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Research Article

Study of the effect of selective inhibitor of Arginase II KUD 975 and of low doses of Acetylsalicylic acid on the functional parameters of the cardiovascular system In experimental preeclampsia

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Abstract

Introduction: Over the past decade, preeclampsia has been the subject of an increased attention, as this complication is the most common cause of maternal mortality, triggering every third case of obstetric morbidity and taking lives of up to 50.000 young women worldwide each year. Despite a large number of ongoing studies, no clear algorithm for monitoring pregnant women with this pathology has been developed yet.

Materials and methods: The study was conducted on 220 female Wistar rats weighing 250–300 g. In the experiment, ADMA-like preeclampsia model was used.

Results and discussion: The introduction of L-NAME to the animals from the 14th till the 20th day of pregnancy causes the following disturbances: a rise in systolic and diastolic blood pressure in 1.4 and 1.5 times, an increase in proteinuria in 3.3 times and an increase in the content of fluid in the greater omentum from 44.39 ± 1.62% to 55.02 ± 1.74%, all of which correspond to the disorders in pregnant women in case of preeclampsia.

The use of the selective inhibitor of arginase II KUD 975 (3mg/kg/day) in combination with acetylsalicylic acid (10mg/kg/day) leads to a pronounced correction in the emerging changes: a decrease in systolic and diastolic blood pressure in 1.2 and 1.3 times, a decrease in proteinuria in 1.9 times and a decrease in the fluid content in the greater omentum.

Conclusion: Selective inhibitor of arginase II KUD 975 and small doses of acetylsalicylic acid have a pronounced positive effect in the correction of morphofunctional disorders in animals with ADMA-like preeclampsia.

Keywords

selective inhibitor of arginase II KUD 975, acetylsalicylic acid, preeclampsia, endothelial dysfunction.

Introduction

Preeclampsia, being the most frequent cause in the structure of maternal mortality over the past decade, has been the object of increased attention, as this complication causes every 3rd case of obstetric morbidity and annually takes up to 50.000 lives of young women around the world (Adamyan et al. 2016, Shakhno et al. 2016, Yakushev et al. 2016). Perinatal mortality rates and the rate of preterm birth (10–12%) in pregnant women with hypertensive disorders largely prevail over the corresponding values in women with physiological pregnancy. The incidence of preeclampsia during pregnancy ranges from 2 to 8% (Gureev et al. 2014, Gureev et al. 2015). Despite such close attention to this problem worldwide, the number of cases of this complication does not tend to decrease (Korokin et al. 2015). In recent years, the number of studies indicating that the main role in the development of preeclampsia is played by a violation of the functional state of the vascular endothelium, which entails the development of a generalized spasm, increased blood pressure and, as a consequence, ischemic disorders in the organs. In addition, especially dangerous and attracting an increased attention are disturbances in the hemostatic system in preeclampsia (AlSheeha et al. 2016, Severinova et al. 2019).

One of the main tasks of modern pharmacology and clinical pharmacology is the search for new drugs for the correction of various pathological conditions (Elagin et al. 2018, Skachilova et al. 2018), including preeclampsia (Gureev et al. 2014, Gureev et al. 2015). Despite a huge number of ongoing studies aimed at examining the etiopathogenetic aspects of preeclampsia, possible methods of drug and non-drug prevention and correction of this complication of pregnancy, no clear algorithm for the management of pregnant women with this pathology has been developed yet. The only effective method of treatment for this condition is a delivery, the purpose of which is primarily to minimize the threat to the life of a pregnant woman. Modern methods of treatment and prevention of preeclampsia, used in the daily practice of obstetricians and gynecologists, are aimed primarily at correcting the dominant syndrome itself, without sufficient consideration of the pathogenetic component. At the same time, it should be noted that in the modern literature there is information about experimental papers on different approaches of correction of simulated preeclampsia. Among the patho-physiologically sound approaches used in them are the methods aimed at: correction of placental ischemia using drugs that improve the depth of placentation and blood flow in the spiral arteries of the uterus (Tyurenkov et al. 2012, Rodger et al. 2014, Khodzhaeva et al. 2018), as well as the use of remote ischemic preconditioning and pharmacological preconditioning as activators of natural cytoprotection processes (Xu et al. 2017), improvement of impaired endothelial function and restoration of eNOS activity (Yakushev et al. 2012, Khodzhaeva et al. 2018), correction of hemostatic system (Roberge et al. 2012, Khodzhaeva et al. 2018), elimination of of oxidative stress manifestations (Memmert et al. 2016), correction of balance of cytokines and other humoral factors (Makarenko 2014, Saito et al. 2007). Arginase inhibitors are a promising group of drugs for the treatment of hypertensive conditions in pregnant women. Currently, there are a lot of data about their pronounced endothelioprotective properties obtained on different experimental models (Pokrovskiy et al. 2012, Yakushev et al. 2012). Arginase II inhibitors are of particular interest as they are more selective.

Recently, in the literature there has been evidence that disturbances in the hemostatic system can be not only a complication of preeclampsia or develop alongside it, but can also precede its manifestation or, to some extent, contribute to its development. The pathogenetic events that explain the role of activated platelets in the development of endothelial dysfunction in various diseases, including preeclampsia, are described in (Sidorenko et al. 2007, Hussien and Emam 2016, Blomqvist et al. 2019, Severinova et al. 2019).

The above call for search for and study the efficacy of new and currently available drugs aimed at the prevention and treatment of preeclampsia and its complications. Considering the fact that preeclampsia is based on a violation of the normal course of the placentation process with incomplete remodeling of the spiral arteries, resulting in placental ischemia and subsequent microcirculation disorders, inflammation, hemostatic disorders, oxidative stress and endothelial dysfunction, followed by an imbalance between vasoconstrictors and vasodilators (Hussien and 2016), the most promising direction in the treatment and prevention of preeclampsia is a combination of drugs that can stop the various above-mentioned pathogenetic aspects of this pathological condition during pregnancy.

