role of endothelium in the pathogenesis of pulmonary hypertension (PH) is fundamental.

Research objective: To study the protective effects of the selective arginase II inhibitors L207-0525 and L327-0346 in combination with tadalafil in a monocrotaline model of pulmonary hypertension in rats.

Materials and methods: Monocrotaline-induced pulmonary hypertension was simulated in 10 animals by a subcutaneous injection of an alcohol-water solution of monocrotaline (MCT) in the dose of 60 mg/kg. Seven days after the injection of MCT, the administration of L207-0525 and L327-0346 in the doses of 1 mg/kg and 3 mg/kg was started. The compounds were administered intragastrically once a day for 21 days.

Results and discussion: It was found that L207-0525 and L327-0346 in the dose of 3 mg/kg and tadalafil in the dose of 1 mg/kg prevented the development of pulmonary hypertension, which was expressed in a statistically significant decrease in the coefficient of endothelial dysfunction (CED, prevention of an increase in systolic pressure in the right ventricle, as well as Fulton, RV/BW and WT indices. The greatest activity was shown by L207-0525 and L327-0346 in the dose of 3 mg/kg in combination with tadalafil in the dose of 0.1 mg/kg.

Conclusions: The received results suggest the dose-dependent protective activity of selective arginase II inhibitors L207-0525 and L327-0346 and the development of the additive effect of their combined use with low doses of PDE-5 inhibitor tadalafil in relation to the development of monocrotaline pulmonary hypertension.

Keywords

L207-0525, L327-0346, monocrotaline-induced pulmonary hypertension.

Introduction

Pulmonary arterial hypertension (PAH) is a group of life-threatening progressive diseases of various origins, characterized by a progressive increase in blood pressure (BP) in the pulmonary artery (PA), remodeling of the pulmonary vessels, which leads to increased pulmonary vascular resistance and pulmonary arterial pressure, and,

as a result, to right ventricular heart failure and premature death. One of the important mechanisms of the pathogenesis of PAH is the reduced formation of nitrogen oxide in endotheliocytes of pulmonary vessels (Félétou et al. 2010, Machado and Gladwin 2005, Morris et al. 2008). Therefore, it is obvious that the PAH therapy should be aimed primarily at elimination of endothelial dysfunction, so in this regard, the attempts to restore the level of nitrogen oxide are pathogenetically justified.

In PH, the content of free NO and its derivatives decreases in whole blood, but increases in the tissues of the heart and lungs (Xu et al. 2004). The authors interpret these changes as a weakening of eNOS function and an increase in inflammation. At the same time, they record the presence of the signs of oxidative stress (an increase in ROS and a decrease in reduced glutathione in lungs) and suppose that this makes an additional contribution to a decrease in the level of free NO and its derivatives in blood. The modern literature confirms the influence of inflammation on the development and progression of pulmonary hypertension (Munder et al. 2005). In addition, Th2, IL-4, and IL-13 cytokines have been reported to induce the expression of arginase II in endothelial cells of the human pulmonary artery (Chang et al. 2000, Munder 2009).

Arginase is an enzyme that participates in the urea cycle and is described in two isoforms: arginase I and arginase II. Arginase II is a mitochondrial enzyme which is expressed in several organs and tissues, including endotheliocytes of pulmonary vessels. Arginase catalyzes the hydrolysis of L-arginine to L-ornithine and urea, and thus competes with NOS for a common substrate, L-arginine. In accordance with the foregoing, in the endothelial layer, the competition between arginase II and eNOS reduces the bioavailability of NO, which leads to impaired vasodilation and, as a consequence, to endothelial dysfunction (Pernow and Jung 2013, Romero et al. 2008).

Materials and methods

The experiments were carried out in compliance with the requirements of the federal law of the Russian Federation *On Protection of Animals Against Cruel Treatment* dated June 24, 1998, the rules of laboratory practice in preclinical studies in the Russian Federation (GOST 3 51000.3-96 and GOST R 53434-2009), European Community directives (86/609 EU), the rules and international recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1991).

