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Pharmacological effects of a new soluble guanylate cyclase stimulator in experimental pulmonary arterial hypertension

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ABSTRACT

Aim. To assess the effect of an indolinone derivative (2-[2-[(5RS)-5-(hydroxymethyl)-3-methyl-1,3-oxazolidine-2-yliden]-2-cyanoethylidene]-1H-indole-3(2H)-one (codename – GRS) on right ventricular contractility, endothelial vasodilator function, and histologic changes in the lungs and heart in a rat model of monocrotaline-induced pulmonary hypertension.

Materials and methods. Pulmonary arterial hypertension (PAH) was induced in Wistar rats by a single subcutaneous administration of monocrotaline at a dose of 60 mg / kg. Starting from day 15 after PAH induction, the rats received either GRS at a dose of 10 mg / kg or riociguat at a dose of 1 mg / kg orally once a day. Blood pressure in the right ventricle, right ventricular weight, endothelial vasodilator function, and the histologic structure of the lungs and heart were studied after the last administration of test substances.

Results. Twenty-eight days after monocrotaline administration, the rats developed PAH, as shown by the increase in the maximal blood pressure in the right ventricle and the right ventricular weight / total heart weight ratio. GRS after multiple administration reduced the maximal blood pressure in the right ventricle, had no significant effect on its contractility, improved endothelial vasodilator function, and normalized blood pressure. Riociguat had a hypotensive effect and did not alleviate endothelial dysfunction in experimental PAH.

Conclusion. The indolinone derivative GRS and riociguat, both soluble guanylate cyclase stimulators, lowered blood pressure in the right ventricle. GRS also alleviated endothelial dysfunction in animals with experimental PAH.

Keywords: pulmonary arterial hypertension model, soluble guanylate cyclase stimulators, indolinone derivative GRS, riociguat

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Фармакологические эффекты нового стимулятора растворимой гуанилатциклазы при экспериментальной легочной артериальной гипертензии

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РЕЗЮМЕ

Цель исследования – изучить влияние GRS на сократительную активность правого желудочка сердца, вазодилатирующую функцию эндотелия и гистологические изменения в легких и сердце на модели легочной гипертензии, вызванной введением монокроталина у крыс.

Материалы и методы. У самцов крыс линии Wistar воспроизводили легочную артериальную гипертензию (ЛАГ) однократным подкожным введением монокроталина в дозе 60 мг/кг. Начиная с 15-х сут после моделирования ЛАГ крысам в течение 14 сут вводили в желудок соединение GRS в дозе 10 мг/кг или риоцигуат в дозе 1 мг/кг. После последнего введения веществ измеряли давление крови в правом желудочке сердца, массу правого желудочка, оценивали вазодилатирующую функцию эндотелия и изучали гистологическое строение легких и сердца.

Результаты. Через 28 сут после введения монокроталина у крыс развивалась модель ЛАГ: повышались максимальное давление крови в правом желудочке сердца и отношение массы стенки правого желудочка к массе сердца. Соединение GRS при курсовом введении уменьшало максимальное давление крови в правом желудочке сердца, не оказывало статистически значимого влияния на его сократительную активность, улучшало вазодилатирующую функцию эндотелия, нормализовало системное артериальное давление. Риоцигуат оказывал гипотензивный эффект и не устранял дисфункцию эндотелия при экспериментальной легочной артериальной гипертензии.

Заключение. Стимуляторы растворимой гуанилатциклазы, производное индолинона GRS и риоцигуат снижают давление крови в правом желудочке сердца, соединение GRS устраняет проявления эндотелиальной дисфункции у животных с моделью ЛАГ.

Ключевые слова: модель легочной артериальной гипертензии, стимуляторы растворимой гуанилатциклазы, производное индолинона GRS, риоцигуат

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Pulmonary arterial hypertension (PAH) increases the mean blood pressure in the pulmonary circulation to ≥ 25 mm Hg, causes hyperplasia of endothelium and smooth muscle cells in the pulmonary artery and perivascular inflammatory infiltrates and fibrosis in the lungs, and leads to right ventricular hypertrophy [1].

