



# Correction of morphofunctional disorders of the cardiovascular system with asialized erythropoietin and arginase II selective inhibitors KUD 974 and KUD 259 in experimental preeclampsia

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## Abstract

**Introduction:** Preeclampsia remains one of the most common causes of maternal and perinatal mortality worldwide. A significant role in the pathogenesis of this pathology is assigned to placental ischemia and endothelial dysfunction. Therefore, the aim of the present study was to study the effectiveness of asialized erythropoietin and arginase II selective inhibitors: KUD-259 and KUD-974 in the correction of morphofunctional disorders of the cardiovascular system in experimental preeclampsia.

**Materials and methods:** The study was performed in 260 female Wistar rats, each weighing 250–300 g. An ADMA-like preeclampsia was reproduced in the experiment. To assess the emerging morphofunctional disorders, the following parameters were used: blood pressure, coefficient of endothelial dysfunction, microcirculation in the placenta, proteinuria, fluid content in the omentum, concentration of terminal metabolites in the blood plasma, and morphometric parameters of fetuses.

**Results and discussion:** The administration of arginase II selective inhibitor KUD-974 in combination with asialized erythropoietin leads to a pronounced correction of emerging changes: a decrease in systolic and diastolic blood pressure in 1.5 and 1.7 times, a decrease in proteinuria in 3.6 times and a decrease in fluid content in the omentum. When arginase II selective inhibitor KUD 974 and asialized erythropoietin are used with [methyldopa](#), the positive effects of the former are enhanced.

**Conclusion:** Arginase II selective inhibitors KUD-259 and KUD-974 and asialized erythropoietin have a pronounced positive effect on the correction of morphofunctional disorders in animals with ADMA-like preeclampsia.

## Keywords

arginase II selective inhibitor, asialized erythropoietin, preeclampsia, endothelial dysfunction.

## Introduction

The frequency of hypertensive pregnancy complications is 5–30%, according to the Ministry of Health of the Russian Federation, while they occupy the fourth place in the structure of maternal mortality rate. From 2 to 8% of pregnancies are complicated by preeclampsia in the world, according to rough estimates (Alkema et al. 2016; Roberge et al. 2017; Conti-Ramsden et al. 2019). Hypertensive disorders are the cause of the 16% of maternal losses in countries with high levels of socio-economic development (Pankiewicz et al. 2019).

According to modern concepts, the development of preeclampsia is based on the disorder of the endothelium structural and functional state (Abalos et al. 2013; Kittur et al. 2013; Yanagawa et al. 2013). It arose as a result of placentation disorder on the background of incomplete remodeling of the uterine arteries, which ultimately leads to a decrease in blood supply and placental ischemia (George et al. 2017; Tomimatsu et al. 2019).

In spite of a huge amount of the studies conducted, the only method of treating this condition recognized by all experts is still timely delivery, aimed primarily at minimizing the threat to pregnant woman's life. All other recommendations for the management of patients with preeclampsia can be reduced to the following principles: correction of hypovolemia, normalization of blood pressure, restoration of the blood rheological properties, and prevention of the progression of endothelial dysfunction (Pankiewicz et al. 2019; Tomimatsu et al. 2019).

Nowadays, the only proven effective method for the prevention of preeclampsia is the administration of low doses of aspirin. A number of international recommendations, including those by WHO, state that after 12 weeks of pregnancy, aspirin is required at a dose of 75–100 mg per day. More recent studies concluded that the dose of aspirin should be more than 100 mg per day, and its administration after 16 weeks of pregnancy had more advantages for the prevention of preeclampsia (Topozada et al. 1991; Montfort et al. 2020).

Preclinical studies in **L-NAME** induced preeclampsia in rats demonstrated the positive effects of resveratrol (Zou et al. 2014), recombinant erythropoietin (Gureev 2016) and tadalafil (Yoshikawa et al. 2017). Another promising group of drugs for the treatment of hypertensive conditions in pregnant women is arginase inhibitors (Nguyen et al. 2016; Severinova et al. 2019). At the same time, arginase II inhibitors as being more selective are of particular interest (Pokrovskii et al. 2017; Gureev et al. 2015).

So, despite a wide range of drugs, objective reality dictates the need to search for and study the effectiveness of new and currently existing drugs aimed at the prevention and treatment of preeclampsia and its complications. There is evidence of endothelial protective properties of selective arginase II inhibitors KUD-259 and KUD-974 (Pokrovskii et al. 2017); however, no studies of their effectiveness in experimental preeclampsia have been conducted yet.

In view of the fact that placental ischemia is another component of the pathogenesis of preeclampsia (George et al. 2017; Tomimatsu et al. 2019), erythropoietin derivatives not having an erythropoietic effect are a promising group of drugs in the treatment and prevention of preeclampsia (Mofidi et al. 2011; Ishii et al. 2012; Kaneko et al. 2013). These circumstances created the prerequisites for the present study.

**Objective:** To prove the effectiveness of asialized erythropoietin and selective arginase II inhibitors: KUD-259 and KUD-974 in the correction of morphofunctional disorders of the cardiovascular system arising from experimental preeclampsia.

## Materials and methods

The experimental study was conducted at the Research Institute of Pharmacology of Living Systems of Belgorod State National Research University. The study was performed in compliance with the General Requirements for the Competence of Testing and Calibration Laboratories 17025-2009, GOST R ISO 5725-2002 and the Rules of Laboratory Practice, approved by Order of the Ministry of Healthcare and Social Development of the Russian Federation dated August 23<sup>rd</sup>, 2010 № 708n. All the experiments were approved by the Ethical Committee of Belgorod State National Research University. Vivisection was performed in compliance with the ethical principles of treating laboratory animals of the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes. CETS No. 123 (European Treaty Series 1986).

**ADMA-like preeclampsia was simulation.** The study was performed in 260 female Wistar rats, each weighing 250–300 g. ADMA-like preeclampsia was simulated by administration of a nonselective eNOS blocker N-nitro-L-arginine methyl ether (**L-NAME**) at a dose of 25 mg/kg/day intraperitoneally from the 14<sup>th</sup> to 20<sup>th</sup> day of pregnancy. One day after the last injection under anesthesia (**chloral hydrate** 300 mg/kg), hemodynamic parameters were recorded, and the endothelial function was studied using a sensor and a hardware complex for invasive measurement of hemodynamic parameters by Biopac (USA) and the AcqKnowledge 3.8.1 computer software (Stupakova et al. 2019).