Materials and methods

The research was conducted on 220 female Wistar rats weighing 250–300 g. 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 requirements of *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 23rd, 2010 № 708n, in compliance with the *European Convention for the Protection of Vertebrates Used for Experiments or Other Scientific Purposes* [Directive 2010/63/EU]. All the experiments were approved by the Ethical Committee of Belgorod National Research University. Vivisection was performed in compliance with the ethical principles of treating laboratory animals in *European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes. CETS No. 123.*

The experimental animals were kept in individually ventilated cells “Tecniplast” for small laboratory animals. Bed-

ding was made of sawdust sterilized under UV irradiation. The animals were fed with granulated feedstuff for small laboratory animals-rodents. Water was purified and sterilized by UV irradiation. The microclimate was created and maintained by a system of individually ventilated cells. Acclimatization and selection of animals for research consisted of a quarantine for at least 10 days. The animals were divided into groups according to their body weight. The animal were identified by applying individual labels on their bodies.

At the time of the research, the animals were healthy, without any changes in behavior, appetite, sleep and wakefulness. For 18 h before the experiments, the animals had been completely deprived of food, with free access to water.

In order to form groups of pregnant animals, males (2 animals), which had been kept separately, were put into cages to females (3 animals) for a day. Then the animals were separated again, and 10–14 days later, in a drug-induced sleep, by palpating the anterior abdominal wall, the fact of pregnancy was established. The pregnant rats were then randomized into 11 groups:

- Group 1 – intact (animals with physiological pregnancy);
- Group 2 – control (simulation of ADMA-like preeclampsia in the studied animals was performed by administration of a nonselective NOS blocker N-nitro-L-arginine-methyl ether (L-NAME) 25 mg/kg/day intraperitoneally from the 14th to the 20th day of pregnancy);
- Group 3 – L-NAME + L-Norvaline at a dose of 10 mg/kg/day orally from the 14th to the 20th day of pregnancy;
- Group 4 – L-NAME + methyldopa at a dose of 0.043 g/kg/day twice a day orally from the 14th to the 20th day of pregnancy;
- Group 5 – L-NAME + selective inhibitor of arginase II, KUD 975, at a dose of 1 mg/kg/day orally from the 14th to the 20th day of pregnancy;
- Group 6 – L-NAME + selective inhibitor of arginase II, KUD 975, at a dose of 3 mg/kg/day orally from the 14th to the 20th day of pregnancy;
- Group 7 – L-NAME + acetylsalicylic acid (ASA) at a dose of 7 mg/kg/day orally from the 14th to the 20th day of pregnancy;
- Group 8 – L-NAME + acetylsalicylic acid at a dose of 10 mg/kg/day orally from the 14th to the 20th day of pregnancy;
- Group 9 – L-NAME + selective inhibitor of arginase II, KUD 975, at a dose of 3 mg/kg/day orally + acetylsalicylic acid at a dose of 10 mg/kg/day orally from the 14th to the 20th day of pregnancy;
- Group 10 – L-NAME + acetylsalicylic acid at a dose of 10 mg/kg/day + methyldopa at a dose of 0.043 g/kg/day twice a day orally from the 14th to the 20th days of pregnancy;
- Group 11 – L-NAME + KUD 975 at a dose of 3 mg/kg/day + methyldopa at a dose of 0.043 g/kg/day twice a day orally from the 14th to the 20th day of pregnancy.

On the 21st day of the gestation, the experimental animal was anesthetized by intraperitoneal injection of chloral hydrate at a dose of 300 mg/kg of body weight, after which the functional tests were performed.

The hemodynamic parameters registered, and then the study of the endothelial function was carried out by means of a sensor and a hardware complex for invasive measurement of hemodynamic parameters Biopac (USA) and computer software ACQKNOWLEDGE 3.8.1. To evaluate the functional state of the endothelium, a calculated index was used – the coefficient of endothelial dysfunction (CED), which is the ratio of endothelium-dependent vasorelaxation (acetylcholine) and endothelium-independent vasorelaxation (sodium nitroprusside).

The final level of NO metabolites (total concentration of nitrate and nitrite, NOx) in blood plasma of the studied animals was measured using a colorimetric method by evaluating the development of staining in the reaction of diazotization by sulfanilamide nitrite.

The evaluation of the microcirculation state in the placenta was carried out using equipment from Biopac systems: a MP100polygraph with a laser Doppler flowmetry module (LDM) LDF100C and an invasive needle sensor TSD144, which was placed directly on the projection of the placental disc. Registration and processing of LDM results were performed using AcqKnowledge version 3.8.1; the microcirculation values were expressed in perfusion units (PU).

Collection of daily urine was carried out in metabolic cages. Evaluation of protein level in daily urine was carried out by the pyrogallol method on spectrophotometer PE-5400 V.

Descriptive statistics was applied to all the data: the data were checked for normality of distribution. The type of distribution was determined by the Shapiro-Wilk criterion. In the case of normal distribution, the mean (M) and the standard error of the mean (m) were calculated. The ontergroup differences were analyzed by methods depending on a type of distribution (Student's t-test or Mann-Whitney test). All the calculations were performed using a statistical software package Microsoft Excel 2010.