One of the causes of PAH is dysfunction of pulmonary vascular endothelium accompanied by a decrease in the production of vasodilator and antithrombotic factors, namely nitric oxide (NO) and prostacyclin [2]. NO deficiency disrupts activation of soluble guanylate cyclase (sGC) in the vascular smooth muscles and synthesis of cyclic 3',5'-guanosine monophosphate (cGMP), which is the secondary messenger [3, 4]. In patients over 18 years old, PAH and chronic thromboembolic pulmonary hypertension are treated with the NO-independent sGC stimulator riociguat. It increases sensitivity of sGC heme to low NO concentrations by stabilizing NO – sGC binding and increases cGMP production. This cyclic nucleotide activates calcium-dependent ATPase in the sarcoplasmic reticulum of the vascular smooth muscle cells, leading to subsequent deposition of calcium ions, pulmonary artery dilation, and an increase in pulmonary circulation and lung functioning [5].

The new indolinone derivative (2-[2-[(5RS)-5-(hydroxymethyl)-3-methyl-1,3-oxazolidine-2-yliden]-2-cyanoethylidene]-1H-indole-3(2H)-one (codenamed GRS) increases sGC activity independent of NO, exerts antiplatelet effects, normalizes increased blood pressure, and restores endothelial dysfunction [6, 7].

The aim of the study was to assess the effect of the indolinone derivative (2-[2-[(5RS)-5-(hydroxymethyl)-3-methyl-1,3-oxazolidine-2-yliden]-2-cyanoethylidene]-1H-indole-3(2H)-one (codename – GRS) on right ventricular contractility, endothelial vasodilator function, and histologic changes in the lungs and heart in a rat model of monocrotaline-induced pulmonary hypertension.

The study focused on the effect of GRS on blood pressure in the right ventricle, endothelial function, and histologic structure of the lung and heart in the rat model of PAH induced by the administration of monocrotaline, a pyrrolizidine alkaloid found in the Crotalaria spectabilis Roth plant. Monocrotaline pyrrole, which is its active metabolite produced in the liver, activates the extracellular calcium-sensing receptor of vascular endothelial cells, binds to DNA, inhibits cell division, and increases membrane permeability, causing pulmonary and alveolar endothelial cell apoptosis [8–10]. Riociguat was used as a reference listed drug.

MATERIALS AND METHODS

The study used 60 male Wistar rats weighing 250–320 g obtained from the Department of Experimental Biological Models of Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center of the Russian Academy of Sciences. The rats were kept in groups of 5–8 animals in standard plastic cages (VELAZ, Czech Republic) at 20–23 °C, relative humidity of no more than 50 %, exhaust – supply ratio of 8 :10, with a 12 : 12 light / dark cycle. Animal handling was performed in accordance with the European Convention for the Protection of Vertebrate Animals (Directive 2010/63/EU). The study was performed in accordance with the principles of Good Laboratory Practice and was approved by the Ethics Committee at Siberian State Medical University (Protocol No. 5378 of 24.10.2016), IPHAR LLC (Protocol No. 113/2019 of 28.09.2021), and Goldberg Research Institute of Pharmacology and Regenerative Medicine of Tomsk National Research Medical Center (Protocol No. 185092021 of 11.10.2021).

The rats were divided into 4 groups: group 1 – intact animals (n = 12), groups 2–4 – animals with PAH (n = 16 in each group); group 2 was the control group which did not receive the test substances, group 3 received GRS, group 4 received the reference listed drug riociguat (Selleckchem, USA).

PAH was simulated by a single subcutaneous administration of monocrotaline at a dose of 60 mg / kg (Sigma-Aldrich, USA). Monocrotaline was dissolved in 0.5 N HCl, then the pH was adjusted to 7.4 using 0.5 N NaOH [11].

