UDC:618.3-06-08:577.112.385.2

Рус. Eng.

Gureev V.V.

NEW APPROACHES OF MORFOFUNKTIONAL PHARMACOLOGICAL CORRECTION OF VIOLATIONS OF CARDIOVASCULAR SYSTEM IN EXPERIMENTAL PREECLAMPSIA

Ph.D. Associate Professor, Department of Pharmacology of the Federal State Autonomous Educational Institution of Higher Education "Belgorod State National Research University" of the Ministry of Education and Science of the Russian Federation 85, str. Pobedy, Belgorod, 308015, Russia, e-mail: *gureev@bsu.edu.ru*

Abstract. Experimental Modeling ADMA-like preeclampsia administration to rats was performed by N-nitro-L-arginine methyl ester, from 14 to 20 days of pregnancy. The animals were observed increase in blood pressure, proteinuria, impaired microcirculation in the placenta, the violation of the regulation of vascular tone and destructive changes in the placenta of ischemic origin. Introduction of tetrahydrobiopterin, a selective inhibitor of arginase II of, recombinant erythropoietin, tadalafil, erythromycin, azithromycin, and playback systems polivitaminnomineralnyh distant ischemic preconditioning resulted in a pronounced correction of morphological and functional disorders arise when modeling the experimental preeclampsia. This was reflected in the reduction of blood pressure, reduction of proteinuria, increase of microcirculation in the placenta, the restoration of vasodilator function of blood vessels and prevent destructive phenomena in the placenta compared with a group of untreated animals, increasing the concentration of NO end-metabolites in plasma. These data suggest a pronounced correction of morphological and functional disorders arise when modeling ADMA-like preeclampsia study medication and the prospects for further research to find new drugs have endoteleoprotektivnymi properties for correcting the second half of pregnancy preeclampsia.
Keywords: ADMA, preeclampsia, endothelial dysfunction, rats, endotelioprotektory.

INTRODUCTION

Preeclampsia is the most common obstetric pathology. In certain regions of the country, its frequency can be more than 30% of all cases of pregnancy [1, 2]. In preeclampsia maternal mortality is one of the first places. It accounts for up to 25% of cases [3, 4]. Children born to mothers who have had preeclampsia, perinatal morbidity is 30%, and the mortality rate is 3-4 times higher than the population-based [5]. In addition, suffering mental and physical development of the child, while women are more likely development of chronic kidney disease and hypertension [4, 6].

The pathogenesis of preeclampsia is still far from a complete understanding, but more and more researchers are paying attention disangiogenesis in the placenta and impaired regulation of the tone of small arteries [7, 8, 9]. A morphological study of the placenta, many authors describe a specific histology, which is the imbalances of its border area between the maternal and fetal parts [10, 11, 12, 13]. Incomplete cytotrophoblast invasion occurs in maternal spiral arteries, leading to ischemic events in the placenta and increase the permeability barrier foetoplacental [11, 14].

Release in the systemic circulation of the ischemic placenta free radicals, hormones, growth factors, pro-inflammatory cytokines, fetal antigens and other humoral factors causes an increase in the content of cell adhesion molecules and blood accumulation of endogenous inhibitors of eNOS – methylated analogs of L-arginine asymmetric dimethylarginine (ADMA) and monometilarginina (L-NMMA). The end finale is a complex pathophysiological mechanisms and a violation of the rheological properties of blood coagulation and endothelial dysfunction, which cause multiple organ failure [14, 15, 16].

Thus, at the present time it has accumulated quite a lot of information on the impact of various factors on the course of preeclampsia and their role in the development of endothelial dysfunction as the main pathogenetic link of this terrible disease and the possibility of correction of endothelial dysfunction pharmacological agents of different groups [17, 18, 19]. However, the available literature there is no information about the study endothelioprotektive action in terms of ADMA-like preeclampsia tetrahydrobiopterin, tadalafil, vitamin B6 and folic acid, multivitamins and mineral complexes, erythromycin, azithromycin, recombinant erythropoietin, as well as on the effectiveness of the direction, which is based on distant ischemic preconditioning.

MATERIALS AND METHODS

The experiment was performed on female white rats of Wistar line weighing 250-300 g ADMA-like Gesto modeoirovali by administration of non-selective NO-synthase blocker of N-nitro-L-arginine methyl ester (L-NAME) was administered intraperitoneally in a dose of 25 mg / kg / day for seven days (day 14-20 of pregnancy) [10, 20, 21, 22]. To simulate the reduced

blood flow to the uterus on day 14 of gestation in 10 animals were silver overlay clips directly above the bifurcation of the abdominal aorta (0.2 mm) on both ovarian artery (0.1 mm). In 10 animals on day 14 of gestation performed silver overlay clip on the right iliac artery (0.1 mm) and the right ovarian artery (0.1 mm) [23, 24].

The degree of endothelial dysfunction by calculating the ratio of endothelial dysfunction (CED) [10, 20].

The level of NO metabolites (m. E. The total concentration of nitrite and nitrate, NOx) was measured colorimetrically using a color development reaction diazotization of sulfanilamide nitrite forming part of the reagent. eNOS levels were determined in the cell lysate by the method of R.J. Hendrickson with minor modifications [10, 25].

Measurement of the microcirculation in the placenta was performed using equipment companies Biopacsystems: polygraph MP100 module with laser Doppler flowmetry (LDF) LDF100C and invasive needle sensor TSD144 [10, 20, 21].

Collection of urine was performed for 12 hours using a special metabolic cages. The method Brandberg-Roberts-Stolnikova ring is sample Geller.

To investigate the liquid in the greater omentum was performed weighing it, followed by drying at 37 0C for 24 hours and weighed again [5, 26].

Morphological studies were conducted under the guidance of prof. AA Dolzhikov and prof. VS Barsukov

The embrions was removed from the uterine cavity, weighed, measured growth (craniocaudal size) followed by calculation of height-for-weight [27].

1. COMPARATIVE ASSESSMENT OF MORPHOFUNCTIONAL DISTURBANCES AT ADMA-MODELING OF PREECLAMPSIA AND REDUCED BLOOD FLOW TO THE UTERUS

With the introduction of ADMA-like agents – L-NAME to pregnant females (25 mg / kg once daily from 14 to 20 days of pregnancy) on day 21 we observed the development of pathology in its manifestations corresponding criteria of clinical manifestations of preeclampsia. There was a statistically significant increase in systolic and diastolic blood pressure to 134,5 ± 2,3 and 92,0 ± 2,1 to 186,3 ± 7 and 145,0 ± 5,0 mm Hg. Art., respectively, and an increase in CED with 1,1 ± 0,11 to 3,12 ± 0,17 (p <0,05) (Table. 1.1).

The value of the level of the microcirculation in the placenta intact pregnant rats on day 21 amounted to 446,3 ± 27,5 ped (perfusion units). In the simulation, ADMA-like preeclampsia, it found a significant decrease to 218,3 ± 13,7 ped. In intact pregnant rats 12-hour urine output was 5.8 ± 0.3 ml, which corresponded to the normal performance of animals weighing 250-300 g at proteinuria, which values correspond to 0.93 ± 0.06 g / l, ie to 1.0 g / l. The introduction of L-NAME had no significant effect on 12-hour urine output. In the analysis of urine revealed a moderately pronounced increase in proteinuria with values of 2,04 ± 0,22 g / l (p <0,05). However, it should be noted that in the measurement of the microcirculation in the renal tissue is statistically significant difference between the group of intact animals, and pregnant animals with simulated ADMA-like preeclampsia was not observed.

Examination of endothelial NO-producing function showed that the simulation L-NAME-induced deficit of NO resulted in a sharp decrease in expression of eNOS and content of nitrite ion (NOx), respectively, 1.8 and 1.7-fold (Table 1.1.).

Histology of the kidneys intact pregnant rats (21 days gestation) were as follows: glomeruli moderately congested, the basement membrane is thickened, inflammatory changes in the interstitium and glomeruli are no pyramids; spastic and hypertrophic changes in the small arteries and arterioles were detected (Fig. 1.1).

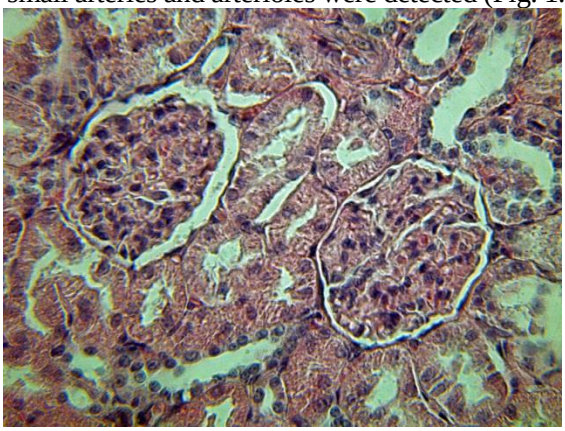


Figure 1.1. Kidney pregnant rats normal: small artery in the cortex is intact with no signs of hypertrophy of the walls, its endothelium; glomerular capillary basement membrane is thickened, the cellular inflammatory response is absent. H & E stain. X 280.

Placenta rats on day 21 of pregnancy with no signs of damage (Fig. 1.2).

In rats with ADMA-like preeclampsia found to increase the fluid content in the tissue of the greater omentum with 49,89 ± 0,82% to 58,09 ± 1,73% (p <0.05). The glomeruli showed signs of ischemic damage in the form of anemia, and degenerative changes in the basal membrane of the glomerular capillaries with thickened in its form of wire loops (Fig. 1.3 (b)). A notable reaction mesangium were observed: the average number of cells per glomerulus was 39.20 ± 2.99 (40.70 ± 3.80 normally). These changes may explain the glomerular apparatus available to the rats in this group hyperfiltration with severe proteinuria. In the small arteries and arterioles spasm observed and marked hypertrophy of the walls (Fig. 1.3 (a)).