Results and discussion

Experimental study of the effect of selective inhibitor of arginase II KUD 975 and low doses of acetylsalicylic acid on functional disorders in ADMA-like preeclampsia

The simulation of ADMA-like preeclampsia resulted in an increase in systolic and diastolic blood pressure from 127.6±1.5 mmHg and 91.9±5.63 mmHg up to 200.5±6.32 mmHg and 151±5.69 mmHg, respectively. There was a distortion of the ratio of endothelium-dependent and endothelium-independent vasorelaxations, which resulted in an increase in CED from 1.32±0.8% c.u. to 3.13±0.21 c.u.

There was a decrease in microcirculation in the placenta from 487.9±22.56 PU to 210.2±11.18 PU.

The introduction of selective inhibitor of arginase II KUD 975 at a dose of 3 mg/kg/day in rats with experimental preeclampsia led to a statistically significant ($p<0.05$) decrease in systolic and diastolic blood pressure to 167.4±4.84 mmHg and 126.6 ± 4.85 mmHg, respectively. The introduction of selective inhibitor of arginase II KUD 975 at a dose of 1 mg/kg/day, as well as the introduction of acetylsalicylic acid at doses of 7 mg/kg/day and 10 mg/kg/day in rats with experimental preeclampsia did not lead to a statistically significant ($p<0.05$) decrease in systolic and diastolic blood pressure (Table 1).

Table 1. Results of Correcting ADMA-like Preeclampsia with Selective Inhibitor of Arginase II KUD 975 and Acetylsalicylic Acid in Rats (M±m; N=10).

Group \ Indicator	SBP (mmHg)	DBP (mmHg)	CED (conv.)	Microcirculation (PU)
Intact animals	127.6±1.5^{y}	91.9±5.63^{y}	1.32±0.8^{y}	487.9±22.56^{y}
L-NAME	200.5±6.32*	151±5.69*	3.13±0.21*	210.2±11.18*
L-NAME + L-Norvaline	201.2±6.92*	146.1±5.19*	1.84±0.11^{*y}	345.8±18.25^{*y}
L-NAME + Methyldopa	138.8±3.27^{*y}	99.4±4.36^{y}	2.53±0.18^{*y}	275.3±15.71^{*y}
L-NAME + KUD 975 1 mg/kg/day	192.7±5.07*	147.1±4.91*	2.02±0.07^{*y}	329.4±17.03^{*y}
L-NAME + KUD 975 3 mg/kg/day	167.4±4.84^{*y}	126.6±4.85^{*y}	1.69±0.08^{*y}	420.2±13.19^{*y}
L-NAME + ASA 7 mg/kg/day	198.0±7.53*	146.2±5.19*	2.18±0.10^{*y}	359.4±20.38^{*y}
L-NAME + ASA 10 mg/kg/day	192.3±7.62*	146.5±4.19*	1.78±0.11^{*y}	414±14.7^{*y}

Notes: SBP – systolic blood pressure (mmHg); DBP – diastolic blood pressure (mmHg); CED – coefficient of endothelial dysfunction (c.u.); PU – perfusion units; ASA – acetylsalicylic acid; * – $p<0.05$ compared to the intact animal group; ^{y} – $p<0.05$ compared to the L-NAME group.

The ratio of endothelium-dependent vasorelaxation and endothelium-independent vasorelaxation, expressed by CED, selective inhibitor of arginase II KUD 975 and acetylsalicylic acid in the studied doses had a statistically significant ($p<0.05$) positive effect. The greatest effect was observed at high doses, reducing CED to 1.69=0.08 c.u. and 1.78=0.11 c.u, respectively, but it did not reach the target level (Table 1).

In rats with ADMA-like preeclampsia with the administration of selective inhibitor of arginase II KUD 975 at doses of 1 mg/kg/day and 3 mg/kg/day and with the administration of acetylsalicylic acid at doses of 7 mg/kg/day and 10 mg/kg/day, a statistically significant ($p<0.05$) increase in microcirculation in the placenta was observed, with the greatest effect at higher doses (Table 1).

In rats with ADMA-like preeclampsia, an increase in the edema of the greater omentum was observed, which resulted in an increase in the fluid content in its tissues from 44.39±1.62% to 55.02±1.74%. In addition, there was a decrease in the content of final NO metabolites in blood plasma from 2.28±0.05 mmol/DL to 1.3±0.02 mmol/DL and an increase in proteinuria from 0.23±0.051 g/L to 2.2±0.177 g/L.

In rats with experimental preeclampsia, there was a statistically significant ($p<0.05$) decrease in fluid content in the tissue of the greater omentum when administering selective inhibitor of arginase II KUD 975 and acetylsalicylic acid in comparison with the group of the untreated animals (Fig. 1A). This may indicate a decrease in edema.

A biochemical study of blood plasma revealed a statistically significant increase in the concentration of final NO metabolites in blood plasma under the influence of selective inhibitor of arginase II KUD 975 and acetylsalicylic acid in the correction of functional disorders in rats with ADMA-like preeclampsia ($p<0.05$) compared with the group of the untreated animals (Fig. 1B). The greatest effect was observed at high doses (1.93±0.03 μmol/DL and 1.75±0.03 μmol/DL, respectively), but the target level was not achieved.

When ADMA-like agent was administered to the pregnant rats from the 14th to the 20th day of pregnancy, there was no change in the amount of urine excreted compared to the intact pregnant animals; the administration of selective inhibitor of arginase II KUD 975 and low doses of acetylsalicylic acid to the rats with experimental preeclampsia had no effect on the level of diuresis in experimental animals either (Fig. 1C).

The administration of selective inhibitor of arginase II KUD 975 and acetylsalicylic acid to the animals with experimental preeclampsia caused a statistically significant ($p<0.05$) decrease in protein concentration in urine of the experimental animals (Fig. 1D).