The indolinone derivative (codenamed GRS) at a dose of 10 mg / kg and riociguat at a dose of 1 mg / kg were administered orally once a day for 14 days, starting from Day 15 after the monocrotaline administration. Pilot studies showed that the GRS dose was close to ED_{50} in terms of its antithrombotic activity [6]. The dose of riociguat (1 mg / kg) was close to its highest tolerated dose (0.03–3 mg / kg) having an antihypertensive effect. Intact and control group animals received 1 % starch solution using the same regimen as for the test substances.

On Day 28 after PAH modeling, blood pressure in the right ventricle was measured in half of the rats in each group. Then they were euthanized, so that their right ventricle weight and histologic structure of the lungs and right ventricle were studied. In the other half of the animals, we measured changes in blood pressure in response to endothelium-dependent and endothelium-independent vasodilators.

Blood pressure in the right ventricle was measured using the MP150 high-speed data acquisition system (BIOPAC Systems Inc., USA) and the TSD282 micro pressure sensor (OpSens, Canada). The rats were anesthetized by isoflurane inhalation, then the micro pressure sensor was introduced into the right ventricle of the animals through the jugular vein. The data were registered and processed using AcqKnowledge 4.2 software for the MP150 system (BIOPAC Systems Inc., USA). The maximum (P_{max}) and minimum (P_{min}) blood pressure in the right ventricle was measured in mm Hg, the maximal rate of pressure rise during one cardiac cycle (dP / dt_{max}) was measured in mm Hg / sec, the contractility index (CI) was calculated (1 / sec).

After registering the blood pressure in the right ventricle, the rats were euthanized in the carbon dioxide chamber. The heart and right ventricular wall were weighed. Right ventricular hypertrophy was calculated in mg / mg as the right ventricular wall weight / total heart weight ratio (RVWW / THW).

The functional state of endothelium was assessed as follows: the rats were anesthetized by isoflurane inhalation, then an intra-arterial catheter was implanted into the right carotid artery to measure blood pressure. Bolus doses of pharmacological agents were administered into the right femoral vein. Mean blood pressure (MBP) was registered continuously using the MP150 high-speed data acquisition system, the DA100C module, and the TSD104A sensor (AcqKnowledge 4.2.0 software, USA). Endothelium-dependent vasodilation was registered as reduction of MBP in response to intravenous acetylcholine chloride (AC) administration at a dose of 5 mcg / kg [12]. Endothelium-independent vasodilation was registered as a fall in MBP in response to sodium nitroprusside dihydrate (SN) administration at a dose of 10 mcg / kg [13]. The degree of vasodilation was determined based on the area of the triangle above the curve of MBP restoration after AC or SN administration. The short leg of the triangle represented a decrease in MBP (Δ MBP) in response to a vasodilator agent (mm Hg), while the long leg was the time of MBP restoration (sec) after the test. Endothelial dysfunction coefficient (EDC) was calculated by dividing the triangle area above the MBP restoration curve after SN administration by the triangle area after AC administration [14].

We conducted a histologic examination of deparaffinized tissue sections obtained from the lung and right ventricle stained with hematoxylin and eosin. The histologic samples were examined using the Zeiss Axio Lab.A1 microscope (Carl Zeiss AG, Germany) at 50× magnification and photographed using ZEN software (Carl Zeiss AG, Germany).

The results were statisticallyprocessed using Statistica 8.0 software (StatSoft, USA). The data were presented as $M \pm m$, where M is the mean value, m is the standard error of the mean. Multiple comparison and intergroup differences were assessed using the Kruskal – Wallis test and the Mann – Whitney test, respectively.

RESULTS AND DISCUSSION

P_{max} in the right ventricle of intact rats was 24.0 ± 2.9 mm Hg, while P_{min} was –3.3 ± 1.1 mm Hg. The values of dP / dt_{max} and CI were 53.7 ± 4.4 mm Hg / sec and 596 ± 76 1 / sec, respectively (Table 1). These values did not differ from the normal ones [15, 16].