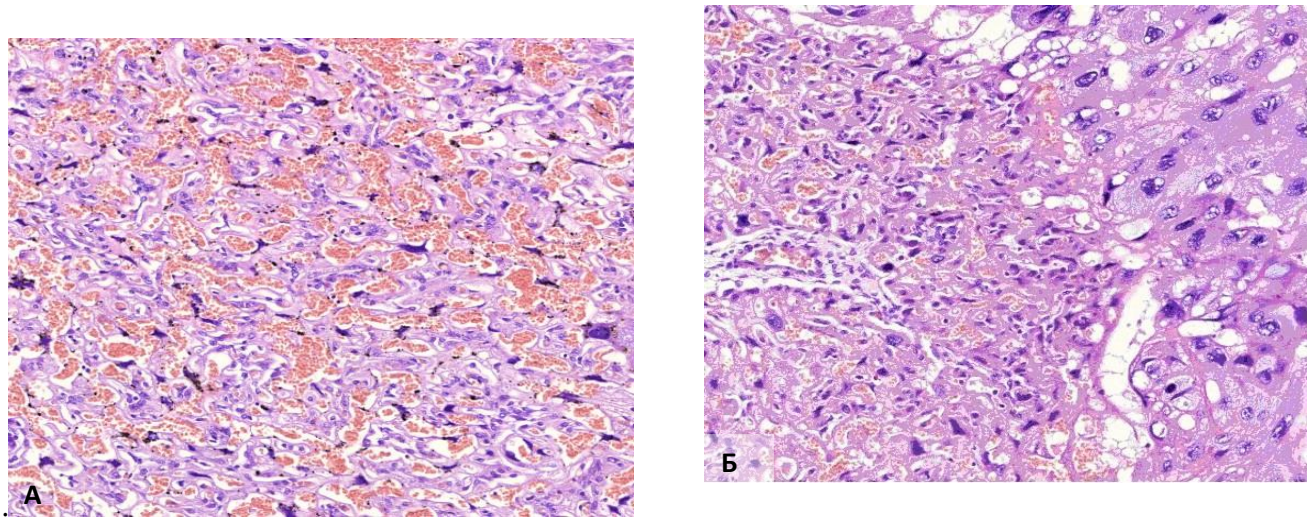


Figure 1.2. The structure of an intact placenta. spongy structure (villous) layer (a) and the border area between naps and a layer of giant trophoblast (b) of the placenta intact animals: uniform blood circulation capillaries of the villi and mezhkapillyarnyh spaces, relatively monomorphic structure trophoblast, the continuity of the transition from villous trophoblastic layer in the layer; Ochre. hematoxylin and eosin. Fig-. X 200

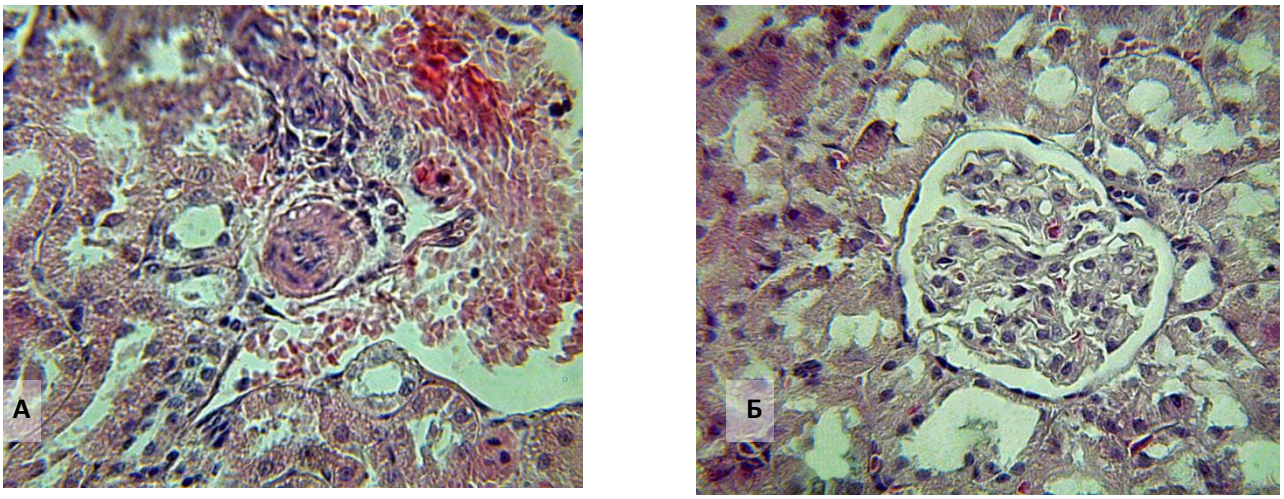


Figure 1.3. Kidney of pregnant rats with preeclampsia (pregnancy day 21). and – a spasm and hypertrophy of arterioles; b – a ball of anemia, capillary basement membrane thickened dramatically and have the form of wire loops (membranous glomerulopathy); Ochre. hematoxylin and eosin. Fig-. X 280

Morphology of the placenta at ADMA-like preeclampsia manifested as ischemia villi and intervilleznyh spaces and desquamation of the endothelium in the vessels of the placenta (Fig. 1.4). In some cases, this resulted in the emergence of necrosis of decidual cells of placenta tissue. On the outer surface of the placenta was observed massive deposits of fibrin.

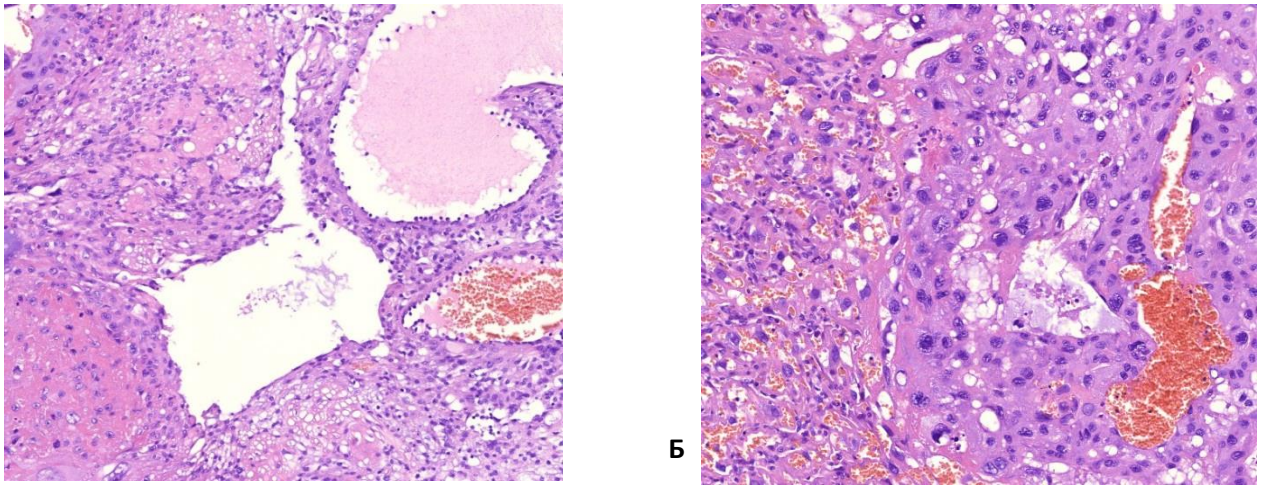


Figure 1.4. Pathological changes in the placenta in modeling ADMA-like preeclampsia. A – uneven blood filling spongy layer; B – vacuolar degeneration of giant trophoblast; foci of necrosis on the border of giant trophoblast and decidua tissue; degenerative changes, anemia decidual layer. Ochre. hematoxylin and eosin. Fig-. X 200.

Silver overlay clips above the aortic bifurcation and 2 ovarian artery led to a rise in blood pressure up to 155,4 ± 3,6 and 109,3 ± 5,7 mm Hg. Art. Factor of endothelial dysfunction increased to 2,02 ± 0,23 ($p < 0,05$). In addition, there was a decrease of microcirculation in the placenta of both uterine horns to 229,7 ± 9,9 446,3 ± 27,5 at ped in intact pregnant females. Simulation of blood flow reduction in both uterine horns resulted in a statistically significant as compared to intact pregnant animals increase in proteinuria to 1,34 ± 0,11 g / l ($p < 0,05$).

NO-producing endothelial function tests in animals with reduced blood flow in both uterine horns showed no statistically significant difference in the expression of eNOS and endothelial final concentration of NO metabolites in blood plasma compared with intact pregnant animals.

In the simulation, the reduced blood flow in both uterine horns in the placenta were observed pronounced changes in ischemic (Fig. 1.5).

Overlay clip on the artery supplying the right uterine horn only led to a decrease in microcirculation in the placenta right before the horn and 204,4 ± 14,3 to 309,0 ± 15,8 ped in the left horn of the uterus ($p < 0,05$). No statistically significant changes in blood pressure, CED, diuresis, proteinuria and NO – synthesizing endothelial function have been identified in these animals.

In the simulation, the reduced blood flow in the right horn in the right uterine horn placentas appeared ischemic changes and morphological picture is not fundamentally different from the placentas condition in a group of animals with the simulation of a reduced blood flow in both uterine horns

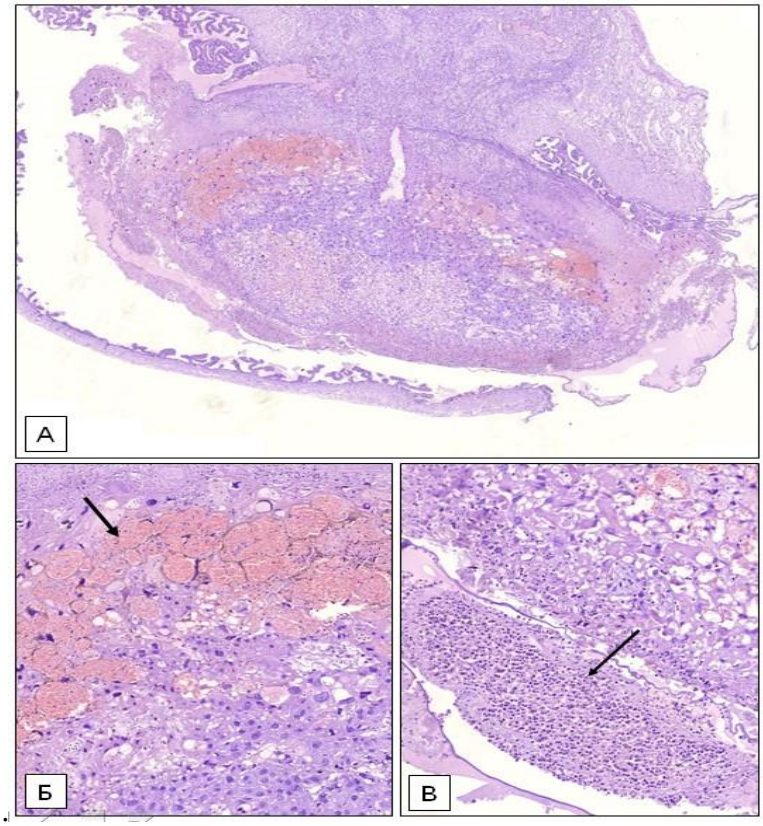


Figure 1.5. Pathological changes in placental ischemia. A – a general view with extensive necrosis and hemorrhage in the labyrinth zone (LZ), B – and hemorrhagic necrosis of the lake in the LZ, B – a major focus of purulent detsiduita, tissue separation at the boundary of the placenta and the uterine wall. H & E stain. Fig-. X50 (A), X200 (B, C)

Integral characteristics of scoring changes in morphological parameters for modeling ADMA-like preeclampsia and simulate the reduced blood flow to the uterus is presented in Table. 1.1.