The statural-weight values of fetuses in ADMA-like preeclampsia and its corrections have to be highlighted. The administration of L-NAME led to a decrease in fetuses' weight and had almost no effect on their growth, so there was a statistically significant increase in the height/weight ratio of the fetuses. With the administration of a selective inhibitor of arginase II KUD 975 and acetylsalicylic acid, the loss of fetuses' weights was stopped, except in the groups with acetylsalicylic acid at a lower dose (Table 2). However, it should be noted that the height-weight ratio of the fetus is the most accurate indicator, since it has a smaller spread. In the groups using selective inhibitor of arginase II KUD 975 and acetylsalicylic acid, this indicator decreased due to an increase in the weight of fetuses, but it did not reach the target level.

Table 2. Effect of Selective Inhibitor of Arginase II KUD 975 and Acetylsalicylic Acid on Fetal Heightand Weight Parameters in Rats at ADMA-like Preeclampsia (M±m; n=10).

Group \ Indicator	Fetal weight, gm	Fetal height, mm	Height/weight, mm/gm
Intact animals	1.56±0.03^{y}	23.00±0.47	14.77±0.15^{y}
L-NAME	1.44±0.03*	23.3±0.45	16.2±0.13*
L-NAME + L-Norvaline	1.53±0.04^{y}	23.4±0.52	15.31±0.13^{y*}
L-NAME + Methyldopa	1.53±0.03^{y}	24.05±0.41	15.74±0.16^{y*}
L-NAME + KUD 975 1 mg/kg/day	1.54±0.03^{y}	23.5±0.43	15.26±0.13^{y*}
L-NAME + KUD 975 3 mg/kg/day	1.57±0.02^{y}	23.8±0.41	15.19±0.12^{y*}
L-NAME + ASA 7 mg/kg/day	1.49±0.03	23.4±0.43	15.72±0.16^{y*}
L-NAME + ASA 10 mg/kg/day	1.54±0.03^{y}	23.75±0.48	15.47±0.13^{y*}

Note: * – $p<0.05$ compared to intact pregnant females; ^{y} – $p<0.05$ compared to L-NAME group; ASA – acetylsalicylic acid.

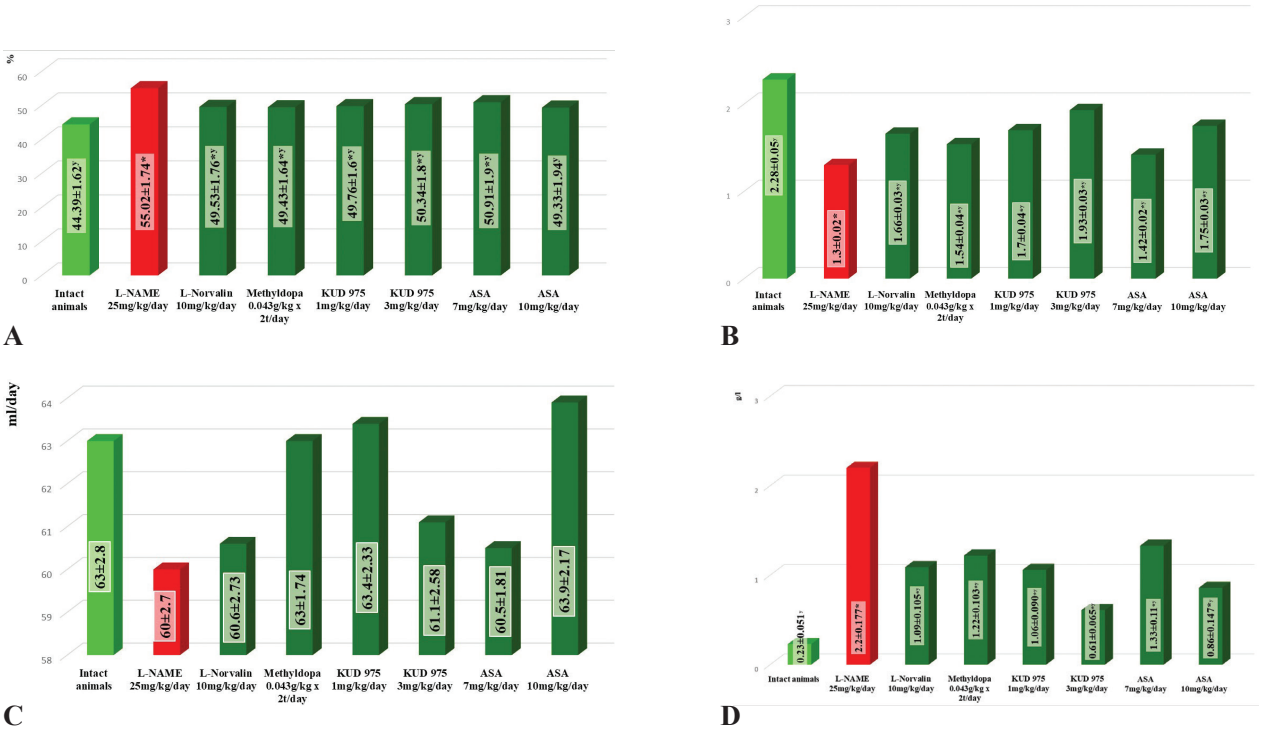

Figure 1. Effect of selective inhibitor of arginase II KUD 975 and low doses of acetylsalicylic acid on edema of the greater omentum (**A**), the content of final nitric oxide metabolites in plasma (**B**), diuresis (**C**) and proteinuria (**D**) in rats at ADMA-like preeclampsia. Note: * – p<0.05 compared to intact pregnant females; ^{y} – p<0.05 compared to L-NAME group; ASA – acetylsalicylic acid.