The study was performed on 100 sexually mature Wistar male rats weighing 200–220 g. Seven days after the injection of MCT, the administration of selective arginase II inhibitors L207-0525 and L327-0346 in the doses of 1 mg/kg and 3 mg/kg, tadalafil in the dose of 10 mg/kg in monotherapy and in the dose of 1 mg/kg in combination with L207-0525 and L327-0346 was started; all the compounds were administered intragastrically once a day for 21 days. Thus, 10 experimental groups were formed: 1 – intact animals; 2 – control (MCT) (0.5 ml, 60% alcohol solution once subcutaneously); 3 – MCT + L207-0525 in the dose of 1 mg/kg; 4 – MCT + L207-0525 in the dose of 3 mg/kg; 5 – MCT + L327-0346 in the dose of 1 mg/kg; 6 – MCT + L327-0346 in the dose of 3 mg/kg; 7 – MCT + tadalafil in the dose of 0.1 mg/kg; 8 – MCT + tadalafil in the dose of 1 mg/kg; 9 – MCT + L207-0525 in the dose of 3 mg/kg + tadalafil in the dose of 1 mg/kg; and 10 – MCT + L327-0346 in the dose of 3 mg/kg + tadalafil in the dose of 1 mg/kg.

Monocrotaline pulmonary hypertension was simulated by subcutaneous injection of an alcohol-water solution of MCT in the dose of 60 mg/kg in the volume of 0.5 ml per animal (Kaminskii et al. 2011).

Seven days after MCT injection, the administration of the test compounds once a day for 21 days was started. Four weeks after the start of the experiment, the animals were anesthetized (chloral hydrate 150 mg/kg + zoletil 60 mg/kg); the left carotid artery was catheterized to record blood pressure (BP), and the necessary pharmacological agents were bolus injected into the femoral vein. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured with the use of a Biopac hardware-software complex. In addition to the study of blood pressure, a number of functional tests were carried out with the subsequent assessment of changes in the parameters of systolic and diastolic blood pressure, as well as the heart rate in response to intravenous administration of the solution of acetylcholine (AC) in the dose of 40 μg/kg at 0.1 ml per 100g of animal body weight as well as changes in hemodynamic parameters in response to intravenous administration of the solution of sodium nitroprusside (SN) in the dose of 30 μg/kg at 0.1 ml per 100g of animal body weight (Denisyuk et al. 2016, Ivlitskaya et al. 2016).

The level of development of endothelial dysfunction in the experimental animals, as well as a degree of its correction by the studied pharmaceutical substance, was estimated by the calculated coefficient of endothelial dysfunction (CED), calculated by the formula: CED = SBP SN/SBP AC, where SBP SN is the area of the triangle above the curve of blood pressure recovery in response to intravenous administration of sodium nitroprusside; SBP AC is the area of the triangle above the curve of blood pressure recovery in response to intravenous administration of AC (Korokin et al. 2009, Molchanova et al. 2016).

The hemodynamic parameters were determined with the use of the Biopac MP-150 system and AcqKnowledge 3.8.1 software (USA). After measurement of hemodynamic parameters, the animal was withdrawn from the experiment; blood sample was drawn for analysis of the gas composition (partial pressure of oxygen and carbon dioxide) with the use of the Micro-Astrup apparatus (Denmark); then the heart was removed for weighing and estimating the absolute and relative mass of the right ventricle (Korokina et al. 2019)

After the physiological experiment, the pulmonary heart of animals was weighed as an indicator of the development of hypertrophy, and histological preparations of the pulmonary vessels were made.

The following parameters were used to reflect the development of pulmonary hypertension:

- systolic pressure in the right ventricle (mm Hg);
- Fulton index – the ratio of right ventricular weight over left ventricular septal weight (%);
- RV/BW index (mg/g);
- wall thickness index of the pulmonary artery (%).

Results and discussion

Modelling of monocrotaline pulmonary hypertension showed that the animals after 4 weeks reduced body weight gain compared with the control.

The study of the histological pattern of MCT pulmonary hypertension discovered hyperplasia of the muscle layer of the pulmonary vasculature (Fig. 1). This makes it possible to conclude that on the 28th day of the simulation of MCT pulmonary hypertension, hypertrophy of the pulmonary artery vascular wall develops, which is confirmed by the results of the assessment of the functional indicators.