Table 1.1

Values of morphological and functional parameters in pregnant rats at modeling ADMA-like preeclampsia and reduced blood flow in the uterus , (M ± m; n = 10)

Group / Index	Pregnant	Pregnant intact + L-NAME	Pregnant (reduced blood flow to the uterine horns 2)	Pregnant (reduced blood flow in the right uterine horn)
SBP mm Hg.	134,5±2,3^{y}	186,3±7*	155,4±3,6*	138,8±4,4^{y}
DBP, mm Hg.	92,0±2,1^{y}	145,0±5,0*	109,3±5,7*	89,8±5,8^{y}
CED, cond. u	1,10±0,11^{y}	3,12±0,17*	2,02±0,23*	1,29±0,08^{y}
microcirculation, per. u	446,3±27,5^{y}	218,3±13,7*	229,7±9,9*	204,4±14,3^{*R} 309,0±15,8^{*yL}
NOx, umol/dL	2,28±0,11^{y}	1,28±0,08*	2,12±0,11^{y}	2,15±0,09^{y}
eNOS, %	113,2±5,1^{y}	68,5±3,3*	104,5±6,6^{y}	105,4±3,2^{y}
Assessment of pathomo–rphological shanges, in points	0-1	5-6*	5-6*	5-6^{*P} 0-1^{L}
The liquid content in tissues gland %	49,89±0,82^{y}	58,09±1,73	52,86±2,66	48,78±2,00^{y}
Weight , g	1,73±0,06^{y}	1,52±0,06*	1,16±0,04*	1,13±0,06^{*R} 1,31±0,08^{*L}
The growth, mm	24,59±0,42^{y}	22,91±0,34*	20,10±0,27*	20,89±0,45^{*R} 22,90±0,45^{*L}
Height/weight mm/g	14,91±0,28^{y}	15,93±0,31*	18,25±0,55*	19,36±0,76^{*R} 18,63±0,68^{*L}
Postimplantation death,%	0	0	36,53±2,86*	34,16±3,37^{*R} 18,66±5,66^{*L}

Note: * – p <0.05 comparison with intact pregnant rats; y- at p <0.05 in comparison with pregnant rats with L-NAME, R- microcirculation in the right uterine horn, L – microcirculation in the left uterine horn.

Comparative assessment of the fruit revealed a decrease in fetal weight at all modeled pathological conditions as compared to the intact group of pregnant animals. In groups of animals with reduced uterine blood flow malnutrition fruit it was more pronounced compared to the group with ADMA – similar to preeclampsia. In addition, in animals with reduced uterine blood flow was observed postimplantation fetal death (Table 1.1.).

Thus, a comprehensive analysis of morphological and functional changes in the simulation of various pathologies of pregnant women leads to the conclusion that the ADMA-like model is closest to the second half of pregnancy gestosis. This is supported by: high blood pressure, disturbance of the relationship vasoconstrictor and vasodilating mechanisms, reduction of microcirculation in the placenta, increasing proteinuria, violation of NO-synthesizing endothelial function, a set of morphological changes in the kidney and placenta, that was the reason for using it as a base model.

2. RESEARCH EFFICIENCY OF PHARMACOLOGICAL AGENTS AFFECTING THE WAY L-ARGININE – NO – CGMP, THE CORRECTION OF ADMA-LIKE PREECLAMPSIA

Investigation of the effectiveness of pharmacological agents that affect the way L-arginine – NO – cGMP: tetrahydrobiopterin (BH4), a selective inhibitor of arginase 2 (ZB49-0010) and a PDE-5 inhibitor tadalafil in the correction of ADMA-like preeclampsia showed a statistically significant reduction in blood pressure under the influence BH4, tadalafil and drug included with standard therapy of preeclampsia methyldopa and improving endothelial dysfunction (CED) under the influence of all studied pharmacological agents affect the way L-arginine – NO – cGMP (Table 2.1.).

When microcirculation research in placenta were detected its improvement under the influence of tetrahydrobiopterin, a selective inhibitor of arginase 2 and tadalafil (Table 2.1.). In addition, under the influence of pharmacological agents that affect the way L-arginine – NO – cGMP methyldopa and a decrease in proteinuria (see Table 2.2.).

In the study of the effect of tetrahydrobiopterin, ZB49-0010 and tadalafil on the NO-synthesizing endothelial function in the correction of ADMA-like preeclampsia was found a statistically significant improvement ($p < 0,05$) under the influence of methyldopa, tetrahydrobiopterin and ZB49-0010 (Fig. 2.1). This applies to both end metabolites content NO, and the level of expression of eNOS.

Morphological and immunohistochemical study revealed kidney and placenta protective effect of the application of methyldopa and pharmacological agents that affect the metabolic pathway of L-arginine – NO – cGMP, which resulted in an integrated approach to the assessment of the group intact pregnant animals (Table 2.3.).

Table 2.1

Effect of pharmacological agents that affect the way L-arginine – of NO – cGMP in the blood pressure, CED and microcirculation in the placenta in the correction of ADMA-like preeclampsia in rats (M ± m; n = 10)

Group / Index	SBP, mmHg.	DBP, mmHg.	CED , cond. u	Microcirculation
intact	134,5±2,3^{y}	92,0±2,1^{y}	1,10±0,11^{y}	446,3±27,5^{y}
L-NAME	186,3±7*	145,0±5,0*	3,12±0,17*	218,3±13,7*
L-NAME + methyldopa (2 x 0,43) mg / kg / day	135,8±2,9^{y}	103,8±3,8^{y}	3,16±0,33*	248,4±10,7*
L-NAME + tetrahydro-biopterin (10 mg / kg)	157,4±7,9^{*y}	116,7±8,8^{*y}	1,73±0,24^{*y}	402,0±26,2^{y}
L-NAME + ZB49-0010 (5 mg / kg)	192,5±8,7*	150,2±6,7*	1,49±0,14^{y}	435,4±27,4^{y}
L-NAME + tadalafil (0.9 mg / kg)	149,7±2,2^{y}	97,6±3,2^{y}	1,85±0,08^{*y}	398,7±24,9^{y}

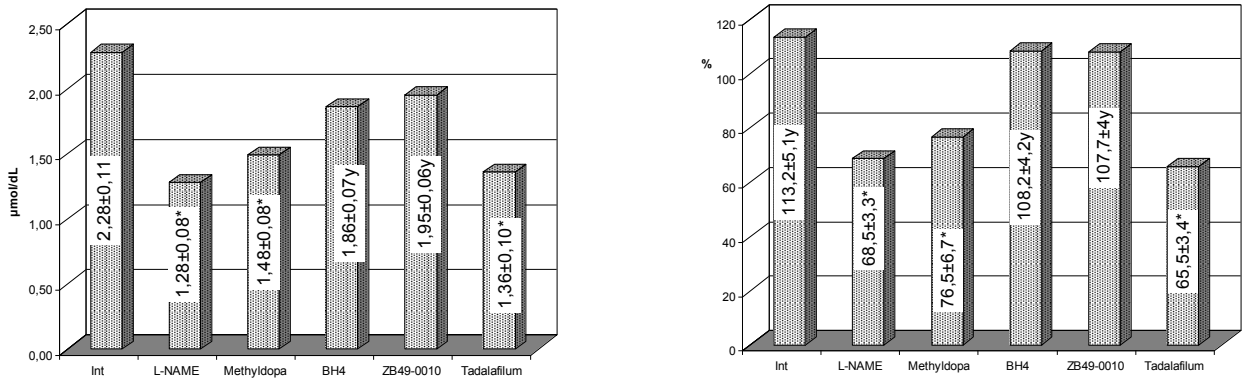
Note: * – $p < 0,05$ – compared to intact; y – $p < 0.05$ – in comparison with L-NAME.

Table 2.2

Effect of pharmacological agents that affect the way L-arginine – NO – cGMP values of parameters a 12-hour proteinuria and urine output correction ADMA-like preeclampsia (M ± m; n = 10)

Index \ Group	Number of 12-hour urine (mL)	Proteinuria (G / l)
intact pregnant	5,8±0,3	0,93±0,06^{y}
L-NAME	5,6±0,3	2,04±0,22*
L-NAME + methyldopa (2 x 0,43) mg / kg / day	5,4±0,3	1,45±0,14$^{* y}$
L-NAME + tetrahydrobiopterin (10 mg / kg)	5,6±0,3	1,16±0,09^{y}
L-NAME + ZB49-0010 (5 mg / kg)	5,6±0,3	1,19±0,10^{y}
L-NAME + tadalafil (0.9 mg / kg)	5,7±0,3	0,99±0,05^{y}

Note: * – p <0,05 – compared with intact pregnant animals; y – p <0.05 – compared with pregnant females receiving L-NAME.



Note: * – p <0.05 compared with intact pregnant rats; Y- p <0.05 compared with the group of pregnant animals treated with L-NAME.