Experimental study of the effect of combined use of selective inhibitor of arginase II KUD 975 with acetylsalicylic acid on functional disorders in ADMA-like preeclampsia

When selective inhibitor of arginase II KUD 975 is administered in combination with acetylsalicylic acid (a drug included in the standard regimen of the preventive therapy of hypertensive states and preeclampsia in pregnant women) to the rats with experimental ADMA-like preeclampsia, there was a statistically significant (p<0.05) decrease in systolic and diastolic blood pressure (166.5±6.5 mmHg and 123.6±6.8 mmHg, respectively) in comparison with the group of the untreated rats, but the target level could not be achieved (Table 3).

The ratio of endothelium-dependent vasorelaxation and endothelium-independent vasorelaxation, expresse by CED, was positively affected by the combined administration of selective inhibitor of arginase II KUD 975 and acetylsalicylic acid to the rats with experimental preeclampsia, which was expressed in reaching a level statistically indistinguishable from the level of the intact animals (1.39±0.1 c.u. (Table 3).

In the rats with experimental preeclampsia, the combined administration of selective inhibitor of arginase II KUD 975 and acetylsalicylic acid resulted in a statistically significant (p<0.05) increase in microcirculation in the placenta (478.4±13.3 PU), which reached the level of the intact animals (Table 3).

Table 3. Results of Correction of ADMA-like Preeclampsia in Rats by Combined Administration of Selective Inhibitor of Arginase II KUD 975 and ASA (M±m; N=10).

Indicator / Group	SBP (mmHg)	DBP (mmHg)	CED (conv.)	Microcirculation (PU)
Intact animals	127.6±1.5^{y}	95.36±2.82^{y}	1.32±0.8^{y}	487.9±22.56^{y}
L-NAME	200.5±6.32*	151±5.69*	3.13±0.21*	210.2±11.18*
L-NAME + KUD 975 3 mg/kg/day	167.4±4.84*^{y}	126.6±4.85*^{y}	1.69±0.08*^{y}	420.2±13.19^{y*}
L-NAME + ASA 10 mg/kg/day	192.3±7.62*	146.5±4.19*	1.78±0.11*^{y}	414±14.7*^{y}
L-NAME + KUD 975 3 mg/kg/day + ASA 10 mg/kg/day	166.5±6.5*^{y}	123.6±6.8*^{y}	1.39±0.1^{y}	478.4±13.3^{y}

Notes: SBP – systolic blood pressure (mmHg); DBP – diastolic blood pressure (mmHg); CED – coefficient of endothelial dysfunction (c.u.); PU – perfusion units; ASA – acetylsalicylic acid; * – p<0.05 compared to the intact animal group; ^{y} – p<0.05 compared to the L-NAME group.

The study of the concentration of final metabolites of nitric oxide in blood plasma when administering selective inhibitor of arginase II KUD 975 with acetylsalicylic acid to the rats with ADMA-like preeclampsia revealed their statistically significant(p<0.05) increase to the level of the intact animals (Fig. 2A).

The study of the content of fluid in the tissues of the greater omentum in the rats with experimental preeclampsia revealed that there was a statistically significant (p<0.05) decrease of this indicator when administering selective inhibitor of arginase II KUD 975 in combination with acetylsalicylic acid (45.28±1.96%), compared to the group of the untreated rats, with this indicator achieving the level of the intact animals (Fig. 2B).