Four weeks after monocrotaline administration (control group), P_{max} in the right ventricle increased by 1.5 times, which indicated the development of PAH, while P_{min} decreased by 1.7 times compared with blood pressure in the intact animals. The dP / dt_{max} value increased by 1.8 times ($p < 0.05$). CI was the same as in intact rats. This indicated a compensatory increase in cardiac contractions.

After continuous GRS administration, P_{max} in the right ventricle was 42.6 ± 3.1 mm Hg, which was significantly lower than in the control group ($p < 0.05$), although it did not fully improve. P_{min} was –3.4 ± 0.8 mm Hg, which was 1.6 times lower than the blood pressure in the control group and did not differ from that in the intact group.

Table 1

Effect of GRS (10 mg / kg) and riociguat (1 mg / kg) on blood pressure in the right ventricle of the heart of rats with simulated PAH, *M ± m*

Group	P_{max}, mm Hg	P_{min}, mm Hg	CI, 1 / sec	dP / dt_{max}, mm Hg / sec
Intact rats, $n = 6$	24.0 ± 2.3	–3.3 ± 1.1	53.7 ± 4.4	596 ± 76
Rats with PAH (control), $n = 8$	54.3 ± 3.2*	–5.6 ± 0.3*	45.6 ± 1.4	1, 082 ± 62*
Rats with PAH, receiving:				
– GRS, $n = 8$	42.6 ± 3.1*+	–3.4 ± 0.8+	49.6 ± 3.6	902 ± 60*
– riociguat, $n = 8$	42.1 ± 4.0*+	–3.0 ± 1.5	45.8 ± 1.5	930 ± 72*

$p < 0.05$ * compared with the intact animals; + compared with the control animals.

Differences in the values of CI and dP / dt_{max} in the control and PAH groups were not statistically significant.

After continual riociguat administration, P_{max} in the right ventricle was 1.3 times lower (42.1 ± 4.0 mm Hg) than in the control group ($p < 0.05$), although its value was significantly higher than that in the intact group. The values of dP / dt_{max} and CI did not differ from those in the rats treated only with monocrotaline. The values of P_{max}, CI, and dP / dt_{max} did not have significant differences in the GRS and riociguat groups ($p > 0.05$) (Table 1).

The indolinone derivative GRS and riociguat were equally effective in lowering P_{max} in the right ventricle of the rats with simulated PAH. The GRS compound also normalized P_{min}.

In the intact animals, the RVWW / THW ratio was 0.222 ± 0.006 mg / kg; in the rats with PAH, it increased by 1.5 times ($p < 0.05$). These changes indicated right ventricular hypertrophy, which developed due to increased blood pressure in the pulmonary circulation. The RVWW / THW ratio was still increased in the GRS and riociguat groups ($p > 0.05$) (Table 2).

Table 2

Effect of GRS (10 mg / kg) and riociguat (1 mg / kg) on the right ventricular weight to total heart weight ratio in rats with simulated PAH, *M ± m*

Group	RVWW / THW, mg / mg
Intact rats, $n = 6$	0.222 ± 0.006
Rats with PAH (control), $n = 8$	0.332 ± 0.013*
Rats with PAH, receiving:	
GRS, $n = 8$	0.306 ± 0.020*
riociguat, $n = 8$	0.314 ± 0.020*

* $p < 0.05$ compared with the intact animals.

After AC administration, MBP decreased from 111 ± 4 to 42 ± 2 mm Hg in the intact rats, and from 92 ± 3 to 34 ± 2 mm Hg in the rats with PAH. These changes indicated a weakened response of blood vessels to endothelium-dependent vasodilator AC ($p < 0.05$) (Table 3).