Figure 2.1. Study of the influence on NOx concentration in plasma and the expression of eNOS pharmacological agents that affect the way L-arginine – of NO – cGMP in the correction of ADMA-like preeclampsia

Table 2.3

Effect of pharmacological agents that affect the way L-arginine – NO – cGMP to integral evaluation of the complex pathological changes in the kidney and placenta eNOS expression and the correction ADMA-like preeclampsia (n = 10)

A series of experiments	Comprehensive assessment in points	The expression of eNOS placenta, %
Intact pregnant females	0-1	5,4±0,21
L-NAME	5-6*	0,04±0,01*
L-NAME + methyldopa (2 x 0,43) mg / kg / day	3*	2,07±0,16*
L-NAME + tetrahydrobiopterin (10 mg / kg)	2-3*	4,01±0,26
L-NAME + ZB49-0010 (5 mg / kg)	2*	4,45±0,21
L-NAME + tadalafil (0.9 mg / kg)	3*	5,04±0,26

***Note.* *** – P <0,05 compared with intact pregnant females.

Microscopic study of the kidneys of animals treated, showed the same kind of picture that is sharply different from the control group of pregnant rats. Small artery kidney cortex with signs of mild hypertrophy of the muscular layer. The capillaries glomeruli congested, thickened basement membranes is not a symptom of "wire loops" were absent. Mesangial significant reaction was observed. Thus, the average number of cells per glomerulus in the group using Tadalafil was 39.80 ± 2.31 (40.70 ± 3.80 normally).

Morphology of the placenta at the L-NAME-induced preeclampsia, the treatment of methyldopa and pharmacological agents affect the metabolic pathway of L-arginine – NO – cGMP, I approached the group of intact animals. It noted monomorphic layer structure of the placenta without focal destructive changes, mild hyperemia spongy layer. On the outer surface of the placenta were observed traces of fibrin deposits.

Thus, the use of tetrahydrobiopterin, a selective inhibitor of arginase 2 and tadalafil has a marked protective effect on the model of ADMA-like preeclampsia, which was reflected in a statistically significant antihypertensive effect, reducing the rate of endothelial dysfunction (CED), the prevention of reduction of microcirculation in the placenta and in full prevention NOx reduction of nitrite ions and eNOS expression under the influence of tadalafil. At the same time, the results of the placenta urine studies showed a reduction in proteinuria. Morphological studies of kidney and placenta in these groups showed maximal approach to histology group intact pregnant animals.

The results of correction of morphological and functional disorders of the cardiovascular system in such of ADMA-eclampsia suggest a promising approach for the treatment and prevention of preeclampsia aimed at overcoming of ADMA-mediated inhibition of eNOS and correction caused by its pathophysiological changes.

3. RESEARCH EFFICIENCY SHORT EPISODE ISCHEMIA-REPERFUSION AND RECOMBINANT ERYTHROPOIETIN OF ADMA-IN LIKE PREECLAMPSIA AND ROLE AND INOS ATP-DEPENDENT K + CHANNELS IN THE REALIZATION OF THEIR POSITIVE EFFECT

Investigation of the effectiveness of short episodes of ischemia-reperfusion, and recombinant erythropoietin for the correction of morphological and functional disorders of ADMA-while similar preeclampsia

Playing a single ischemic episode, for 90 minutes before removing the samples did not lead to any significant change in blood pressure.

10x playback ischemic episode and recombinant erythropoietin administration resulted in a statistically significant reduction in blood pressure: Systolic to 141.6 ± 5.5 and 143.5 ± 4.0 mmHg, diastolic to 104.2 ± 5.7 and 98,1 ± 5,9 mm Hg (P <0.05), respectively (Table. 3.1).

Playing as a single ischemic episode in 90 minutes to remove the samples and 10 times an episode of ischemia-reperfusion 10 to 20 days of gestation, and the introduction of recombinant erythropoietin resulted in normalization of relations between vasodilating and vasoconstrictor responses in of ADMA-like preeclampsia, as evidenced by CED reduction to 1,52 ± 0,09, 1,56 ± 0,13 and 1,67 ± 0,15 mustache. u, respectively (Table. 3.1).

It is noteworthy that a single playback of ischemia-reperfusion episodes decreased CED on a background of L-NAME-induced pathology, without affecting blood pressure. This is indicative of the distinguishing features of the mechanisms of action of single and multiple play episodes of ischemia-reperfusion and their different effects on the process involved in the pathogenic elements of humoral and neurogenic contours regulation of the circulatory system.

Playing a single, 10-fold episode ischemia-reperfusion and administration of recombinant erythropoietin caused a significant improvement in microcirculation to 327,3 ± 17,2, 339,6 ± 20,4 and 296,6 ± 27,1 cond. u, respectively, which was significantly higher than that of pregnant females with preeclampsia (p <0,05).

Playing 10-multiple episodes of ischemia-reperfusion 10 to 20 hours and the introduction of a recombinant erythropoietin in pregnant animals with simulation of ADMA-like preeclampsia indicators normalized protein in the urine, the values of which do not go beyond the norm and amounted to 1,02 ± 0,04 and 1 01 ± 0,06 g / l, respectively, is statistically distinguishable (p <0.05) compared to untreated group of animals pregnant (Table 3.2.).

Effect of short episodes of ischemia-reperfusion, and recombinant erythropoietin for NO-producing endothelial function studied on the basis of data eNOS expression and increase NOx content of nitrite ions (Fig. 3.1). It is found that after a single playback of ischemia-reperfusion episodes no increase expression or eNOS, any level of nitrite ions in the blood plasma. Increased expression of eNOS parameters occurred under the influence of recombinant erythropoietin, and playing back 10-fold episode of ischemia-reperfusion. Increased content of final NO metabolites in blood plasma occurred only at 10-fold playback episodes of ischemia-reperfusion.

Table 3.1

Effect of short episodes of ischemia-reperfusion and recombinant erythropoietin on blood pressure, CED, and on placenta mikrocirculation (M ± m; n = 10)

Index \ Group	SBP, mmHg.	DBP, mmHg.	CED , cond. u	Microcirculation
intact	134,5±2,3^{y}	92,0±2,1^{y}	1,10±0,11^{y}	446,3±27,5^{y}
L-NAME	186,3±7*	145,0±5,0*	3,12±0,17*	218,3±13,7*
L-NAME + ischemia-reperfusion (1)	177,1±9,8*	124,9±8,4*	1,52±0,09^{y}	327,3±17,2^{y}
L-NAME + ischemia-reperfusion (10)	141,6±5,5^{y}	104,2±5,7^{y}	1,56±0,13^{y}	339,6±20,4^{y}
L-NAME + recombinant erythropoietin (50 U / kg)	143,5±4,0^{y}	98,1±5,9^{y}	1,67±0,15^{y}	296,6±27,1^{y}

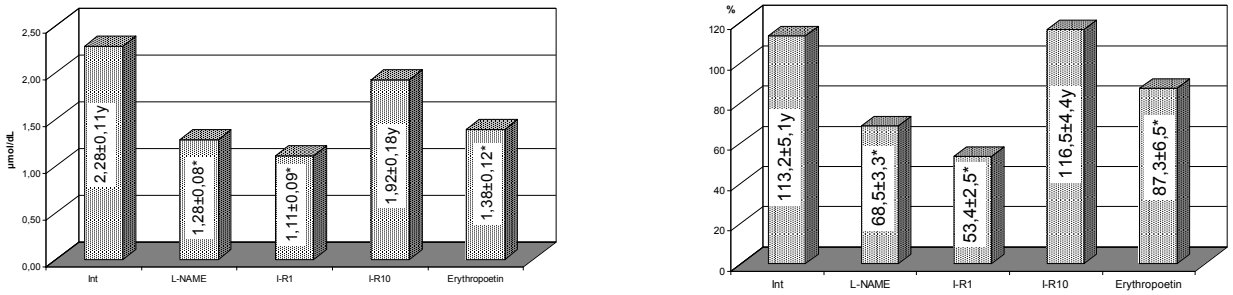
Note: SBP, DBP – systolic and diastolic blood pressure; CED – rate of endothelial dysfunction; microcirculation in the placenta; Ped – perfusion unit; * – P <0.05 – in comparison with a group of pregnant intact animals; y- P <0.05 – in comparison with a group of pregnant rats treated L-NAME..

Table 3.2

Effect of short episodes of ischemia-reperfusion and recombinant erythropoietin values of parameters a 12-hour proteinuria and diuresis in modeling ADMA-like preeclampsia (M ± m; n = 10)

Index \ Group	Number of 12-hour urine (mL)	Proteinuria (G / l)
Intact	5,8±0,3	0,93±0,06^{y}
L-NAME	5,6±0,3	2,04±0,22*
L-NAME + ischemia-reperfusion (10)	5,5±0,3	1,02±0,04^{y}
L-NAME + recombinant erythropoietin (50 U / kg)	5,3±0,2	1,01±0,06^{y}

Note: * – p <0,05 – compared with intact pregnant animals; y – p <0.05 – compared with pregnant females receiving L-NAME.



Note: * – p <0.05 comparison with intact pregnant rats;
Y- p <0.05 compared with the group of pregnant rats treated with L-NAME.

Figure 3.1. Effect of short episodes of ischemia-reperfusion, and recombinant erythropoietin for NOx concentration in the plasma and in eNOS expression in endothelial correction ADMA-like preeclampsia

Morphologic study of kidney and placenta also found a protective effect of 10-fold playback ischemia-reperfusion episodes and administration of recombinant erythropoietin. In animals with 10x playback of short episodes of ischemia-reperfusion picture dramatically different from the control group of pregnant rats. Small artery kidney cortex with mild signs of hypertrophy and spasm of the muscle layer. The capillaries glomeruli congested, thickened basement membranes is not a symptom of "wire loops" is missing. A notable reaction mesangium were observed: the average number of cells per glomerulus was 36.80 ± 3.16 (normally 40.70 ± 3.80).

Morphological examination of the placenta in the correction of L-NAME-induced preeclampsia 10x playback episode ischemia-reperfusion revealed the elimination of ischemic damage in the placental tissue (Fig. 3.3). There was a relatively even layer of spongy blood supply, no damage layer of giant trophoblast and decidua. On the outer surface of the placenta observed weak fibrin deposits.