When assessing the results of MCT pulmonary hypertension in the animlas in the control group, there was a statistically significant increase in blood pressure (BP) (Fig. 2).

L207-0525 and L327-0346 had a dose-dependent antihypertensive effect expressed in a statistically significant decrease in blood pressure in the dose of 3 mg/kg, compared with the MCT group ($p < 0.05$) (Fig. 2).

L207-0525 and L327-0346 in the dose of 3 mg/kg in combination with tadalafil in the dose of 0.1 mg/kg reduced blood pressure indicators to a greater extent, providing an additional hypotensive effect (Fig. 2).

The results of the functional tests for endothelium-dependent (AC 40 μg/kg iv) and endothelium-independent (nitroprusside 30 mg/kg iv) vessel dilatation, expressed in the calculated coefficient of endothelial dysfunction, are presented in Figure 3.

When assessing the protective activity of tadalafil in the doses of 0.1 mg/kg and 1 mg/kg, the CED values were 2.0±0.1 and 1.3±0.1, respectively. At the same time, the administration of the combination of the lower dose of tadalafil (0.1 mg/kg) with L207-0525 (3 mg/kg) and L327-0346 (3 mg/kg) showed better endothelioprotective activity than monotherapy of L207-0525 (3 mg/kg) and L327-0346 (3 mg/kg), where the CED indicators were 1.2±0.1 and 1.1±0.1, respectively (Fig. 3).

L207-0525 and L327-0346 in the doses of 1 mg/kg and 3 mg/kg intragastrically once a day showed a dose-dependent protective activity, expressed in a significant decrease in CED ($p<0.05$) (Fig. 3).

At the same time, the statistically significant positive influence of L207-0525 (3 mg/kg) and L327-0346 (3 mg/kg) in combination with tadalafil (0.1 mg/kg) was established on the blood gas composition.

Direct measurement of pressure in the right ventricle showed that with the development of MCT pulmonary hypertension, systolic pressure in the right ventricle increased to 41.3±2.3 mm Hg, while in the control series it was 23.0±1.2 mm Hg ($p<0/05$). At the same time, the Fulton index increased from 23.5±1.2% to 32.1±1.3%, the RV/BW index from 0.6±0.02 mg/g to 0.8±0.02 mg/g, and the average wall thickness of the pulmonary artery – from 0.18±0.01% to 0.23±0.01, respectively, when simulating MCT pulmonary hypertension. The influence of selective arginase II inhibitors L207-0525 and L327-0346, tadalafil on the development of pulmonary hypertension is presented in Table 2.

It was found that L207-0525 and L207-0525 in the doses of 1 and 3 mg/kg and tadalafil in the dose of 1 mg/kg prevented the development of pulmonary hypertension, which was expressed in preventing an increase in systolic blood pressure in the right ventricle, Fulton, RV/BW and WT indices. The greatest activity was shown by L207-0525 and L207-0525 in the dose of 3 mg/kg in combination with tadalafil in the dose of 0.1 mg/kg.