**Assessment of an endothelial dysfunction degree in the simulated pathology.** Endothelial dysfunction was evaluated by calculating a coefficient of endothelial dysfunction (CED), which is the ratio of endothelium-dependent vaso-relaxation (acetylcholine) and endothelium-independent vaso-relaxation (sodium nitroprusside) (Korokin et al. 2019).

**Estimation of the terminal NO metabolites.** The level of NO metabolites (that is the total concentration of nitrates and nitrites, NOx) was determined by the colorimetric method, according to the staining pattern in the diazotization reaction of sulfonamide nitrite, which is a part of the reagent (Stupakova et al. 2019).

**Analysis of the placental microcirculation.** Microcirculation in the placenta was measured using Biopac systems equipment: an MP100 polygraph with laser Doppler flowmetry module (LDF) LDF100C and an invasive needle probe TSD144 (Korokin et al. 2015), which was mounted directly in the projection of the placental disk. Registration and processing of LDF results were carried out using AcqKnowledge 3.8.1 software; microcirculation values were expressed in perfusion units (PU) (Gureev 2016).

**The study of swelling of the omentum.** To study the liquid content in the omentum, it was weighed, followed by drying at 37 °C for 24 hours and another weighing.

**Morphological methods for assessing changes in the placenta and kidneys in ADMA-like preeclampsia.** A histological examination (in all series of the experiment) of the kidneys and placenta was performed for morphological confirmation of the development of simulated pathological processes and in a comprehensive assessment of the drugs effectiveness. The material was fixed in 10% formalin, followed by paraffin embedding. The kidneys were sliced perpendicular to the main axis of the organ through the pelvis. Histological sections of the placenta were performed in a strictly vertical direction through the middle of the placental disc, capturing all layers of the placenta and the walls of the uterine horn. The study of the slides, photoprotocoling and morphometry were carried out using a Leica DM4000B microscope with a video recording and image processing system. For all the morphological studies, hematoxylin and eosin staining was used.

**The study of embryos.** The embryos were removed from the uterine cavity; their weight and growth (cranio-caudal size) were measured, followed by calculating the growth-weight coefficient.

**Statistical processing of the research results.** Descriptive statistics were applied to all the data. The data were checked for normal distribution. The type of distribution was determined by the Shapiro-Wilk criterion. In the case of a normal distribution, the mean value (M) and the standard error of the mean (m) were calculated. The intergroup differences were analyzed using Student's t-test or Mann-Whitney U-test, depending on the type of distribution. The statistical significance of the differences between morphological changes after their ranking was evaluated using Mann-Whitney nonparametric method of data analysis. All the calculations were performed using a Microsoft Excel 7.0 statistical package.

## Experiment design

According to the objective, all animals were divided into the following groups:

- Control group (animals with oral administration of NaCl at equivalent doses from the 14<sup>th</sup> to 20<sup>th</sup> -day of pregnancy).
- Simulation of experimental preeclampsia (**L-NAME** (25 mg/kg once daily intraperitoneally) from the 14<sup>th</sup> to the 20<sup>th</sup> day of pregnancy).
- Simulation of experimental preeclampsia + **methyldopa** (2 × 0.043 g/kg twice a day intragastrically)
- Simulation of experimental preeclampsia + recombinant erythropoietin (50 IU/kg/day intraperitoneally, on days 7, 10, 13, 16, and 19).
- Simulation of experimental preeclampsia + asialized erythropoietin (0.4 µg/kg/day intraperitoneally).
- Simulation of experimental preeclampsia + asialized erythropoietin (2.4 µg/kg/day intraperitoneally).
- Simulation of experimental preeclampsia + **L-Norvaline** (10 mg/kg/day intragastrically).
- Simulation of experimental preeclampsia + selective arginase II inhibitor KUD-259 (1 mg/kg/day intragastrically).
- Simulation of experimental preeclampsia + selective arginase II inhibitor KUD-974 (1 mg/kg/day intragastrically).
- Simulation of experimental preeclampsia + **methyldopa** (2 × 0.043 g/kg twice a day intragastrically) + asialized erythropoietin (2.4 µg/kg/day intraperitoneally).
- Simulation of experimental preeclampsia + **methyldopa** (2 × 0.043 g/kg twice a day intragastrically) + selective arginase II inhibitor KUD-974 (1 mg/kg/day intragastrically).
- Simulation of experimental preeclampsia + asialized erythropoietin (2.4 µg/kg/day intraperitoneally) + selective arginase II inhibitor KUD-974 (1 mg/kg/day intragastrically).

## Results and discussion

### Effect of asialized erythropoietin on morphofunctional disorders resulting from experimental preeclampsia

Female white Wistar rats, each weighing 250–300 g, were used in the experiment. To simulate experimental preeclampsia, N-nitro-L-arginine methyl ether (**L-NAME**), which has the biological properties similar to those of asymmetric dimethylarginin (ADMA) – an eNOS blockade – was administered at a dose of 25 mg/kg intraperitoneally from the 14<sup>th</sup> to 20<sup>th</sup> day of pregnancy. This resulted in a statistically significant ( $p < 0.05$ ) increase in systolic and diastolic blood pressure from  $123.2 \pm 3.46$  and  $76.3 \pm 5.71$  to  $194.8 \pm 7.88$  and  $149.8 \pm 4.73$  mmHg, respectively, in comparison with the group of the intact animals (Table 1).

Administration of asialized erythropoietin to the animals with experimental preeclampsia at a dose of 0.4 µg/kg/day intraperitoneally did not lead to a statistically significant decrease in blood pressure. A statistically significant ( $p < 0.05$ ) decrease in systolic and diastolic blood pressure to  $167.3 \pm 3.43$  and  $129.4 \pm 4.17$  mmHg was observed when administering asialized erythropoietin intraperitoneally at a dose of 2.4 µg/kg/day to the animals with experimental preeclampsia, but it did not

**Table 1.** Experimental results of the correction of experimental preeclampsia with asialized erythropoietin in rats (M ± m; N = 10).