Integral characteristics of scoring morphological parameters and results of immunohistochemical study are presented in Table. 3.3, which implies that a 10-fold playback brief episode of ischemia-reperfusion injury and administration of recombinant erythropoietin significantly reduced cumulative evaluation score and reduced in eNOS expression in placenta compared to ADMA-like preeclampsia.

Table 3.3

Effect of 10 min ischemia-reperfusion episodes of pathological changes of the complex and expression of eNOS in modeling ADMA-like preeclampsia (n = 10)

№ п/п	A series of experiments	Comprehensive assessment in points	The expression of eNOS placenta, %
1	Intact pregnant females	0-1	5,4±0,21
2	ADMA-like preeclampsia	5-6*	0,04±0,01*
3	L-NAME + ischemia-reperfusion (10)	2-3*	5,18+0,38
4	L-NAME + recombinant erythropoietin (50 U / kg)	3*	5,18+0,27

Note. * – P <0.05 compared to intact pregnant females.

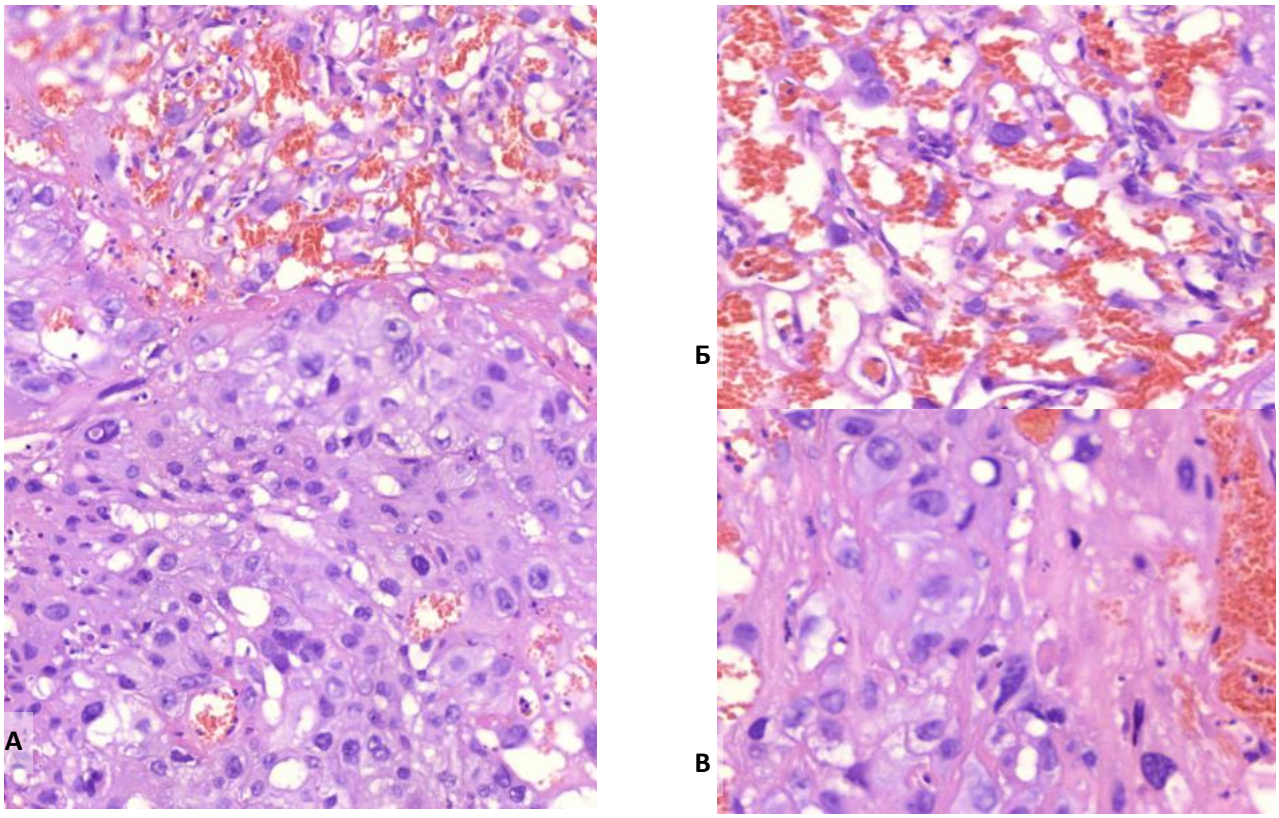


Figure 3.2. The placenta in animals with 10x playback of transient ischemic episodes on the background of ADMA-like preeclampsia. A – decidual layer: monomorphic structure, the absence of degenerative changes; B – even krovenapolnenie spongy layer; In – No degenerative changes trophoblast; Ochre. hematoxylin and eosin. Fig-. X 200

Study the role of iNOS and ATP-sensitive K^+ channels in the implementation of the positive effects of brief ischemia-reperfusion episodes in the correction of ADMA-like preeclampsia

Introduction of aminoguanidine, which is a selective inhibitor of iNOS, or glibenclamide, which is a blocker of ATP-sensitive K^+ channels, healthy pregnant animals did not result in a statistically significant change in blood pressure, CED, microcirculation in the placenta and proteinuria.

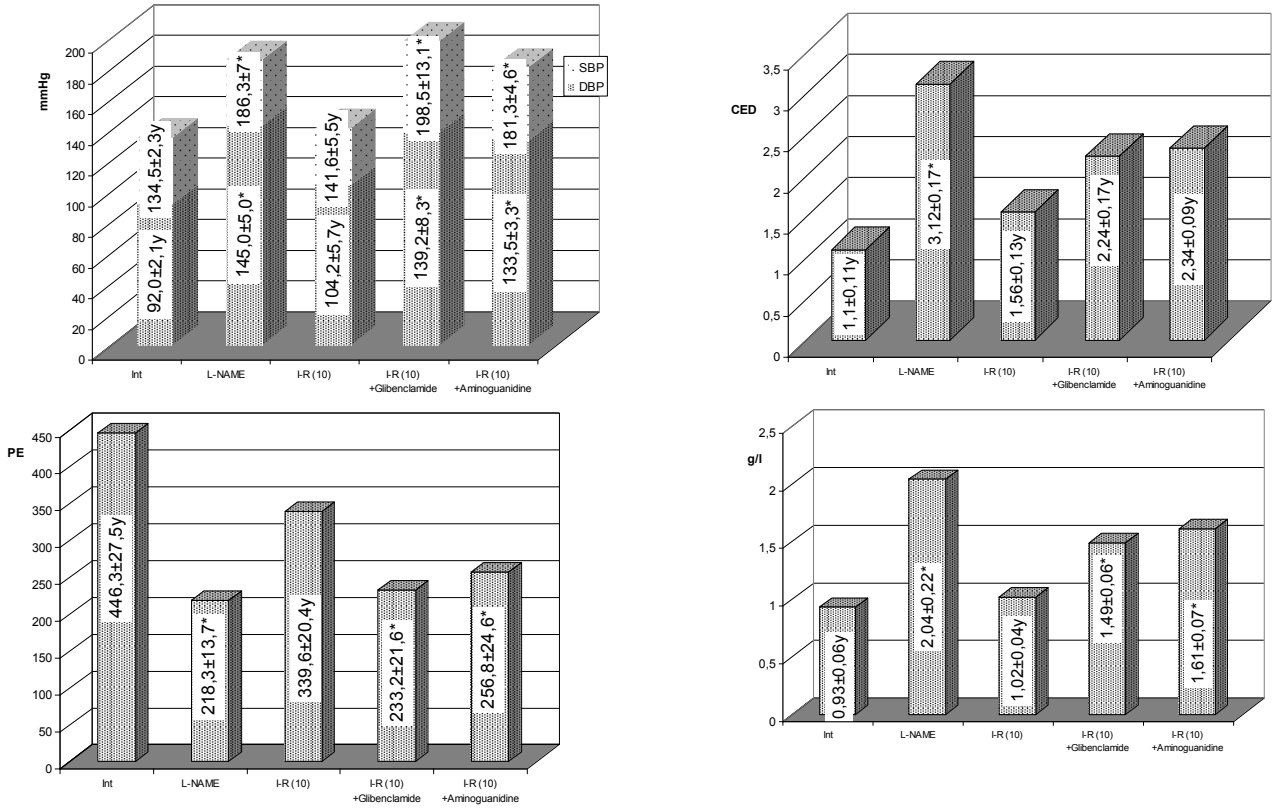
Introduction aminoguanidine or glibenclamide pregnant animals with experimental preeclampsia, the treatment of short episodes of ischemia-reperfusion injury, resulted in the total elimination of their antihypertensive effect, worsened circulation in the placenta (Fig. 3.3), and increased to the level of proteinuria in untreated animals. Only CED, despite a statistically significant increase in its remained below the group of untreated animals (p <0,05).

When administered aminoguanidine or glibenclamide pregnant animals fundamentally significant reduction of nitrite ions in the plasma and the activity of eNOS did not happen. When administered to animals with them of ADMA-like preeclampsia, the treatment of short episodes of ischemia-reperfusion, increased levels of nitrite ions in the blood plasma of the final metabolites of NO and eNOS expression under the influence of the latter did not occur (Fig. 3.4).

Noteworthy is that the negative effect of aminoguanidine and glibenclamide on functional indicators only appears when you attempt to correct

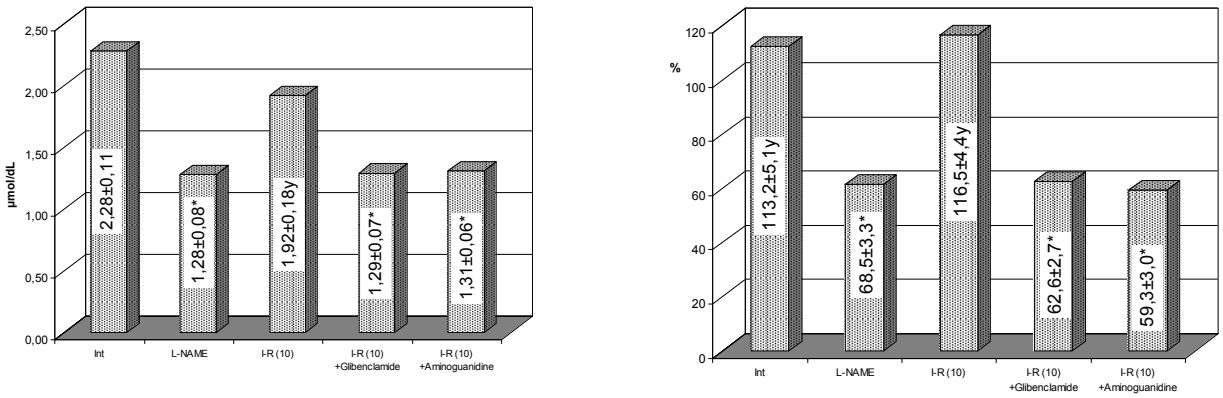
the modeled pathology, indicating the important role of iNOS and ATP-sensitive K^+ channels in the implementation endothelioprotektive effect of short episodes of ischemia-reperfusion.