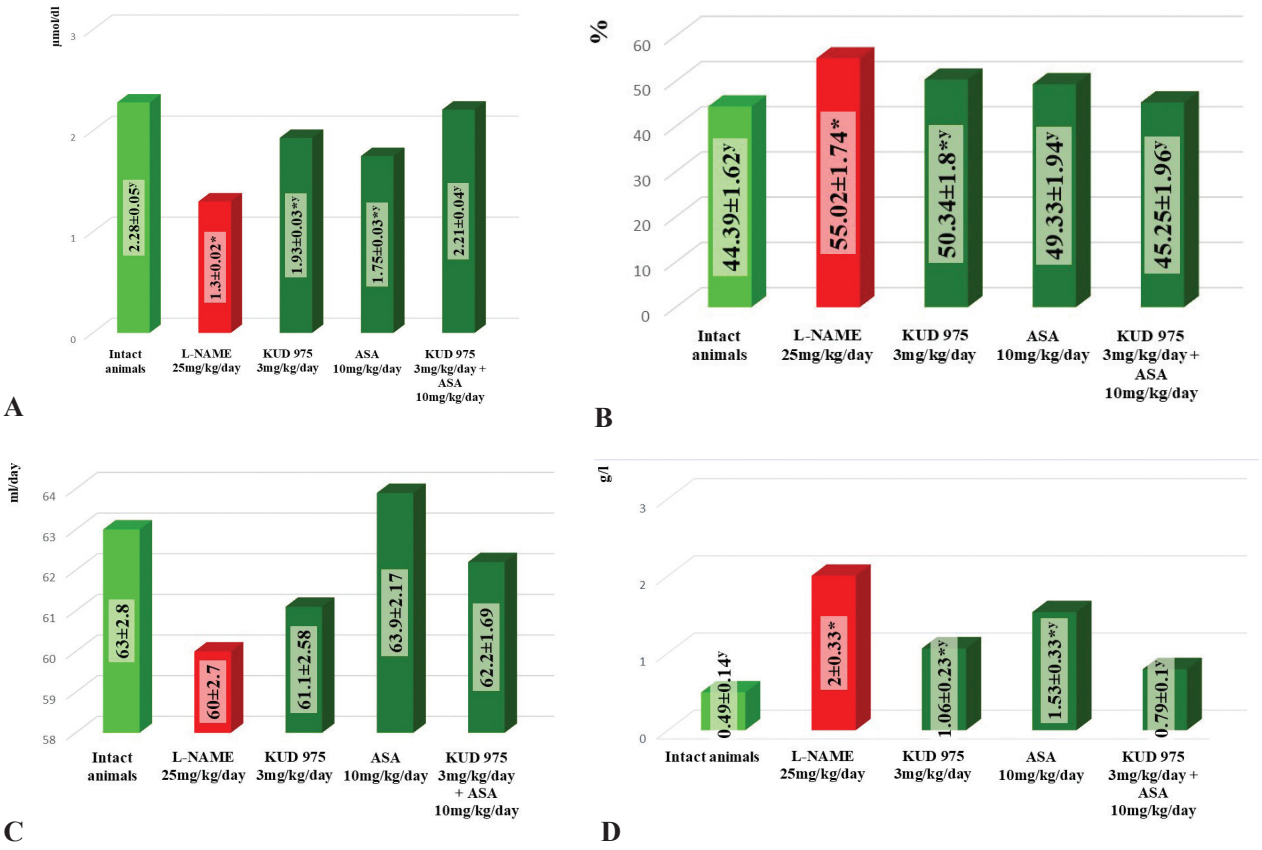

Figure 2. Effect of combined administration of selective inhibitor of arginase II KUD 975 with a low dose of acetylsalicylic acid on the content of final metabolites of nitric oxide in plasma (**A**), edema of the greater omentum (**B**), diuresis (**C**) and proteinuria (**D**) of rats in ADMA-like preeclampsia. Note: * – $p<0.05$ compared to intact pregnant females; ^{y} – $p<0.05$ compared to the L-NAME group.

The combined administration of selective inhibitor of arginase II KUD 975 in combination with acetylsalicylic acid to the rats with experimental preeclampsia results in a decrease in proteinuria to 0.79±0.1 g/L, which is statistically indistinguishable from the level of the intact animals (Fig. 2D). There was no effect on the level of diuresis (Fig. 2).

The study of the results of correction of height-weight parameters in the fetuses in experimental preeclampsia revealed the following pattern. The combined administration of selective inhibitor of arginase II KUD 975 and acetylsalicylic acid resulted in stopping the featuses' weight loss (Table 4). At the same time it should be noted that the ratio of height and weight of the fetus approached the level of statistically indistinguishable from the level of intact rats.

Table 4. Effect of Combined Use of Selective Inhibitor of Arginase II KUD 975 with Acetylsalicylic Acid on Height and Weight Parameters of Fetal Development in Rats with ADMA-like Preeclampsia (M=m; n=10).

Group \ Indicator	Fetal weight, gm	Fetal height, mm	Height/weight, mm/gm
Intact animals	1.56±0.0^{y}	23.00±0.47	14.77±0.15^{y}
L-NAME	1.44±0.0*	23.3±0.45	16.22±0.13^{y}
L-NAME + KUD 975 3 mg/kg/day	1.57±0.02^{y}	23.8±0.41	15.19±0.12^{*y}
L-NAME + ASA 10 mg/kg/day	1.54±0.03^{y}	23.75±0.48	15.47±0.13^{*y}
L-NAME + KUD 975 3 mg/kg/day + ASA 10 mg/kg/day	1.57±0.04^{y}	23.4±0.55	14.91±0.10^{y}

Note: * – $p<0.05$ compared to the intact animal group; ^{y} – $p<0.05$ compared to the L-NAME group; ASA – acetylsalicylic acid.

Experimental study of the effect of the combination of acetylsalicylic acid and selective inhibitor of arginase II KUD 975 with methyldopa on functional disorders and height-weight parameters of fetuses in ADMA-like preeclampsia

The administration of acetylsalicylic acid and selective inhibitor of arginase II KUD 975 in combination with methyldopa to the rats with experimental preeclampsia led to a statistically significant ($p<0.05$) decrease in systolic and diastolic blood pressure levels, reaching the levels of those in the animals with physiological pregnancy at 131.0±2.0 mmHg and 92.4±2.8 mmHg, respectively, and 127.3±1.5 mmHg and 88.3±2.1 mmHg, respectively (Table 5).

The study of the ratio of endothelium-dependent vasorelaxation and endothelium-independent vasorelaxation, the expression of which is CED, showed that the administration of acetylsalicylic acid and selective inhibitor of arginase II KUD 975 in combination with methyldopa

Table 5. Results of Correction of ADMA-like Preeclampsia by Administration of Acetylsalicylic Acid and Selective Inhibitor of Arginase II KUD 975 in Combination with Methyldopa to Rats (M±m; N=10).

Group \ Indicator	SBP (mmHg)	DBP (mmHg)	CED (conv.)	Microcirculation (PU)
Intact animals	127.6±1.5^{y}	91.9±5.63^{y}	1.32±0.8^{y}	487.9±22.56^{y}
L-NAME	200.5±6.32*	151±5.69*	3.13±0.21*	210.2±11.18*
L-NAME + Methyldopa 0.043 g/kg x 2 times/day	138.8±3.27^{*y}	99.4±4.36^{y}	1.84±0.11^{*y}	345.8±18.25^{*y}
L-NAME + ASA 10 mg/kg/ day	192.7±5.07*	147.1±4.91*	2.02±0.07^{*y}	329.4±17.03^{*y}
L-NAME + KUD 975 3 mg/kg/day	167.4±4.84^{*y}	126.6±4.85^{*y}	1.69±0.08^{*y}	420.2±13.19^{*y}
L-NAME + ASA 10 mg/kg/day + Methyldopa 0.043 g/kg x 2 times/day	131.0±2.0^{y}	92.4±2.8^{y}	1.51±0.1^{y}	469.8±15.44^{y}
L-NAME + KUD 975 3 mg/kg/day + Methyldopa 0.043 g/kg x 2 times /day	127.3±1.5^{y}	88.3±2.1^{y}	1.41±0.1^{y}	483.0±14.20^{y}

Notes: SBP – systolic blood pressure (mmHg); DBP – diastolic blood pressure (mmHg); CED – coefficient of endothelial dysfunction (c.u.); PU – perfusion units; ASA – acetylsalicylic acid; * – p<0.05 compared to the intact animal group; ^{y} – <0.05 compared to the L-NAME group.

to the rats with experimental preeclampsia had a positive effect, statistically significantly (p<0.05) reducing this ratio to the level of the intact animals (1.51±0.1 c.u. and 1.41±0.1 c.u., respectively) (Table 5).