Group	Indicator			
	SBP, mm hg	DBP, mm hg	CED, cond. un.	Microcirculation, PU
Intact	123.2 ± 3.46 <sup>γ</sup>	76.3 ± 5.71 <sup>γ</sup>	1.21 ± 0.08 <sup>γ</sup>	493 ± 21.1 <sup>γ</sup>
L-NAME	194.8 ± 7.88*	149.8 ± 4.73*	3.17 ± 0.22*	212 ± 6.0*
Methyldopa (2 × 0.043 g/kg)	134.9 ± 1.89 <sup>γ*</sup>	102.0 ± 3.71 <sup>γ*</sup>	2.51 ± 0.18 <sup>γ*</sup>	272 ± 12.2 <sup>γ*</sup>
EPo (50 IU/kg)	179.3 ± 3.84 <sup>γ*</sup>	133.4 ± 2.62 <sup>γ*</sup>	2.12 ± 0.21 <sup>γ*</sup>	301 ± 10.1 <sup>γ*</sup>
AsEPo (0.4 μg/kg)	183.1 ± 6.71 <sup>γ*</sup>	139.7 ± 3.72 <sup>γ*</sup>	2.09 ± 0.14 <sup>γ*</sup>	357 ± 22.6 <sup>γ*</sup>
AsEPo (2.4 μg/kg)	167.3 ± 3.43 <sup>γ*</sup>	129.4 ± 4.17 <sup>γ*</sup>	1.67 ± 0.12 <sup>γ*</sup>	413 ± 20.0 <sup>γ*</sup>

Note: SBP, DBP – systolic and diastolic blood pressure (mmHg); CED – coefficient of endothelial dysfunction; EPo – recombinant erythropoietin; AsEPo – asialized erythropoietin; PU – perfusion units; \* –  $p < 0.05$  in comparison with the group of intact animals; <sup>γ</sup> –  $p < 0.05$  compared with the L-NAME group.

reach the target level. It is worth noting that the intensity of the hypotensive effect of asialized erythropoietin was comparable with the hypotensive effect of recombinant erythropoietin, but was inferior to the hypotensive effect of **methyldopa**, a drug used in the treatment of hypertensive conditions in pregnant rats.

Simulation of experimental preeclampsia was accompanied by a change in the rate of reactions to vasodilating humoral factors, which indicated the disorder of the regulatory mechanisms of vascular tone and is explained by the resulting endothelial dysfunction. The coefficient of endothelial dysfunction (CED) increased from  $1.21 \pm 0.08$  (intact animals) to  $3.17 \pm 0.22$  relative units. Administration of asialized erythropoietin to the animals with experimental preeclampsia at a dose of  $0.4 \mu\text{g}/\text{kg}/\text{day}$  and  $2.4 \mu\text{g}/\text{kg}/\text{day}$  intraperitoneally led to a statistically significant ( $p < 0.05$ ) decrease in CED to  $2.09 \pm 0.14$  and  $1.67 \pm 0.12$  relative units, respectively, but CED did not reach the target level. This indicates a decrease in violations of the mechanisms controlling blood pressure.

It is important to emphasize that a decrease in CED under the influence of asialized erythropoietin at a dose of  $0.4 \mu\text{g}/\text{kg}/\text{day}$  was comparable with the effect of recombinant erythropoietin and exceeded the effect of **methyldopa**, a comparative drug for the treatment of hypertensive disorders in pregnant rats. The decrease in CED under the influence of asialized erythropoietin at a dose of  $2.4 \mu\text{g}/\text{kg}/\text{day}$  was more pronounced than the effect of both comparison drugs.

In the animals with experimental preeclampsia, a statistically significant ( $p < 0.05$ ) decrease in the placenta microcirculation was observed from  $493 \pm 21.1$  perfusion units (PU) (intact animals) to  $201 \pm 6.0$  PU. The administration of asialized erythropoietin to the animals with experimental preeclampsia at a dose of  $0.4 \mu\text{g}/\text{kg}/\text{day}$  and  $2.4 \mu\text{g}/\text{kg}/\text{day}$  intraperitoneally led to a statistically significant ( $p < 0.05$ ) increase in the placenta microcirculation to  $357 \pm 22.7$  PU and  $413 \pm 20.0$  PU, respectively, but did not reach the target level. However, it is of importance that under the influence of asialized erythropoietin at both doses, the improvement in microcirculation was more pronounced in comparison with the effects of both comparison drugs (Table 1).

A biochemical study showed that the simulation of experimental preeclampsia led to a decrease in the final NO

metabolites in plasma from  $2.23 \pm 0.04 \mu\text{mol}/\text{dl}$  (in intact animals) to  $1.25 \pm 0.01 \mu\text{mol}/\text{dl}$  (Fig. 1A). The administration of asialized erythropoietin to the animals with experimental preeclampsia at a dose of  $0.4 \mu\text{g}/\text{kg}/\text{day}$  and  $2.4 \mu\text{g}/\text{kg}/\text{day}$  intraperitoneally led to a statistically significant ( $p < 0.05$ ) increase in this indicator to  $1.49 \pm 0.02 \mu\text{mol}/\text{dl}$  and  $1.61 \pm 0.02 \mu\text{mol}/\text{dl}$ , respectively, but it did not reach the target level.

Simulated preeclampsia did not change the daily diuresis, but it led to a statistically significant ( $p < 0.05$ ) increase in proteinuria compared with the intact pregnant animals from  $0.18 \pm 0.05 \text{ g}/\text{L}$  to  $2.02 \pm 0.20 \text{ g}/\text{L}$  (Fig. 1B). The administration of asialized erythropoietin at a dose of  $0.4 \mu\text{g}/\text{kg}/\text{day}$  and  $2.4 \mu\text{g}/\text{kg}/\text{day}$  intraperitoneally led to a statistically significant ( $p < 0.05$ ) decrease in protein in the urine to  $1.4 \pm 0.06 \text{ g}/\text{L}$  and  $1.03 \pm 0.08 \text{ g}/\text{L}$ , respectively.