Morphologic kidneys and placenta study animals administered aminoguanidine or glibenclamide revealed the absence of any effect on their microscopic picture in the bodies that matched the group intact pregnant animals (0-1 points).



Note: * – p <0.05 comparison with intact pregnant rats;
Y- p <0.05 compared with the group of animals treated with L-NAME.

Figure 3.3 Effect of aminoguanidine (300 mg / kg) and glibenclamide (50 mg / kg) on blood pressure, CED, micro-circulation in the placenta and proteinuria in the correction of ADMA-like preeclampsia short episodes of ischemia-reperfusion



Note: * – p <0.05 comparison with intact pregnant rats;
Y- p <0.05 compared with the group of animals treated with L-NAME.

Figure 3.4. Effect of aminoguanidine (300mg / kg) and glibenclamide (50 mg / kg) on the NOx concentration in plasma and the expression of eNOS in the correction ADMA-like preeclampsia short episodes of ischemia-reperfusion

When administered aminoguanidine or glibenclamide experimental animals with preeclampsia, the treatment of short episodes of ischemia-reperfusion, there was almost complete elimination of the positive impact of the latter in the placental tissue and kidney. The kidneys were observed spasm and hypertrophy of the muscle layer of small arteries. Flattened tubular epithelium, on significant areas of desquamated. A notable reactions were observed mesangium.

Morphological picture of the placenta villi and ischemia manifested intervilleznyh spaces and desquamation of the endothelium in the vessels of the placenta. There have been foci of necrosis of decidual cells in the placental tissue. On the outer surface of the placenta was observed massive deposits of fibrin.

Integral characteristics of scoring morphological parameters and results of immunohistochemical study are presented in Table. 3.4, from which it follows that a 10-fold reproduction of short episodes of ischemia-reperfusion on the background of aminoguanidine or glibenclamide positive effect on the morphological and functional disorders of ADMA-while such did not have preeclampsia.

Table 3.4

Effect of aminoguanidine and glibenklamina on complex pathological changes and the expression of eNOS in the correction of short episodes of ischemia-reperfusion of ADMA-like preeclampsia (n = 10)

№	A series of experiments	Comprehensive assessment in points	eNOS expression in the placenta,%
1	Intact pregnant females	0-1	5,4±0,21
2	ADMA-like preeclampsia	5-6*	0,04±0,01*
3	L-NAME + ischemia-reperfusion (10)	2-3*	5,18±0,38
4	L-NAME + Ischemia-reperfusion injury (10) + aminoguanidine (300mg / kg)	5-6*	0,03±0,01*
5	L-NAME + Ischemia-reperfusion injury (10) + Glibenclamide (50 mg / kg)	5-6*	0,04±0,01*

Note. * – P <0.05 compared to intact pregnant females.

Thus, a single and 10x playback of short ischemic episodes and the introduction of recombinant erythropoietin has shown marked endotelioprotektivnoe effect on the model of ADMA-like preeclampsia, which was reflected in the strengthening of endothelium-dependent relaxation of blood vessels and reducing the rate of endothelial dysfunction CED to the level of intact animals, as well as in reducing violations microcirculation in the placenta. In addition, a 10-fold reproduction of short ischemic episodes and the introduction of recombinant erythropoietin resulted in a decrease in blood pressure, normalization of proteinuria, restore function NO-producing system. Morphological study revealed the prevention of destructive phenomena ischemic, caused by the introduction of L-NAME when playing 10-fold ischemic episodes and the introduction of recombinant erythropoietin. However, complete normalization of morphological picture in the kidney and placenta did not come.

It should be noted that the effects of a single playback and 10x playback ischemic episodes NO-synthesizing function multidirectional. This fact shows the different mechanisms for the implementation of their action endotelioprotektivnogo.

In the presented experiments showed that the administration of aminoguanidine, an inhibitor of iNOS, or glibenclamide, which is a blocker of ATP-sensitive K + channels, the experimental animals of ADMA-like preeclampsia, treatment playback ischemia-reperfusion episodes, almost completely eliminates the positive effect of the latter. This is manifested in the removal of gipotezivnogo effect, reducing the severity of a positive effect on endothelial function, reduction of microcirculation in the placental tissue and indicators of endothelial NO-producing function to the level of untreated animals, as well as in the restoration of destructive damage to the placental tissue and ischemic kidneys. This demonstrates the important role of iNOS and ATP-sensitive K + channels in the implementation of the protective effects of ischemia-reperfusion episodes in the correction of ADMA-like preeclampsia in the experiment.

4. STUDY OF EFFICIENCY ERYTHROMYCIN AND AZITHROMYCIN IN ADMA-LIKE PREECLAMPSIA

Introduction of azithromycin (30 mg / kg) resulted in a statistically significant reduction in both systolic and diastolic blood pressure in animals with experimental ADMA-like preeclampsia (Fig. 4.1). Introduction erythromycin (30 mg / kg) did not have any significant effect on these parameters. Both drugs approximately equally CED significantly reduced to 1.88 ± 0.16 and 1.82 ± 0.09, respectively (p <0.05), whereas in the group of untreated pregnant rats given

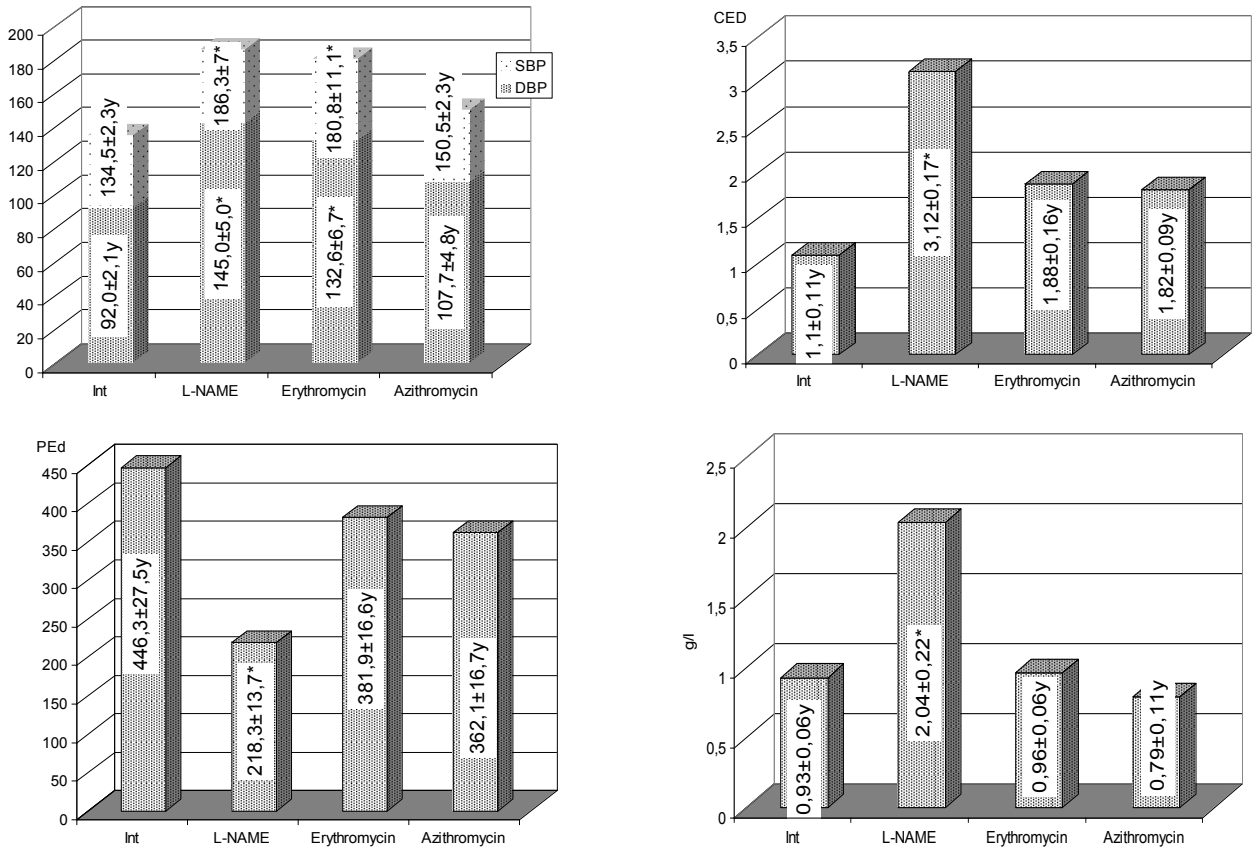
L-NAME, it was 3,12 ± 0,17. However, the target level of reduction of CED is not reached.

Course administration of erythromycin and azithromycin caused a significant improvement in microcirculation to 381,9 ± 16,6 and 362,1 ± 16,7 perfusion units, respectively, which was significantly higher than that of pregnant females with preeclampsia (p <0,05), and statistically It did not differ from the values of the microcirculation in intact pregnant rats. In addition, there was a decrease in urine protein to the level of intact animals (Fig. 4.1).

Introduction of erythromycin and azithromycin caused improvement in NO-synthesizing endothelial function, which resulted in an increase in the level of nitrite ions in the plasma by the action of erythromycin and statistically significant increase in eNOS expression under the influence of both drugs (Fig. 4.2).