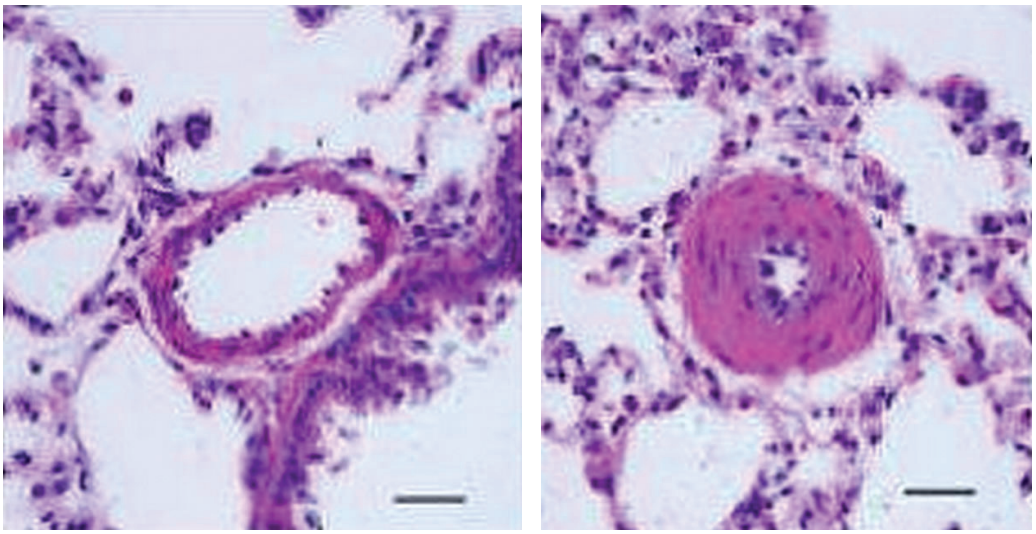

Figure 1. Hyperplasia of the smooth muscle wall of the pulmonary artery (on the left – control; on the right – monocrotaline pulmonary hypertension).

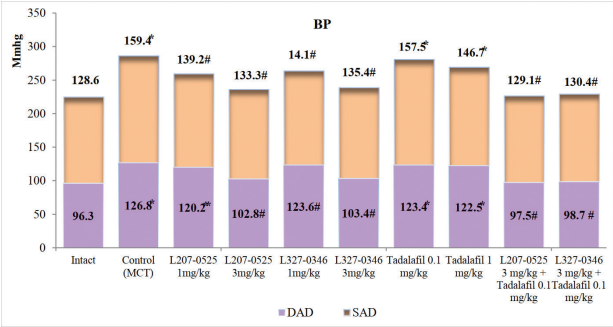

Figure 2. Indicators of systolic and diastolic blood pressure in the simulation of monocrotaline pulmonary hypertension and its correction by L207-0525 and L327-0346, tadalafil. **Note:** BP – blood pressure; DBP – diastolic blood pressure; SBP – systolic blood pressure, (MCT) – monocrotaline; *p <0.05 in comparison with the intact group, # – p <0.05 in comparison with the MCT group.

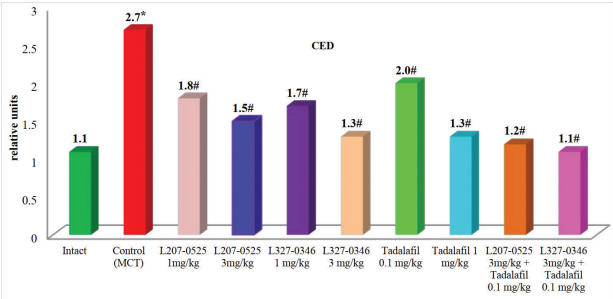

Figure 3. The influence of MCT on the coefficient of endothelial dysfunction and its correction by L207-0525 and L207-0525, tadalafil. **Note:** CED – coefficient of endothelial dysfunction, (MCT) – monocrotaline; * – p <0.05 in comparison with the intact group, # – p <0.05 in comparison with the MCT group.

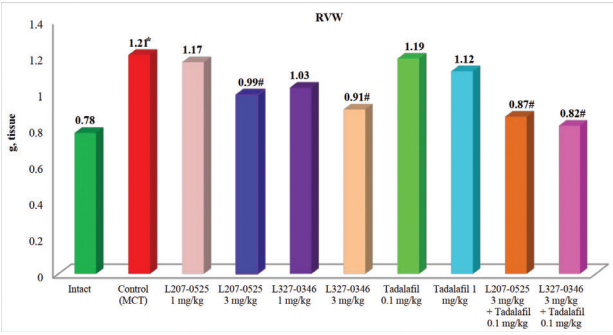

Figure 4. Relative right ventricle weight (RVW) of the rats' heart on the background of the simulation of MCT pulmonary hypertension and its correction by L207-0525 and L327-0346, tadalafil. **Note:** RVW – relative right ventricle weight; MCT – monocrotalin; * – p <0.05 in comparison with the intact group, # – p <0.05 in comparison with the MCT group.