A study of the liquid content in the omentum of the animals with experimental preeclampsia revealed its increase ( $p < 0.05$ ) compared with the intact pregnant animals from  $44.20 \pm 1.08\%$  to  $54.27 \pm 0.64\%$ . The administration of asialized erythropoietin at the studied doses led to a decrease in the level of swelling of the omentum tissues ( $p < 0.05$ ) to  $49.89 \pm 0.75\%$  and  $48.73 \pm 0.81\%$ , respectively, but it did not reach the target level. The liquid content in the omentum decreased in the animals with ADMA-like preeclampsia treated with **methyldopa** and recombinant erythropoietin ( $p < 0.05$ ) to  $50.51 \pm 0.57\%$  and  $50.74 \pm 0.51\%$ , respectively.

A histological study of placental microsections in the group of intact animals on the 21<sup>st</sup> day of gestation revealed two well-visualized parts of the placenta: fetal and maternal. A microscopic examination of the histological sections of the placenta in the animals with experimental preeclampsia revealed distinct signs of destructive-dystrophic changes in the placenta. The administration of the studied pharmacological agents for the correction of experimental preeclampsia led to positive dynamics with the pronounced effect in the group of animals treated with asialized erythropoietin at a dose of  $2.4 \mu\text{g}/\text{kg}/\text{day}$ .

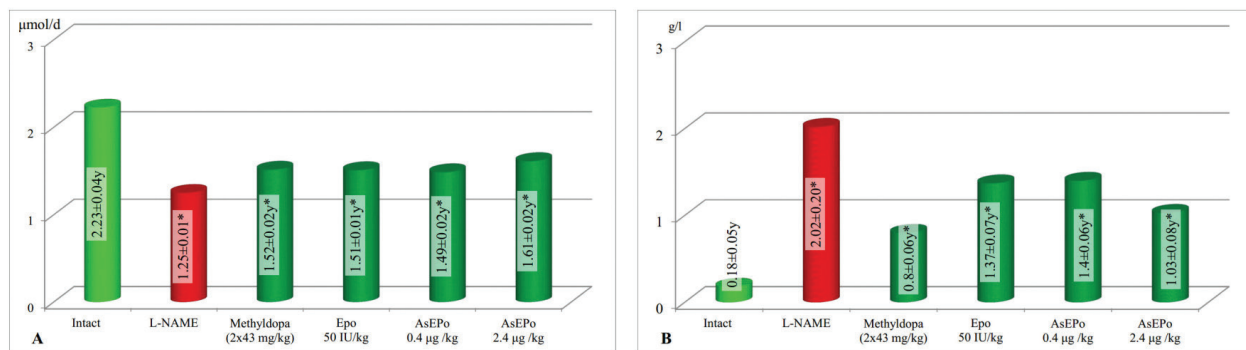
The results of the study of the features of fetal development in the animals with ADMA-like preeclampsia revealed fetal malnutrition (Table 2). The fetal mass changed to a greater extent. The most accurate indicator reflecting this pattern is the fetal height-to-weight ratio, which statistically significantly increased for these em-



**Table 2.** Effect of asialized erythropoietin on statural-weight values of fetal development in ADMA-like preeclampsia ( $M \pm m$ ;  $N = 20$ ).

Group	Indicator		
	Weight of embryos, g	Stature of embryos, mm	Statural- weight value, mm/g
Intact	$1.69 \pm 0.03^y$	$24.65 \pm 0.37$	$14.57 \pm 0.13^y$
L-NAME	$1.47 \pm 0.03^*$	$23.95 \pm 0.40$	$16.33 \pm 0.10^*$
Methyldopa ( $2 \times 0.043$ g/kg)	$1.52 \pm 0.02^*$	$23.80 \pm 0.28$	$15.65 \pm 0.08^{y*}$
EPo (50 IU/kg)	$1.54 \pm 0.03^*$	$24.25 \pm 0.47$	$15.73 \pm 0.09^{y*}$
AsEPo (0.4 $\mu$ g /kg)	$1.54 \pm 0.03^*$	$24.20 \pm 0.45$	$15.72 \pm 0.09^{y*}$
AsEPo (2.4 $\mu$ g /kg)	$1.63 \pm 0.03^y$	$24.80 \pm 0.40$	$15.20 \pm 0.09^{y*}$

Note: \* –  $p < 0.05$  in comparison with the group of intact animals;  $y$  –  $p < 0.05$  compared with the L-NAME group; EPo – recombinant erythropoietin; AsEPo – asialized erythropoietin.



**Figure 1.** Effect of asialized erythropoietin on the concentration of final nitric oxide metabolites in plasma during experimental preeclampsia (A), proteinuria (B). Note: \* –  $p < 0.05$  in comparison with the group of intact animals;  $y$  –  $p < 0.05$  compared with the L-NAME group; EPo – recombinant erythropoietin; AsEPo – asialized erythropoietin.

bryos. No post-implantation deaths were observed in any groups. The administration of the studied pharmacological agents led to positive dynamics, namely an increase in the mass of the fetus, though the level of the intact animals was not reached.

### Effect of selective arginase II inhibitors: KUD-259 and KUD-974 on morphofunctional disorders in experimental preeclampsia

The administration of arginase II selective inhibitors: KUD-259 and KUD-974 – to the animals with experimental preeclampsia led to a statistically significant ( $p < 0.05$ ) decrease in blood pressure compared with the group of the untreated animals only under the influence of KUD-974 (Table 3).

The use of arginase II selective inhibitors in the animals with ADAM-like preeclampsia: KUD-259 and KUD-974 – at a dose of 1 mg/kg/day orally led to a statistically significant ( $p < 0.05$ ) decrease in CED to  $1.53 \pm 0.10$  and  $1.70 \pm 0.12$  relative units, respectively, but did not reach the target level. This indicates a decrease in violations of the mechanisms controlling blood pressure. It is of importance that a decrease in CED under the influence of arginase II selective inhibitors: KUD-259 and KUD-974 – at a dose of 1 mg/kg/day was comparable with the effect of the non-selective arginase II inhibitor – L-Norvaline – and exceeded the effect of the comparison drug included in the treatment regimen of hypertensive conditions in pregnant – methyldopa.

In the animals with experimental preeclampsia, the administration of arginase II selective inhibitors: KUD-259 and KUD-974 – at a dose of 1 mg/kg/day intragastrically led to a statistically significant ( $p < 0.05$ ) increase in the placenta microcirculation to  $410 \pm 18.7$  PU and  $394 \pm 24.4$  PU, respectively, but did not reach the target level. However, it is worth noting that under the influence of both arginase II selective inhibitors: KUD-259 and KUD-974, the improvement of microcirculation was comparable to the effect of the non-selective arginase II inhibitor – L-Norvaline – and was more pronounced in comparison with the drugs included in the treatment standards for hypertensive conditions in pregnant women (Table 3).