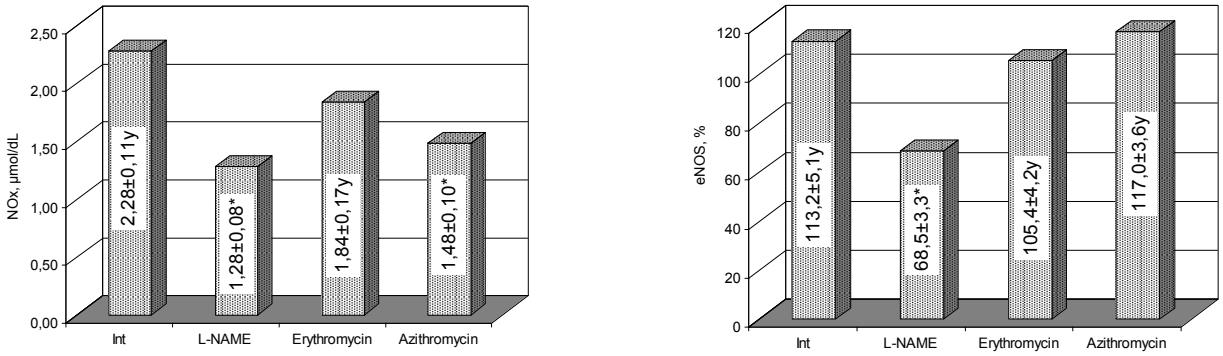
A morphological study of the kidney and the placenta also found a protective effect of erythromycin and azithromycin. The renal vessels in animals treated with L-NAME during treatment with erythromycin and azithromycin, the pattern was different from the control group of pregnant rats. The most pronounced beneficial effects of the therapy was observed in the group of animals with the use of azithromycin. Small artery kidney cortex with moderate signs of hypertrophy of the muscular layer. The capillaries glomeruli congested, slightly thickened basement membranes, symptom "wire loops" is missing. A notable reaction mesangium were observed: the average number of cells per glomerulus was 39.40 ± 2.74 and 39.30 ± 2.55 respectively (normal 40.70 ± 3.80).

Morphological examination of the placenta in the correction of erythromycin and azithromycin L-NAME-induced preeclampsia showed the same type of histology in both groups, but more positive effects were found in a group of animals receiving azithromycin. Placenta without pathological changes, naps and intervilleznye congested space. There are signs of ischemia of the functional nature of the placenta. On the outer surface of the placenta fibrin deposits were observed.



Notes: * – p <0.05 comparison with intact pregnant rats;
Y- p <0.05 compared with the group of pregnant animals treated with L-NAME.

Figure 4.1. Effect of erythromycin (30 mg / kg) and azithromycin (30 mg / kg) on blood pressure values, CED, micro-circulation in the placenta and proteinuria in the correction of ADMA-like preeclampsia



Note: * – p <0.05 compared to intact rat; Y- p <0.05 compared with the group of animals treated with L-NAME

Figure 4.2. Effect of erythromycin (30 mg / kg) and azithromycin (30 mg / kg) on the NOx concentration in the plasma and in eNOS expression in endothelial correction ADMA-like preeclampsia.

Integral characteristics of scoring morphological parameters and results of immunohistochemical study are presented in Table. 4.1, which implies that erythromycin and azithromycin significantly reduced scores in the evaluation of the integral (3.4) compared to ADMA-like preeclampsia and increased eNOS expression in placental vascular endothelium.

Thus, erythromycin and azithromycin, 30 mg / kg showed a clear endothelioprotektive action model L-NAME-induced preeclampsia, as manifested in the reduction rate of endothelial dysfunction up to the level of intact animals, improving microcirculation in the placenta, and to reduce the decrease eNOS expression on background complete prevention of proteinuria. Additionally, azithromycin showed moderate hypotensive effect, erythromycin reduced drop stable metabolites in plasma NOx. Morphological studies have found a decrease under the influence of erythromycin and azithromycin in the indicated doses of disorders of kidney and ischemic changes in the placenta, not reaching the damage to structures.

5. STUDY THE EFFECTIVENESS OF VITAMIN B9, COMBINING THE USE OF VITAMIN B9 AND B6, VITAMIN-MINERAL COMPLEX "COMPLIVIT® TRIMESTRUM TRIMESTER 2" AND "COMPLIVIT® TRIMESTRUM TRIMESTER 3" WITH ADMA-LIKE PREECLAMPSIA

Introduction to the study drugs: folic acid, a combination of folic acid and vitamin B6, multivitamin-mineral complexes "Complivit® trimestrum Trimester 2" and " Complivit® trimestrum Trimester 3" did not have any significant effect on blood pressure in the background of the simulated pilot of ADMA-like preeclampsia (Fig. 5.1).

Introduction of folic acid alone does not result in correction of CED. The introduction of folic acid in combination with vitamin B6, as well as both multivitamin-mineral complexes led to an improvement in endothelial function, which resulted in a statistically significant reduction in CED. Improvement of microcirculation in the placenta occurred in all treatment groups (Fig. 5.1).

The use of vitamin and mineral complexes "Complivit® trimestrum Trimester 2" and "Complivit® trimestrum trimester 3" in pregnant animals with simulation L-NAME-induced preeclampsia statistically significantly reduced the amount of protein in the urine, bringing its value in the last group of animals to the value of intact animals . Using a combination of folic acid and folic acid and vitamin B6 are not resulted in a statistically significant reduction in proteinuria pathology in this model (Fig. 5.1).

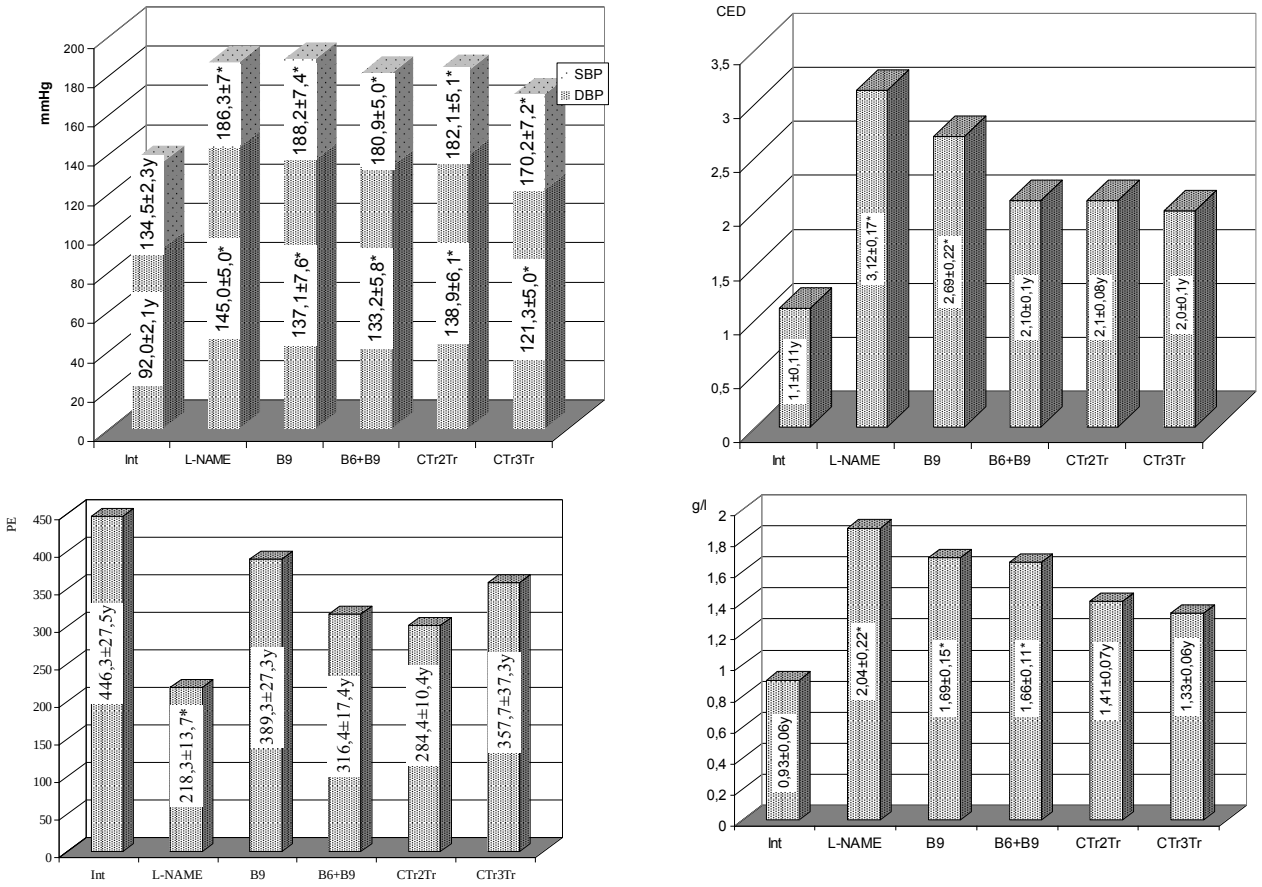
Investigation of the effect of vitamin B9, combined use of vitamins B6 and B9, drugs "Complivit® trimestrum Trimester 2" and "Complivit® trimestrum trimester 3" to the NO-synthesizing endothelial function in the correction of ADMA-like preeclampsia showed that the use of multivitamin-mineral preparations "Complivit® trimestrum trimester 2" and "Complivit® trimestrum trimexter 3" significantly increased the concentration of nitrite ions (Fig. 5.2). Introduction of folic acid and its combination with Vitamin B6 not result in a statistically significant increase in the concentration of nitrite ions in the blood plasma of animals with ADMA-like preeclampsia.