Table 3

Effects of GRS (10 mg / kg) and riociguat (1 mg / kg) on endothelial vasodilator function in the rats with simulated PAH, *M ± m*

Group	Acetylcholine chloride, 5 mcg / kg			
	MBP, mm Hg	Δ MBP, mm Hg	Time of MBP restoration, sec	Area of the triangle above the MBP restoration curve, mm Hg / sec
Intact rats, $n = 6$	111 ± 4	42 ± 2	56 ± 11	1,121 ± 151
Rats with PAH (control), $n = 8$	92 ± 3*	34 ± 2*	58 ± 6	993 ± 115
Rats with PAH, receiving:				
– GRS, $n = 8$	102 ± 5	41 ± 3#	73 ± 5*	1,489 ± 151+
– riociguat, $n = 8$	87 ± 3*	29 ± 2*	98 ± 13+	1,467 ± 228

Here and in Table 4: $p < 0.05$ compared with: * the intact animals; + the control animals; # the animals receiving riociguat.

Most studies observed MBP reduction in systemic circulation in monocrotaline-induced PAH [17]. This effect is caused by a decrease in cardiac output and hypoxemia, leading to vasodilation [18]. PAH also reduces the activity of angiotensin-converting enzyme in the lungs, disrupting the production of angiotensin II and weakening its vasoconstrictive effect [19]. The reduced MBP response to AC in the rats with PAH confirms vascular endothelial dysfunction.

MBP and Δ MBP in the GRS group were higher than in the group with PAH and were similar to those in the intact group. Riociguat administration did not affect MBP and ΔMBP, which remained the same as in the group with PAH. The effect of GRS on Δ MBP

after AC administration was more pronounced than that of riociguat, which is probably associated with its ability to increase NO production in the endothelium.

Riociguat considerably increased the time of MBP restoration both after AC and SN administration. The GRS compound delayed MBP restoration after AC administration, but to a lesser extent than riociguat. We assume that riociguat stimulates sGC more, binds more strongly to enzyme molecules or stabilizes sGC – NO binding more [20]. The protective effect of GRS on the endothelium was shown by a larger area of the triangle above the MBP restoration curve compared with its area after riociguat administration ($p < 0.05$).

After SN administration, MBP in the intact animals decreased from 114 ± 4 to 51 ± 2 mm Hg. In the PAH animals, the MBP response to SN was weaker ($p < 0.05$) (Table 4), indicating a decrease in sGC sensitivity to the NO effect [20].

Table 4

Effects of GRS (10 mg / kg) and riociguat (1 mg / kg) on endothelial dysfunction in the rats with simulated PAH, *M* ± *m*

Group	Sodium nitroprusside dihydrate, 10 mcg / kg				EDC
	MBP, mm Hg	Δ MBP, mm Hg	Time of MBP restoration, sec	Area of the triangle above the MBP restoration curve, mm Hg / sec	
Intact rats, $n = 6$	114 ± 4	51 ± 2	80 ± 11	2,000 ± 244	1.80 ± 0.06
Rats with PAH (control), $n = 8$	97 ± 2*	44 ± 1*	99 ± 7	2,169 ± 158	2.36 ± 0.27
Rats with PAH, receiving:					
GRS, $n = 8$;	103 ± 5	45 ± 4#	105 ± 10#	2,388 ± 340	1.59 ± 0.13+
riociguat, $n = 8$	88 ± 4*+	31 ± 2*+	178 ± 15*+	2,833 ± 398	2.20 ± 0.37

The indolinone derivative GRS after continual administration at a dose of 10 mg / kg to rats with PAH did not reduce MBP to a level lower than the value in the intact animals. In the GRS group, Δ MBP did not improve after SN administration, but it did not become less than in the intact animals. The GRS compound has an antihypertensive effect, while it does not reduce normal blood pressure and maintains its regulation by activating the oxidized form of sGC. EDC in the GRS group was lower than in the control group (Table 4).