The analysis of the relative weight of the heart found the expressed protective activity of L207-0525 and L327-0346 in the dose of 3 mg/kg in combination with small doses of tadalafil of 0.1 mg/kg against the background of the simulation of monocrotalin-induced pulmonary hypertension (Fig. 4).

Table 1. The Influence of L207-0525 and L327-0346, Tadalafil on the Parameters of the Blood Gas Composition on the Background of Modelling Monocrotaline Pulmonary Hypertension.

Experimental group	Partial pressure of oxygen PaO2 (mm Hg)	Partial pressure of carbon dioxide PaCO2 (mmHg)
Intact	84.70±5.36	39.60±1.92
Control (MCT)	63.80±3.71*	52.50±1.27*
MCT + L207-0525 1 mg/kg	69.98±3.15#	48.13±2.78#
MCT + L207-0525 3 mg/kg	73.54±4.09#	45.07±1.35#
MCT + L327-0346 1 mg/kg	70.35±3.37#	46.90±1.86#
MCT + L327-0346 3 mg/kg	74.18±4.22#	44.36±2.49#
MCT + Tadalafil 0.1 mg/kg	70.67±3.88#	46.05±2.55#
MCT + Tadalafil 1 mg/kg	75.32±4.56#	44.07±2.09#
MCT + L207-0525 3 mg/kg + Tadalafil 0.1 mg/kg	79.19±5.05#	43.71±2.11#
MCT + L327-0346 3 мг/кг + Tadalafil 0.1 mg/kg	81.23±3.44#	42.45±2.04#

Note: MCT – monocrotaline; * – p <0.05 in comparison with the intact group, # – p <0.05 in comparison with the MCT group.

Thus, the protective effect of new selective arginase II inhibitors L207-0525 and L327-0346 with respect to hypertrophy of the vascular wall of the pulmonary vasculature was found when simulating monocrotaline pulmonary hypertension.

Based on all the functional vascular and cardiac tests, the assessment of cardiodynamic effects on the model of MCT pulmonary hypertension, L207-0525 and L327-0346 showed a dose-dependent protective activity. PDE-5 tadalafil in the high dose of 1 mg/kg was slightly more effective than L207-0525 (3 mg/kg) and comparable in effectiveness with L327-0346 (3 mg/kg). The cardioprotective effects of the studied compounds involved preventing the development of pulmonary hypertension, a significant decrease in blood pressure and CED. In addition, L207-0525 and L327-0346 in the dose of 3 mg/kg, tadalafil in the dose of 1 mg/kg prevented an increase in Fulton, RV/BW and WT indices. The combination of L207-0525 and L327-0346 in the dose (3 mg/kg) once a day with tadalafil in a small dose (0.1 mg/kg) showed additive both endothelial and cardioprotective effects on the model of MCT pulmonary hypertension. Moreover, an increase in the dose of tadalafil to 1 mg/kg in the combination did not contribute to an improvement in all the studied parameters.

Therefore, the research results indicate the development of the additive effect of the combined use of selective arginase II inhibitors L207-0525 and L327-0346 and small doses of PDE-5=Is in relation to the development of monocrotaline-induced pulmonary hypertension.

As is known, L-arginine is the only substrate for the synthesis of NO, which is actively biotransformed under the influence of the arginase II enzyme. The increased activity of arginase II leads to a decrease in NO and, as a consequence, to the development of ED. According to a number of modern authors, the increased activity of arginase enzymes is observed in various diseases, such as bronchial asthma, arthritis, glomerulonephritis, psoriasis, and diabetic erectile dysfunction. Inhibition of arginases contributes to an increased production of nitrogen oxide and

Table 2. The Influence of the Selective Arginase 2 Inhibitor L207-0525 and L327-0346, Tadalafil on the Development of Monocrotaline-induced Pulmonary Hypertension (M±m; n=10).