A biochemical study found that the administration of arginase II selective inhibitors: KUD-259 and KUD-974 at a dose of 1 mg/kg/day intragastrically to the animals with experimental preeclampsia led to a statistically significant ( $p < 0.05$ ) increase in the content of terminal NO metabolites in blood plasma up to  $1.60 \pm 0.02$   $\mu$ mol/dl and  $1.80 \pm 0.03$   $\mu$ mol/dl, respectively, but it did not reach the target level (Fig. 2A).

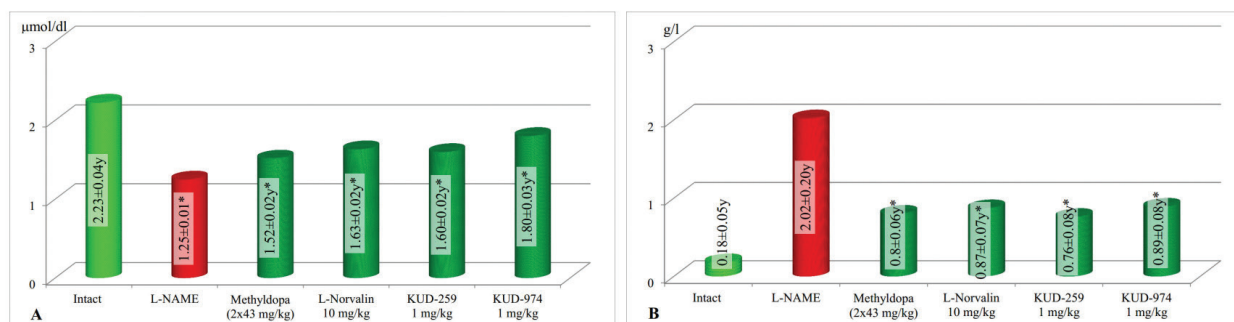
The administration of arginase II selective inhibitors: KUD-259 and KUD-974 – to the pregnant animals with experimental preeclampsia did not lead to a change in diuresis, but led to a decrease in proteinuria ( $p < 0.05$ ) to  $0.76 \pm 0.08$  g/L and  $0.89 \pm 0.08$  g/L, respectively.

The use of arginase II selective inhibitors: KUD-259 and KUD-974 – led to a decrease in the level of swelling of the omentum tissues ( $p < 0.05$ ) to  $49.30 \pm 0.26\%$  and  $48.92 \pm 0.68\%$ , respectively, but the target level was not

**Table 3.** Correction results of experimental preeclampsia with arginase II selective inhibitors: KUD-259 and KUD-974 (M ± m; N = 10).

Group	Indicator			
	SBP, mm Hg	DBP, mm Hg	CED, relative units.	Microcirculation, PU
Intact	123.2 ± 3.46 <sup>y</sup>	76.3 ± 5.71 <sup>y</sup>	1.21 ± 0.08 <sup>y</sup>	493 ± 21.1 <sup>y</sup>
L-NAME	194.8 ± 7.88*	149.8 ± 4.73*	3.17 ± 0.22*	212 ± 6.0*
Methyldopa (2 × 0.043 g/kg)	134.9 ± 1.89 <sup>*</sup>	102.0 ± 3.71 <sup>y*</sup>	2.51 ± 0.18 <sup>*</sup>	272 ± 12.2 <sup>*</sup>
L-Norvaline (10 mg/kg)	201.3 ± 2.89*	153.4 ± 3.27*	1.81 ± 0.12 <sup>y*</sup>	406 ± 19.8 <sup>*</sup>
KUD-259 (1 mg/kg)	195.8 ± 5.52*	144.9 ± 6.88*	1.53 ± 0.10 <sup>y*</sup>	410 ± 18.7 <sup>y*</sup>
KUD-974 (1 mg/kg)	148.1 ± 2.96 <sup>*</sup>	105.7 ± 4.85 <sup>y*</sup>	1.70 ± 0.12 <sup>y*</sup>	394 ± 24.4 <sup>*</sup>

Note: SBP, DBP – systolic and diastolic blood pressure (mmHg); CED – coefficient of endothelial dysfunction; PU – perfusion units; \* – p < 0.05 in comparison with the group of intact animals; <sup>y</sup> – p < 0.05 compared with the L-NAME group.



**Figure 2.** The effect of arginase II selective inhibitors: KUD-259 and KUD-974 – on the concentration of terminal nitric oxide metabolites (A) in plasma and proteinuria during experimental preeclampsia (B). Note: \* – p < 0.05 in comparison with the group of the intact animals; <sup>y</sup> – p < 0.05 compared with the L-NAME group.

reached. When the animals with ADMA-like preeclampsia were administered with L-Norvaline, the fluid content in the omentum tissues was  $49.57 \pm 0.70\%$ .

After the pharmacological correction, there was a positive dynamics of the previously detected reactive morphological changes in the placenta structures on the 21<sup>st</sup> day of gestation during experimental preeclampsia in all the experimental groups. The most pronounced positive effects were observed in the group of the animals treated with arginase II selective inhibitor KUD-794.

The administration of the studied arginase II selective inhibitors: KUD-259 and KUD-974 – led to an increase in fetal weight in the animals with ADMA-like preeclampsia, but the level of the intact animals was not reached (Table 4).

### Effect of the combination of asialized erythropoietin and selective arginase II KUD-974 inhibitor and their combined use with methyldopa on morphofunctional disorders in ADMA-like pre-eclampsia

The administration of asialo-EPO and arginase II selective inhibitor KUD-974 in combination with a standard therapy of hypertensive conditions methyldopa in the pregnant animals with experimental preeclampsia led to a statistically significant (p < 0.05) decrease in systolic and diastolic blood pressure compared with the group of the untreated animals (Table. 5). It is important to note that the use of arginase II selective inhibitor KUD-974 in

combination with methyldopa decreased systolic and diastolic blood pressure to the level not statistically different from that in the group of the intact animals. In the group of the animals treated with asialo-EPO and methyldopa, only systolic pressure decreased to the level statistically indistinguishable in comparison with the group of the intact animals.