As a hypotensive agent [5], riociguat reduced MBP and Δ MBP in response to SN administration ($p < 0.05$). It is possible that in conditions of hypoxemia and endothelial dysfunction, some sGC molecules have lost heme and become oxidized, and riociguat does not stimulate oxidized sGC molecules [20].

The histologic examination showed that interalveolar septa became significantly thicker and were sclerotized in the lungs of animals with PAH. The alveoli were deformed, alveolar type II cells and smooth muscles proliferated. Granulation tissue grew in the alveolar lumen. Endothelial cell hyperplasia and smooth muscle hypertrophy were observed in the pulmonary arteries. Such pathological changes in the lung tissue correspond to interstitial pneumonia (Fig. 1). Focal cardiomyocyte hypertrophy and interstitial myocarditis developed in the myocardium of the right ventricle (Fig. 2).

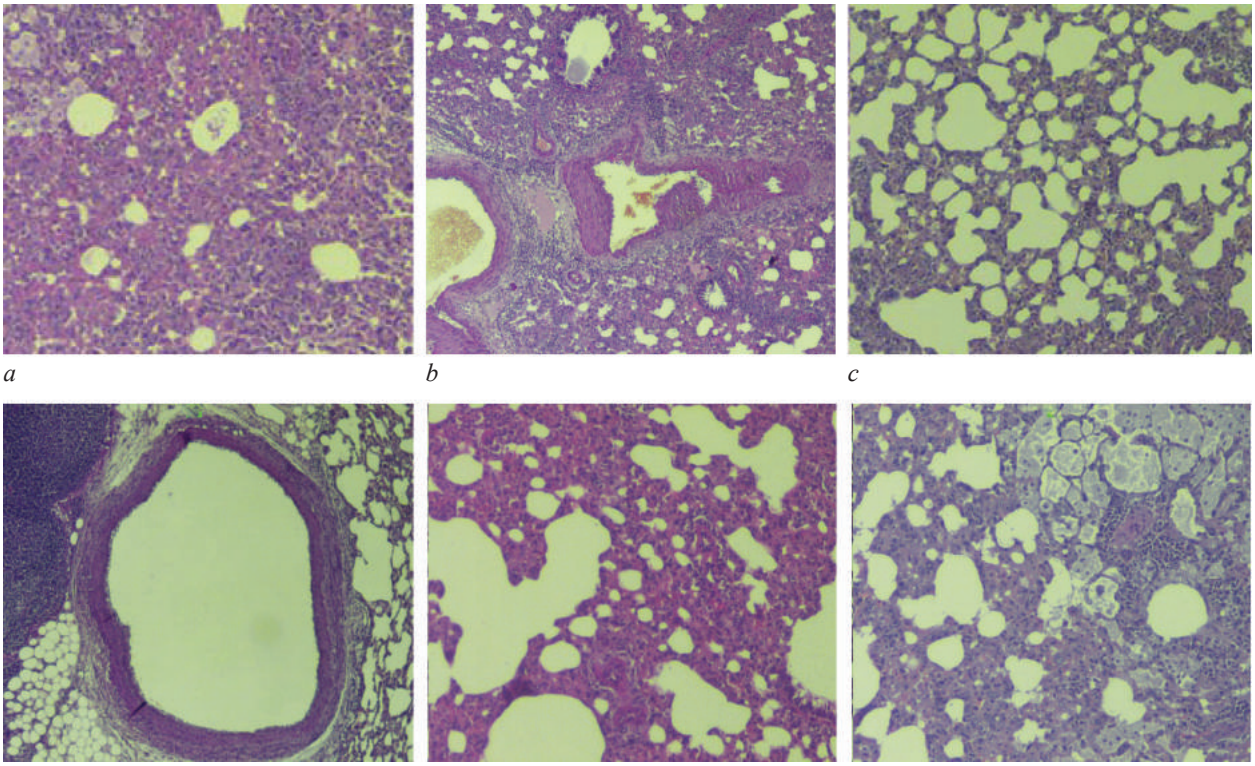
Administration of GRS and riociguat to the rats with PAH considerably decreased the thickness of the interalveolar septum; the alveoli became more open and air-filled. Alveolar type 2 cell and smooth muscle cell proliferation was less pronounced in the alveoli. Endothelial and arterial smooth muscle cells did not proliferate (Fig. 1). GRS administration reduced inflammatory infiltration in the myocardium, but cardiomyocyte hypertrophy persisted. Riociguat did not affect the myocardial pathology in PAH (Fig. 2).