Experimental series	RVSP	Fulton Index	RV/BW Index	WT Index
Intact animals	23.0±1.2	23.5±1.2	0.6±0.02	0.18±0.01
Monocrotaline 60 mg/kg (MCT)	41.3±2.3*	32.1±1.3*	0.8±0.02*	0.23±0.01*
MCT + L207-0525 1 mg/kg	36.4±1.9*	29.1±1.2*	0.7±0.02*	0.22±0.01*
MCT + L207-0525 3 mg/kg	27.1±1.8#	25.5±1.1#	0.6±0.02#	0.20±0.01#
MCT + L327-0346 1 mg/kg	35.2±1.6*	28.1±1.3*	0.7±0.01*	0.21±0.01*
MCT + L327-0346 3 mg/kg	26.7±1.7#	24.9±1.1#	0.6±0.02#	0.19±0.01#
MCT + Tadalafil 0.1 mg/kg	35.3±2.2#	26.4±1.3#	0.7±0.01#	0.21±0.01#
MCT + Tadalafil 1 mg/kg	32.5±2.1#	25.4±1.5#	0.7±0.02#	0.20±0.01#
MCT + L207-0525 3 mg/kg + Tadalafil 0.1 mg/kg	24.0±1.4#	24.5±1.3#	0.6±0.01#	0.18±0.01#
MCT + L327-0346 3 mg/kg + Tadalafil 0.1 mg/kg	23.0±1.2#	23.9±1.1#	0.6±0.02#	0.18±0.01#

Note: MCT – monocrotalin; RVSP – systolic blood pressure in the right ventricle (mm Hg); the Fulton index – weight ratio of the mass of the right ventricle/left ventricle and septum (%); RV/BW – index ventricular weight (mg/g); WT index – the pulmonary artery wall thickness (%). * – p <0.005 compared to control; # – p <0.005 compared to MCT.

the prevention of dysfunctional disorders in the endothelium (Khong et al. 2012, Michell et al. 2011, Shemyakin et al. 2012, Yakushev et al. 2012, Yakushev and Pokrovsky 2016). Therefore, the use of highly selective arginase II inhibitors is the most promising and pathogenetically reasonable in the prevention and complex therapy of PH. As it is known, today there are no drugs from the group of highly selective arginase II inhibitors at the global pharmaceutical market. That is why the search for active candidate molecules, highly selective arginase II inhibitors, is justified.

It was determined that arginase II is expressed in endothelial cells of the human pulmonary artery (Budhiraja et al. 2004). Therefore, it appears to be the main enzyme expressed in the pulmonary vasculature of humans and mice. Based on the analysis of the literature, it can be assumed that arginase can contribute to the development of PH through several mechanisms. Firstly, arginases compete with NOS for the mutual substrate, L-arginine, which leads to a decrease in the bioavailability of NO (Zuckerbraun et al. 2011). Secondly, end products of arginase enzymatic reactions include polyamines and L-proline. Polyamines are known to stimulate cell growth and differentiation, and L-proline is an important component of collagen synthesis (Krystofova et al. 2018, Li et al. 2001). Thus, arginases can contribute to vascular remodeling by proliferation of vascular cells and expansion of the extracellular matrix.

The modern literature confirms the influence of inflammation on the development and progression of pulmonary hypertension (Chicoine et al. 2004). In experimental models of pulmonary hypertension, the increased presence and activity of inflammatory cells (including macrophages, polymorphonuclear neutrophils, lymphocytes and mast cells) is usually observed, which is accompanied by significant activation of inflammatory 16, 17 cytokines and growth factors (TNFα, IL-1β, IL-6, PDGFα, PDGFβ, TGFβ) and adhesion molecules. In addition, Th2, IL-4, and IL-13 cytokines are reported to induce the expression of arginase I and arginase II in various cell types (Nelin et al. 2001).

Since arginase and NOS compete for their common substrate, L-arginine, an increased arginase activity can lead to a decrease in NO production, which contributes to vasoconstriction (Stanley KP et al. 2006). This concept is supported by the observation in a clinic where endothelial cells of pulmonary artery obtained from lungs of patients with PH had higher levels of arginase II protein and lower NO production than similar cells from lungs of patients without PH (Chicoine et al. 2004).