The combined use of asialized erythropoietin and arginase II selective inhibitor KUD-974 led to a statistically significant (p < 0.05) decrease in systolic and diastolic blood pressure compared with the group of the untreated animals, while the level of diastolic pressure was not statistically indistinguishable from the group of intact animals. A decrease in blood pressure in the combined use of the pharmacological agents to the level statistically indistinguishable from the group of the intact animals can be regarded as potentiation of their action, since this was not observed in the monotherapy.

The use of a combination of asialized erythropoietin with arginase II selective inhibitor KUD-974 in the animals with experimental preeclampsia, and their combination with methyldopa, led to a more pronounced decrease in CED compared with groups of the animals where these pharmacological agents were used as a monotherapy (Table 5). In all the groups with combined use of the studied pharmacological agents, CED reached the level of that in the intact animals.

The administration of asialized erythropoietin and arginase II selective inhibitor KUD-974 in combination

**Table 4.** Effect of arginase II selective inhibitors: KUD-259 and KUD-974 – on statural-weight value of fetal development with ADMA-like preeclampsia (M ± m; N = 20).

Group	Indicator		
	Weight of embryos, g	Stature of embryos, mm	Statural-weight value, mm / g
Intact	1.69 ± 0.03 <sup>y</sup>	24.65 ± 0.37	14.57 ± 0.13 <sup>y</sup>
L-NAME	1.47 ± 0.03*	23.95 ± 0.40	16.33 ± 0.10*
L-NAME + L-Norvaline	1.58 ± 0.03 <sup>y*</sup>	24.45 ± 0.53	15.51 ± 0.06 <sup>y*</sup>
L-NAME + Methyldopa	1.52 ± 0.02*	23.80 ± 0.28	15.65 ± 0.08 <sup>y*</sup>
L-NAME + KUD-259 1 mg/kg/day	1.60 ± 0.02 <sup>y*</sup>	24.30 ± 0.31	15.16 ± 0.10 <sup>y*</sup>
L-NAME + KUD-974 1 mg/kg/day	1.61 ± 0.03 <sup>y*</sup>	24.20 ± 0.39	15.07 ± 0.08 <sup>y*</sup>

Note: \* – p < 0.05 in comparison with the group of the intact animals; <sup>y</sup> – p < 0.05 compared with the L-NAME group.

**Table 5.** Effect of asialized erythropoietin and arginase II selective inhibitor KUD-974 Combined with Methyldopa, as well as the combined use of asialized erythropoietin and arginase II selective inhibitor KUD-974 on functional parameters in experimental preeclampsia (M ± m; N = 10).

Group	Indicator			
	SBP, mm Hg	DBP, mm Hg.	CED, relative units	Microcirculation, PU
Intact	123.2 ± 3.46 <sup>y</sup>	76.3 ± 5.71 <sup>y</sup>	1.21 ± 0.08 <sup>y</sup>	493 ± 21.1 <sup>y</sup>
L-NAME	194.8 ± 7.88*	149.8 ± 4.73*	3.17 ± 0.22*	212 ± 6.0*
Methyldopa (2 × 0.043 g/kg)	134.9 ± 1.89 <sup>y*</sup>	102.0 ± 3.71 <sup>y*</sup>	2.51 ± 0.18 <sup>y*</sup>	272 ± 12.2 <sup>y*</sup>
AsEPo (2.4 µg/kg)	167.3 ± 3.43 <sup>y*</sup>	129.4 ± 4.17 <sup>y*</sup>	1.67 ± 0.12 <sup>y*</sup>	413 ± 20.0 <sup>y*</sup>
KUD-974 (1 mg/kg)	148.1 ± 2.96 <sup>y*</sup>	105.7 ± 4.85 <sup>y*</sup>	1.70 ± 0.12 <sup>y*</sup>	394 ± 24.4 <sup>y*</sup>
KUD-974 + Methyldopa	130.9 ± 4.43 <sup>y</sup>	91.4 ± 5.07 <sup>y</sup>	1.36 ± 0.10 <sup>y</sup>	492 ± 15.8 <sup>y</sup>
AsEPo + Methyldopa	130.1 ± 3.70 <sup>y</sup>	97.4 ± 4.93 <sup>y*</sup>	1.44 ± 0.12 <sup>y</sup>	480 ± 10.9 <sup>y</sup>
AsEPo + KUD-974	133.0 ± 2.50 <sup>y*</sup>	88.1 ± 6.45 <sup>y</sup>	1.30 ± 0.12 <sup>y</sup>	481 ± 16.8 <sup>y</sup>

Note: SBP, DBP – systolic and diastolic blood pressure (mmHg); CED – coefficient of endothelial dysfunction (relative units); PU – perfusion units; EPo – recombinant erythropoietin; AsEPo – asialized erythropoietin; \* – p < 0.05 in comparison with the group of intact animals; <sup>y</sup> – p < 0.05 compared with the L-NAME group.

with methyldopa, as well as a combination of asialized erythropoietin and arginase II selective inhibitor KUD-974 to the animals with experimental preeclampsia, led to an improvement in placental microcirculation to the level of the intact animals (Table 5).

A biochemical study of the concentration of terminal NO metabolites in plasma revealed a statistically significant increase (Fig. 3A) with the administration of asialized erythropoietin and arginase II selective inhibitor KUD-974 in combination with methyldopa, as well as with the combined use of asialized erythropoietin and arginase II selective inhibitor KUD-974. Moreover, it is important to note that this indicator in the described experimental groups reached the level of that in the intact animals.

The use of asialized erythropoietin and arginase II selective inhibitor KUD-974 in combination with methyldopa, as well as a combination of asialized erythropoietin and arginase II selective inhibitor KUD-974 in the animals with experimental preeclampsia, led to a statistically significant decrease in protein concentration in the urine. At the same time, proteinuria in the groups with combined use of the studied pharmacological agents decreased to a level comparable to that in the group of the intact animals. No changes in diuresis in the described experimental groups were observed.