CONCLUSION

The need for effective PAH treatment remains urgent [21]. Currently used drugs, such as endothelin receptor antagonists, calcium channel blockers, and iloprost, which is a prostacyclin analog, do not protect endothelium, may lower systemic blood pressure, and induce bleeding and other adverse effects. The SGC stimulator riociguat is the treatment standard for PAH, but it does not alleviate endothelial dysfunction and can cause tachycardia, arterial hypotension, and anemia [22, 23].

The new antithrombotic drug GRS, which is an indolinone derivative and a sGC stimulator, is as potent in lowering blood pressure in the right ventricle as riociguat in experimental PAH; unlike riociguat, it can also alleviate endothelial dysfunction. GRS also prevents pathological remodeling of pulmonary vessels.

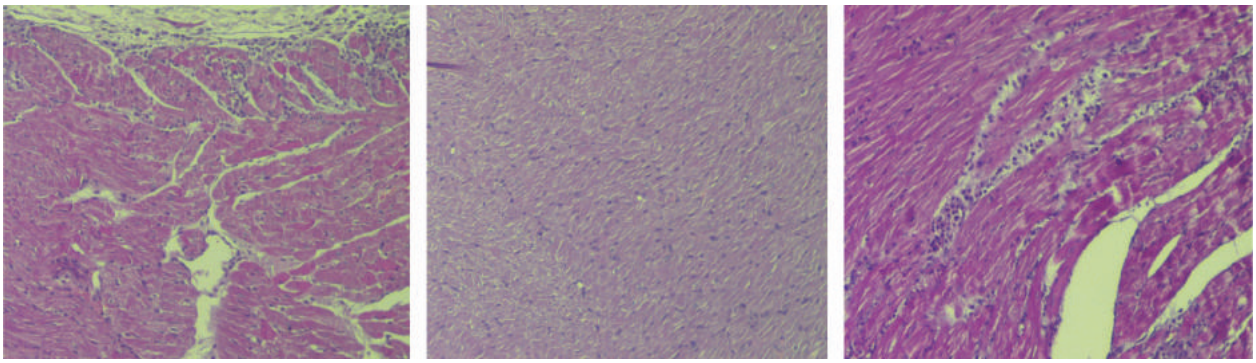
The data obtained in this study indicate the prospects of using the new antithrombotic drug, the indolinone derivative GRS, for the prevention and treatment of pulmonary arterial hypertension.



a *b* *c*

d *e* *f*

Fig. 1. Histologic changes in the lungs of the rats with simulated PAH (*a, b*) and administration of GRS at a dose of 10 mg / kg (*c, d*) and riociguat at a dose of 1 mg / kg (*e, f*). Here and in Fig. 2: staining with hematoxylin and eosin, 50× magnification



a *b* *c*

Fig. 2. Histologic changes in the right ventricular myocardium of the rats with PAH *(a)*, and administration of GRS at a dose of 10 mg / kg (*b*) and riociguat at a dose of 1 mg / kg (*c*)

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Bykov V.V., Bykova A.V. – conception and design. Aliev O.I., Sidikhmenova A.V., Dunaeva O.I. – carrying out of the experiment, analysis and interpretation of the data. Khazanov V.A., Stankevich S.A. – justification of the manuscript, critical revision of the manuscript for important intellectual content. Vengerovskii A.I., Udut V.V. – final approval of the manuscript for publication.

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