In addition to the treatment of erectile dysfunction, PDE-5-Is are widely used in clinical practice for the treatment of both primary and secondary pulmonary hypertension. An important feature in the correction of PDE-5-IS is not only vasodilation, but also the prevention of remodelling vessels (Wang et al. 2018). The controlled randomized trial revealed the presence of a vasorelaxating effect in conditions of pulmonary arterial hypertension in sildenafil, vardenafil, and tadalafil (Mokry et al. 2017)

Due to the accumulation of cGMP, PDE-5 inhibitors may have a stimulating influence on the metabolic pathway of the formation of the nitrogen oxide NO/cGMP/PDE. In addition, PDE-5-Is, due to the activation of K^+ adenosine triphosphatase channels, can reduce the production of VCAM and ICAM adhesion molecules and, therefore, prevent the activation of a endothelial dysfunction neutrophilic link; besides, PDE-5-Is in small doses probably activates protein kinase G, increasing at the same time the activity of eNOS and iNOS (Raina et al. 2017, Schermuly et al. 2011, Zuckerbraun et al. 2011).

Thus, based on studies conducted, it can be assumed that selective arginase II inhibitors L207-0525, L327-0346, and PDE-5-I tadalafil exert the additive effects with the following therapeutic targets involved: arginase II/NO bioavailability and the cGMP/PDE-5 metabolic pathway (Fig.5).

Figure 5 presents the variants of the therapeutic influence of «*L-arginine/NO/NOS*» on NO synthesis through the classical pathway for the formation of nitrogen oxide, with «*L-arginine/NO/NOS*» through the accumulation of *cGMP* (cyclic guanosine monophosphate) stimulating the metabolic pathway «*NO/cGMP/PDE-5*». The first strategy is to increase the availability of the L-arginine substrate for NO synthesis using arginase inhibitors. Alternative strategies involve the therapy that uses an advantage of cyclic-guanosine-monophosphate-dependent signaling, including type 5 phosphodiesterase inhibitors.

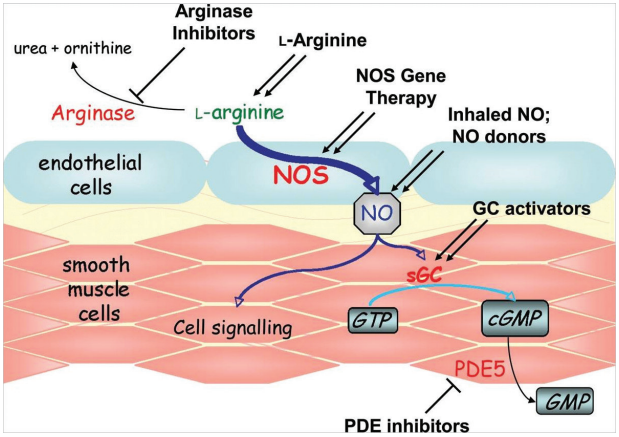

Figure 5. The influence of arginase inhibitors and PDE-5 inhibitors on the formation of NO (Zuckerbraun et al. 2011). **Note:** cGMP – cyclic guanosine monophosphate; GMP – guanosine monophosphate; PDE-5 – type 5 phosphodiesterase inhibitors; sGC – guanylate cyclase.

Summarizing the foregoing, a further experimental and then clinical study of the combined effects of selective arginase II inhibitors L207-0525, L327-0346 and small doses of the PDE-5 inhibitor tadalafil aimed at improvement of the treatment of pulmonary arterial hypertension appears to be necessary.

Conclusions

1. The results indicate the dose-dependent protective activity of selective arginase II inhibitors L207-0525 and L327-0346 in relation to the development of monocrotalin pulmonary hypertension.
2. Further studies of protective activity on the PH model will objectively assess the prospects for the use of new selective arginase inhibitors L207-0525 and L327-0346 in the treatment of PH.
3. The combination of selective arginase II inhibitors L207-0525 and L327-0346 in the dose of 3 mg/kg with tadalafil in a small dose of 0.1 mg/kg once a day intragastrically for 21 days at monocrotaline pulmonary hypertension showed the highest additive effect.

Conflict of interest

The authors have no conflicts of interest to declare.

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