When assessing the fluid content in the omentum, a statistically significant (p < 0.05) decrease was revealed with the administration of asialized erythropoietin and

arginase II selective inhibitor KUD-974 in combination with methyldopa to 44.25 ± 0.87% and 43.24 ± 1.31%, respectively, as well as with the combined use of asialized erythropoietin and arginase II selective inhibitor KUD-974 (41.95 ± 0.88%). Moreover, it is of importance that this indicator in the described experimental groups reached the level of that in the intact animals.

A histological examination of the placenta showed that the administration of asialized erythropoietin and arginase II selective inhibitor KUD-974 in combination with methyldopa, as well as a combination of asialized erythropoietin and arginase II selective inhibitor KUD-974 in the animals with experimental preeclampsia, led to pronounced positive dynamics of the morphological pattern comparable with that in the group of the intact animals.

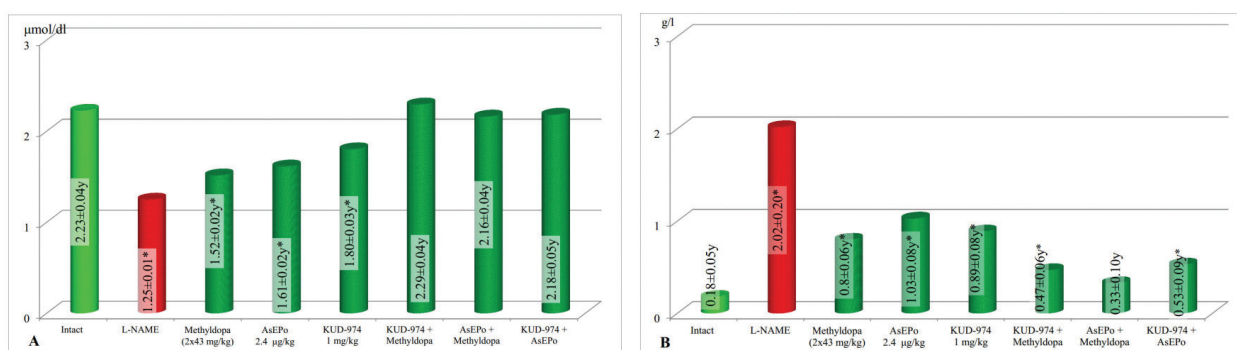
The results of the study of the fetal development in the animals with ADMA-like preeclampsia treated with the combination of asialized erythropoietin and arginase II selective inhibitor KUD-974 with methyldopa, as well as the combination of asialized erythropoietin and arginase II selective inhibitor KUD-974 revealed an increase in embryos' weight (Table 6). In both groups in which the combined therapy included arginase II selective inhibitor KUD-974, the fetal weight reached the level of the intact animals.

Thus, the results of the experiments indicate a pronounced protective activity of asialized erythropoietin and arginase II selective inhibitor KUD-974 in combination with methyldopa, as well as the combined use of asialized

**Table 6.** Effect of asialized erythropoietin and arginase II selective inhibitor KUD-974 in combination with **Methyldopa**, as well as the combined use of asialized erythropoietin and arginase II selective inhibitor KUD-974 on fetal statural-weight values in AD-MAc-like preeclampsia ( $M \pm m$ ;  $N = 20$ ).

Group	Indicator		
	Weight of embryos, g	Stature of embryos, mm	Statural-weight value, mm/g
Intact	$1.69 \pm 0.03^y$	$24.65 \pm 0.37$	$14.57 \pm 0.13^y$
L-NAME	$1.47 \pm 0.03^*$	$23.95 \pm 0.40$	$16.33 \pm 0.10^*$
Methyldopa ( $2 \times 0,043$ g/kg)	$1.52 \pm 0.02^*$	$23.80 \pm 0.28$	$15.66 \pm 0.08^y*$
AsEPo ( $2.4 \mu\text{g}/\text{kg}$ )	$1.63 \pm 0.03^y$	$24.80 \pm 0.40$	$15.20 \pm 0.09^y*$
KUD-974 ( $1 \text{ mg}/\text{kg}$ )	$1.61 \pm 0.03^y*$	$24.20 \pm 0.39$	$15.06 \pm 0.08^y*$
KUD-974 + Methyldopa	$1.62 \pm 0.03^y$	$23.95 \pm 0.52$	$14.81 \pm 0.10^y*$
AsEPo + Methyldopa	$1.62 \pm 0.02^y$	$24.20 \pm 0.40$	$14.94 \pm 0.10^y*$
AsEPo + KUD-974	$1.65 \pm 0.03^y$	$24.30 \pm 0.41$	$14.78 \pm 0.09^y$

Note: \* –  $p < 0.05$  in comparison with the group of intact animals;  $^y$  –  $p < 0.05$  compared with the L-NAME group; EPo – recombinant erythropoietin; AsEPo – asialized erythropoietin.



**Figure 3.** The effect of asialized erythropoietin and arginase II selective inhibitor KUD-974 in combination with **methyldopa**, as well as the combined use of asialized erythropoietin and arginase II selective inhibitor KUD-974 on the concentration of terminal nitric oxide metabolites in plasma (A) and proteinuria (B) in experimental preeclampsia. Note: \* –  $p < 0.05$  in comparison with the group of intact animals;  $^y$  –  $p < 0.05$  compared with the L-NAME group; EPo – recombinant erythropoietin; AsEPo – asialized erythropoietin.

erythropoietin and arginase II selective inhibitor KUD-974 on correction of morphofunctional disorders that occur in animals with simulated preeclampsia. It is worth noting that the used combinations provide a more pronounced correction of morphofunctional disorders compared to using each drug from this combinations as a monotherapy.

## Conclusion

One of the foundations of the modern concept of the development of preeclampsia is a violation of the structural and functional states of the endothelium (Pankiewicz et al. 2019; Tomimatsu et al. 2019) as a result of placental disorder on the background of incomplete remodeling of the uterine arteries, which ultimately leads to a decrease in blood supply and placental ischemia (George et al. 2017; Tomimatsu et al. 2019). The endothelium is a powerful endocrine organ involved in regulating vascular tone and maintaining their normal structure, monitoring the rheological properties of blood and local inflammation. One of the manifestations of the development of endothelial dysfunction is a deficiency of nitric oxide (NO), which is a powerful vasodilator (Aouache et al. 2018; Than et al. 2018).