In the rats with ADMA-like preeclampsia, the administration of acetylsalicylic acid and selective inhibitor of arginase II KUD 975 in combination with methyldopa resulted in a statistically significant (p<0.05) increase in microcirculation in the placenta, reaching the level of that in the intact animals at 469.8±15.44 PU and 483.0±14.20 PU, respectively (Table 5).

Evaluation of the fluid content in the tissues of the greater omentum revealed a statistically significant (p<0.05) decrease in this indicator when administrating acetylsalicylic acid and selective inhibitor of arginase II KUD 975 in combination with methyldopa to the rats with experimental preeclampsia in comparison with the group of the untreated animals, to the level of 44.32±2.1% and 44.65±1.67%, respectively. It should be noted that the edema index reached the level of that in the intact rats (Fig. 3A).

The biochemical study of the concentration of final metabolites of nitric oxide in blood plasma with the administration of acetylsalicylic acid and selective inhibitor of arginase II KUD 975 in combination with methyldopa to the rats with ADMA-like preeclampsia revealed their statistically significant (p<0.05) increase, to a level statistically indistinguishable from the level that in the intact animals (Fig. 3B).

When acetylsalicylic acid and selective inhibitor of arginase II KUD 975 are administered in combination with methyldopa to the rats with ADMA-like preeclampsia, there was a statistically significant (p<0.05) decrease in proteinuria, which reached the level in the intact rats (0.33±0.09 g/L and 0.38±0.09 g/L) (Fig. 3D). No effect on

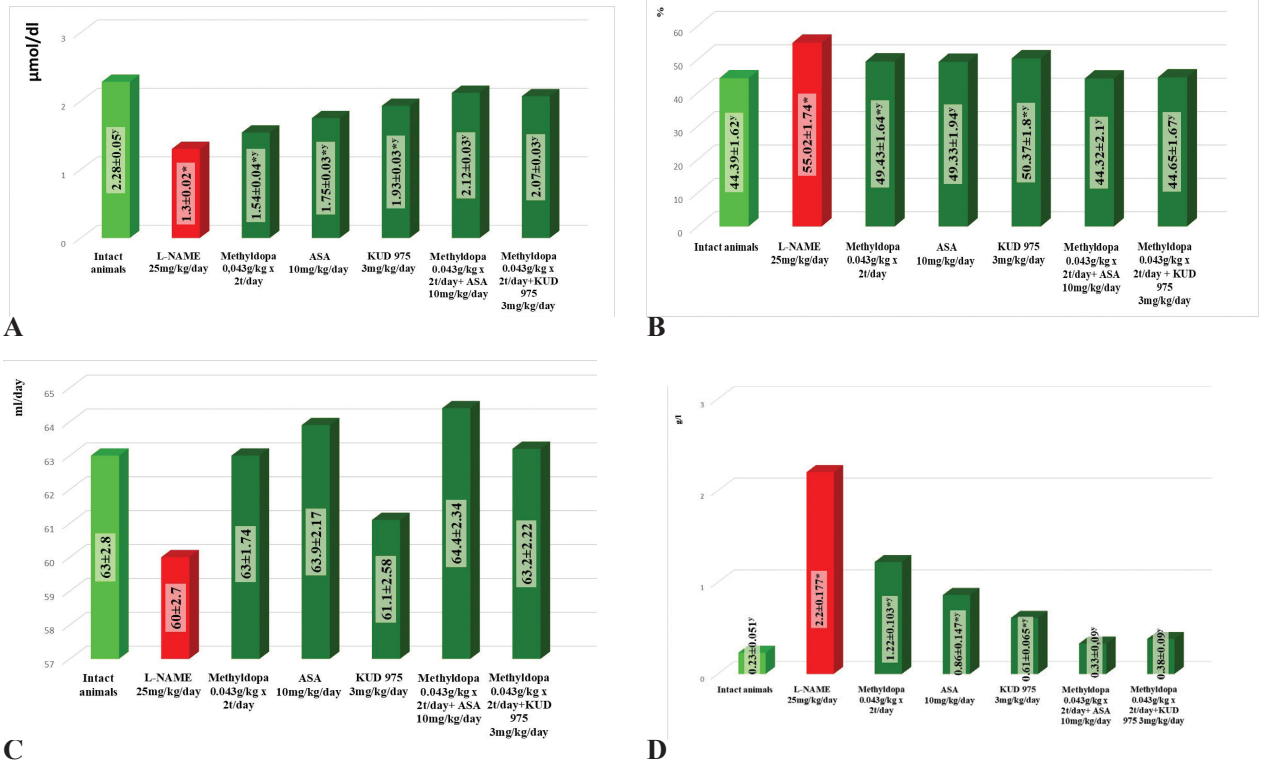

Figure 3. The impact of the administration of a low dose of acetylsalicylic acid and selective inhibitor of arginase II KUD 975 and their combinations with methyldopa on the content of final nitric oxide metabolites in plasma (**A**), edema of the greater omentum (**B**), diuresis (**C**), and proteinuria (**D**) in rats with ADMA-like preeclampsia. Note: * – p<0.05 compared to intact pregnant females; ^{y} – p<0.05 compared to L-NAME group; ASA – acetylsalicylic acid.

the level of diuresis in the experimental rats was recorded (Fig. 3).

The study of the results of correcting the height and weight parameters in fetuses in experimental preeclampsia showed an increase in fetal body weight in the groups with combined use of selective inhibitor of arginase II KUD 975 and acetylsalicylic acid with methyldopa (Table 6). It should be noted that the ratio of height and weight of the fetus approached the level which was statistically indistinguishable from the level in the intact rats.

Summing up all the above, it can be argued that the results of the series of experiments indicate a pronounced protective activity of selective inhibitor of arginase II KUD 975 and low doses of ASA in the correction of morpho-functional disorders in ADMA-like preeclampsia.

The positive effects of selective inhibitor of arginase II KUD 975 in the correction of morpho-functional disorders in ADMA-like preeclampsia can be explained by the available literature data on the ability of arginase II inhibitors to prevent the conversion of L-arginine into ornithine and urea, resulting in the formation of a larger amount of nitric oxide from L-arginine under the action of NO-synthase (Dzugkoev et al. 2018, Gewaltig and Kojda 2002, Miller 2006). The increased production of NO reduces the signs of endothelial dysfunction and contributes to the normalization of the regulatory mechanisms of the vascular tone. Elimination of vasoconstriction leads not only to lower blood pressure, but also improves tissue trophism, relieving ischemic symptoms in them, including in the placenta. Thus, by reducing the release of humoral factors of ischemic origin by the placenta, the vicious circle was broken, in which placental ischemia contributed to endothelial dysfunction, and endothelial dysfunction caused placental ischemia (Rodger et al. 2014). In support of the above, the previously published data on the efficacy of nonselective inhibitor of arginase II L-Norvaline in the correction of morpho-functional disorders in ADMA-like preeclampsia can be cited.

The positive effects of aspirin are related to its ability to irreversibly inhibit cyclooxygenase 1 (COX 1) of platelets (AlSheeha et al. 2016, Khodzhaeva et al. 2018). This reduces the formation of thromboxane A2, which causes vasoconstriction and activation of platelet adhesion and aggregation. Thus, acetylsalicylic acid by inhibiting the synthesis of thromboxane A2 reduces platelet activation (Sahin et al. 2015).

In the literature, there are data on the ability of low doses of acetylsalicylic acid to promote an increase in placental vascularization, normalization of the balance of angiogenic growth factors, such as VEGF, sVEGFR-1, PlGF in the serum of pregnant women. In addition, low doses of aspirin help improve the depth of placentation, microcirculation in the placenta and increase the blood flow in the spiral uterine arteries (Adamyan et al. 2016, Roberge et al. 2012, Roberge et al. 2016). Thus, the mechanisms of positive effects of acetylsalicylic acid include the improvement of microcirculation in tissues, reduction of thrombotic conditions and optimization of neovasculogenesis in the placenta. This leads to a decrease in ischemic conditions in the placenta and the restoration of the endothelial function, which play an important role in the modern understanding of the pathogenetic mechanisms of preeclampsia.

Table 6. Effect of Combined Use of Selective Inhibitor of Arginase II KUD 975 with a Low Dose of Acetylsalicylic Acid and Their Combination with Methyldopa on Height and Weight Parameters of Fetal Development in Rats with ADMA-like Preeclampsia (M±m; n=10).

Group \ Indicator	Fetal weight, gm	Fetal height, mm	Height/weight, mm/gm
Intact animals	1.56±0.03^{y}	23.00±0.47	14.77±0.15^{y}
L-NAME	1.44±0.03*	23.3±0.45	16.22±0.13*
L-NAME + KUD 975 3 mg/kg/day	1.57±0.02^{y}	23.8±0.41	15.19±0.12^{*y}
L-NAME + ASA 10 mg/kg/day	1.54±0.03^{y}	23.75±0.48	15.47±0.13^{*y}
L-NAME+ KUD 975 3 mg/kg/day + Methyldopa 0.043 g/kg x 2 times /day	1.57±0.04^{y}	23.3±0.50	14.81±0.11^{y}
L-NAME+ ASA 10 mg/kg/day + Methyldopa 0.043 g/kg x 2 times /day	1.6±0.04^{y}	23.65±0.54	17.86±0.11^{y}

Note: * – p<0.05 compared to the intact animal group; ^{y} – p<0.05 compared to the L-NAME group; ASA – acetylsalicylic acid.

The most pronounced effects are observed with the combined use of the studied pharmacological agents. This can be explained by the fact that having different mechanisms of action, the studied pharmacological agents affect a greater number of pathogenetic links of the developing pathology.

Conclusion

1. Selective inhibitor of arginase II KUD 975 has a pronounced protective activity in the correction of morpho-functional disorders arising in the simulated ADMA-like preeclampsia.
2. Acetylsalicylic acid in low doses exhibits protective properties in the correction of morpho-functional disorders arising from ADMA-like preeclampsia in rats.
3. The use of methyldopa in combination with a selective inhibitor of arginase II KUD 975 and acetylsalicylic acid leads to an increase in their positive effects in the correction of morpho-functional disorders occurring in ADMA-like preeclampsia in rats.
4. Selective inhibitor of arginase II KUD 975 in combination with low doses of acetylsalicylic acid exhibits more pronounced protective properties compared to a mono therapy.

Conflict of interests

The authors state no conflict of interest to declare.

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