Another important factor in endothelial dysfunction is ischemia. Endothelial cells, in comparison with other cells of the body, are quite resistant to ischemia. It is assumed that during ischemia, endothelial cells switch to anaerobic energy metabolism and also produce heat shock proteins, glyceraldehyde-3-phosphate dehydrogenase enzymes and non-neuronal enolase, which are involved in glycolysis, which in turn increase cell resistance to damage (Lutsenko et al. 2015). However, hypoxia alters the expression of a number of genes involved in the regulation of vascular tone, which leads to a shift in the balance between vasodilation and vasoconstriction towards the latter. Besides, under conditions of hypoxia, the activity of phospholipases A and C, diacylglycerol lipase, increases; the cascade of arachidonic acid is launched, and the concentration of its derivatives increases, which also affect vascular tone.

One of the promising groups of drugs for the treatment of preeclampsia are arginase inhibitors. At present, a great deal of data has been accumulated on their pronounced endothelial protective properties, including those obtained using various experimental models of preeclampsia (Gureev 2016; Nguyen et al. 2016). Arginase II inhibitors are of particular interest, as being more selective. The pre-clinical studies of the selective arginase II inhibitor ZB49-0010 in various pathology models showed its pronounced



endothelial protective properties that are superior to those for the non-selective inhibitor of L-norvaline (Gureev et al. 2015; Nguyen et al. 2016; Severinova et al. 2019). The literature contains data on the endothelial protective properties of arginase II selective inhibitors KUD-259 and KUD-974 on the functional state of vascular endothelium (Pokrovskii et al. 2017); however, to date, studies of their effectiveness have not been conducted in experimental preeclampsia yet.

Thus, it becomes obvious that short-lived erythropoietin derivatives that do not have an erythropoietic effect and selective arginase II inhibitors can serve as another promising area for searching for new drugs to prevent and correct preeclampsia. In this regard, the purpose of this study was to prove the effectiveness of asialized erythropoietin and arginase II selective inhibitors: KUD-259 and KUD-974 – in the correction of morphofunctional disorders of the cardiovascular system arising from experimental preeclampsia.

The course administration of asialized erythropoietin led to dose-dependent protective effects, which resulted in the correction of morphofunctional disorders arising from this experimental pathology. There was a decrease in blood pressure, an improvement in the placental microcirculation, a noticeable restoration of the regulatory mechanisms of vascular tone, a decrease in proteinuria, a decrease in swelling of the omentum, and a decrease in morphological abnormalities of the ischemic genesis in the placenta.

The presence of pronounced protective effects of asialized erythropoietin can be explained by the ability to bind to the erythropoietin heterodimeric receptor in the absence of an erythropoietic effect (Joshi et al. 2010; Mofidi et al. 2011; Kaneko et al. 2013). This is confirmed in the experiments on various organs and tissues and is explained by anti-apoptotic and antioxidant properties, the ability to restore the endothelial function (Joshi et al. 2010; Ishii et al. 2012; Kaneko et al. 2013; Kittur et al. 2013). The pronounced activity during ischemic injuries (Yanagawa et al. 2013) logically follows from the ability to activate, like recombinant erythropoietin, the processes of natural cytoprotection occurring in ischemic preconditioning. Based on this, it is fair to assume that the effects observed upon administration of recombinant erythropoietin to rats with ADMA-like preeclampsia could be expected from asialized erythropoietin (Gureev 2016).

Positive effects are associated with blockade of the enzyme – arginase II. The potential effects of the blockade of this enzyme have been confirmed in many experiments (Xiong et al. 2014; Sobolev et al. 2018). It was fair to expect that the positive effects obtained in the correction of morphofunctional disorders of ADMA-like preeclampsia by other arginase II inhibitors would be found in the studied pharmacological agents (Gureev et al. 2015; Nguyen et al. 2016; Severinova et al. 2019).

The administration of the studied pharmacological agents in various combinations led to a more pronounced correction of morphofunctional disorders in ADMA-like preeclampsia. This is explained by the fact that the used

pharmacological agents, having various mechanisms of action, affect a greater number of pathogenetic points.

Thus, the administration of the drugs with a different mechanism of action to animals with ADMA-like preeclampsia leads to more pronounced protective effects compared to the groups in which these drugs were used in a monotherapy. It is logical that the use of new pharmacological agents in complex therapy for the treatment of preeclampsia is most appropriate and has great prospects for further research.

1. The use of asialized erythropoietin at the doses of 0.4 µg/kg and 2.4 µg/kg is accompanied by a positive dynamics of morphofunctional disorders in experimental preeclampsia, which is expressed in a decrease in blood pressure, a decrease in CED by about 1.5 and 2 times, respectively, an increase in microcirculatory indicators by 1.7 and 2 times, a decrease in proteinuria by 31% and 49%, and an increase in the concentration of terminal metabolites of nitric oxide in blood plasma by 20% and 30%.
2. The use of arginase II selective inhibitors: KUD-259 and KUD-974 – at a dose of 1 mg/kg in experimental preeclampsia is accompanied by a positive dynamics of morphofunctional disorders, which is manifested in a decrease in blood pressure under the influence of KUD-974, a decrease in CED by about 2 times, and a 1.9-time increase in indicators of microcirculation, a decrease in proteinuria by 63% and 56%, and an increase in the concentration of final metabolites of nitric oxide in blood plasma by 25% and 44%.
3. The combined use of arginase II selective inhibitor KUD-974 and asialized erythropoietin with [methyldopa](#) ( $2 \times 0.043$  g/kg/day) is accompanied by a positive dynamics of morphofunctional disorders, which is manifested in a decrease in blood pressure which is more pronounced in the combination of KUD-974 with [methyldopa](#), and a decrease in CED by about 2.2 and 2.4 times, an increase in microcirculation to the level of the intact animals, a decrease in proteinuria and an increase in the concentration of terminal metabolites of nitric oxide in blood plasma to the level of the intact animals.
4. The combined use of arginase II selective inhibitor KUD-974 with asialized erythropoietin in experimental pre-eclampsia is accompanied by a positive dynamics of morphofunctional disorders, which is manifested in a decrease in blood pressure, a decrease in CED by about 2.4 times, an increase in microcirculation, a decrease in proteinuria, and an increase in the concentration of terminal nitric oxide metabolites in blood plasma to the level of the intact animals.

## Conflict of interests

The authors state no conflict of interest to declare.

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