Table 4.1

Effect of erythromycin and azithromycin in the complex pathological changes and the expression of eNOS in placenta in modeling ADMA-like preeclampsia (n = 10)

№	A series of experiments	Comprehensive assessment in points	eNOS expression in the placenta,%
1	Intact pregnant females	0-1	5,4±0,21
2	L-NAME-induced preeclampsia	5-6*	0,04±0,01*
3	L-NAME + erythromycin (30 mg / kg)	3-4*	3,77±0,12*
4	L-NAME + azithromycin (30 mg / kg)	3*	4,28±0,21*

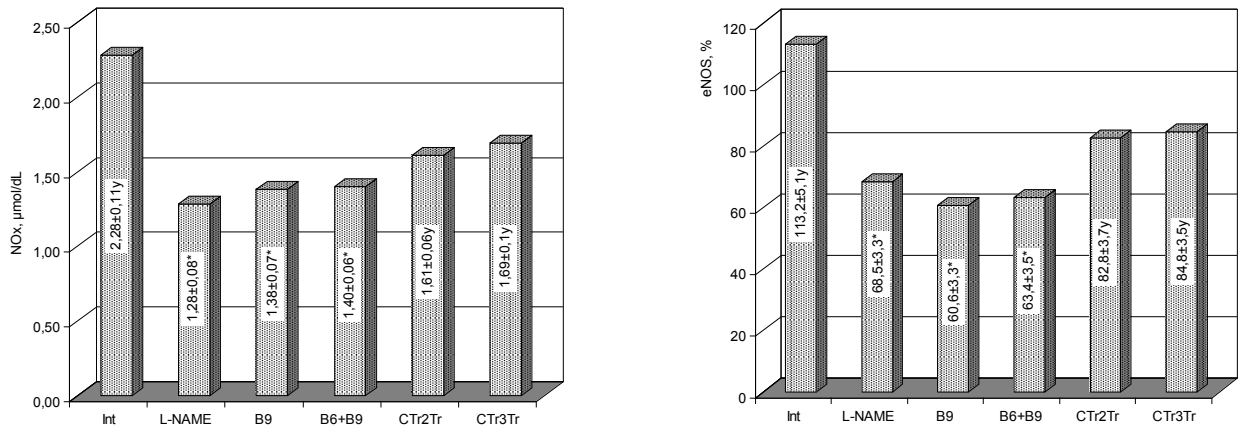
Note. * – P <0.05 as compared with intact pregnant females.



Note: Hereafter – CTr2Tr – «Complivit® trimestrum Trimester 2"; CTr3Tr – «Complivit® trimestrum trimester 3"; * – P <0.05 comparison with intact pregnant rats; Y- p <0.05 compared with the group of pregnant animals treated with L-NAME.

Figure 5.1. Impact of the B9 vitamin (0,2mg / kg) combined use of vitamins B9 (0,2 mg / kg) and B6 (2 mg / kg) of drugs "Complivit® trimestrum 2 Trimester" (0.084 tab/kg) "Complivit® trimestrum 3 trimester" (Table 0.084 tab/kg) on blood pressure, CED, micro-circulation in the placenta and proteinuria in the correction of ADMA-like preeclampsia

Dynamic expression of eNOS in the application of indicators described in this chapter preparations wore the same character as the concentration of stable NO metabolites (Fig. 5.2). There was a statistically significant increase in eNOS expression indices only in groups of animals treated with multivitamin-mineral preparations "Complivit® trimestrum Trimester 2" and "Complivit® trimestrum trimester 3"



Note: Hereafter – CTr2Tr – «Complivit® trimestrum Trimester 2"; CTr3Tr – «Complivit® trimestrum trimester 3"; * – P <0.05 comparison with intact pregnant rats; Y- p <0.05 compared with the group of pregnant animals treated with L-NAME.

Figure 5.2. Effect of vitamin B9 (0,2 mg / kg), the combined use of vitamins B9 (0,2 mg / kg) and B6 (2 mg / kg) of drugs "Complivit® trimestrum Trimester 2" (0.084 tab/kg) and "Complivit® trimestrum 3 trimester" (0.084 tab/kg) in the NOx concentration in plasma and the expression of eNOS in the correction of ADMA-like preeclampsia

A morphological study of the kidney and the placenta was found that administration of folic acid and its combination with vitamin B6 did not result in improvement of histological kidney and placenta. With the introduction of vitamin and mineral complexes "Complivit® trimestrum Trimester 2" and "Complivit® trimestrum Trimester 3" observed moderately positive dynamics in the kidney and in placenta (Table. 5.1). In addition, there was increase in eNOS expression in placental vascular endothelium.

Thus, the data presented studies indicate that the minimum protective properties have been identified by using folic acid as monotherapy and when combined with its use of vitamin B6. Intensity of positive effects from the use of multivitamin-mineral preparations "Complivit® trimestrum Trimester 2" and "Complivit® trimestrum trimester 3" bore significant character and manifested in the improvement of endothelial function, increase microcirculation indices in the placenta and increase of NO-synthesizing endothelial function, reduction of proteinuria and positive dynamics of the morphological picture in the kidney and placenta.

Table 5.1

Effect of vitamin B9, combined use of vitamins B6 and B9, drugs "Complivit® trimestrum Trimester 2" and "Complivit® trimestrum trimester 3" in the complex pathological changes and the expression of eNOS in the correction of ADMA-like preeclampsia (n = 10)

№	A series of experiments	Comprehensive assessment in points	eNOS expression in the placenta,%
1	Intact pregnant females	0-1	5,4±0,21
2	L-NAME	5-6*	0,04±0,01*
3	L-NAME + B_9 (0,2 mg/kg)	5-6*	0,04±0,01*
4	L-NAME + B_9 (0,2 mg/kg)+ B_6 (2 mg/kg)	5-6*	0,03±0,01*
5	L-NAME + CTr2Tr (0.084 tab/kg)	4*	2,02±0,12*
6	L-NAME + CTr3Tr (0.084 tab/kg)	4*	1,83,±0,21*

Note. * – P <0.05 compared to intact pregnant females.

CONCLUSION.

Comparative aspect analyzing the obtained data using an integrated indicator criterion morphological changes, it should be noted that the use of pharmacological agents that affect the path of L-arginine was the most effective – NO – cGMP (Table 6.1.). This can be explained by a direct effect on the main pathogenesis. In addition, special attention should be data obtained by correcting the experimental preeclampsia short episodes of ischemia-reperfusion and recombinant erythropoietin. In clinical situations where significantly increases the role of ischemic events in the placenta of their use will be increasingly important.

Less pronounced therapeutic effect of macrolides and multivitamin-mineral complexes due to their influence on the part of the mediated pathogenesis. However, this is not how does not reduce the significance of the study. Both of these groups of drugs can not be regarded as a drug of choice for targeted treatment of preeclampsia, but that they have of the pleiotropic effects of vitamin increases the relevance of a balanced diet during pregnancy, and in case of need for antibiotic macrolides become an important property for the benefit of their choice.

Table 6.1

Integrated assessment of complex pathological changes in the kidney and placenta in the correction of ADMA-like preeclampsia (n = 10)

A series of experiments	Comprehensive assessment in points
Intact pregnant females	0-1
L-NAME	5-6*
L-NAME + tetrahydrobiopterin (10 mg / kg)	2-3*
L-NAME + ZB49-0010 (5 mg / kg)	2*
L-NAME + tadalafil (0.9 mg / kg)	3*
L-NAME + ischemia-reperfusion (10)	2-3*
L-NAME + recombinant erythropoietin (50 U / kg)	3*
L-NAME + erythromycin (30 mg / kg)	3-4*
L-NAME + azithromycin (30 mg / kg)	3*
L-NAME + CTr2Tr (0.084 tab/kg)	4*
L-NAME + CTr3Tr (0.084 tab/kg)	4*

Note: * – p <0,05 compared with intact pregnant females;

In conclusion, it should be noted that the results of this work provide experimental basis for new approaches of prevention and treatment of preeclampsia pharmacological agents designed to overcome or compensate ADMA-mediated inhibition of NO-synthesizing endothelial function, as well as the activation of biological processes underlying mechanism of the positive effects of ischemic preconditioning.

BIBLIOGRAPHY

1. Bell M. J. A historical overview of preeclampsia-eclampsia. *J. Obstet. Gynecol. Neonatal. Nurs.* 39, №5 (2010): 510-518. [Free PMC]

2. De Falco S. The discovery of placenta growth factor and its biological activity. *Exp. Mol. Med.* 44, № 1 (2012): 1-9. [Free PMC]

3. Karthik P. Pathogenesis late gestosis pregnant. *International Journal of Medicine.* № 1 (2010): 62-66. (In Russian) [Full text]

4. Khetsuriani T. The role of oxygenic stress and σ1-receptors in the development of pre-eclampsia and its pathogenetic treatment: Abstract. Dis. ... Dr. med. Sciences: 14.00.16. – Tbilisi, 2006. – 38 p. (In Russian) [Full text]

5. Ivanova L.B., Karamysheva V.I. Effect of GABA derivatives on endothelial function in rats with experimental preeclampsia. *Problems of reproduction.* № 1 (2012): 28-30. (In Russian) [Abstract]

6. Cohen M., Bischof P. Factors regulating trophoblast invasion. Gynecol. Obstet. Invest. Vol. 64, № 3. (2007): 126-130. [PubMed]

7. Adu-Bonsaffoh K., Antwi D.A. Nitric oxide dysregulation in the pathogenesis of preeclampsia among Ghanaian women. *Integr. Blood Press. Control.* № 8 (2015): 1-6. [Free PMC]

8. Aykas F., Solak Y. Persistence of cardiovascular risk factors in women with previous preeclampsia: a long-term follow-up study. *J. Investig. Med.* 63, № 4 (2015): 641-645. [PubMed]

9. Gureev V.V. Endothelial dysfunction – the central link in the pathogenesis of preeclampsia. *Scientific statements Belgorod State University. Ser. Medicine. Pharmacy.* 17, № 4-1 (123) (2012): 5-12. (In Russian) [eLIBRARY] [Full text]

10. Gureev V.V., Pokrovsky M.V. ADMA – eNOS – deterministic ways of pharmacological correction of preeclampsia. 2014: Belgorod: Publishing house BSU.- 265 p. (In Russian) [Full text]

11. Ducray J.F., Naicker T. Pilot study of comparative placental morphometry in pre-eclamptic and normotensive pregnancies suggests possible maladaptations of the fetal component of the placenta. *European Journal of Obstetrics Gynecology and Reproductive Biology.* 156, №1 (2011): 29-34. [PubMed]

12. Purkerson M.L., Vekerdy L. A history of eclampsia, toxemia and kidney in pregnancy. Am. J. Nephrol. 19, № 2 (1999): 313-319. [PubMed] [Full text]

13. van Oppenraaij R.H., Bergen N.E. Placental vascularization in early onset small for gestational age and preeclampsia. *Reproductive Science.* 18, № 6 (2011): 586-93. [PubMed] [Full text]

14. Wang Q.J., Song B.F. Expression of RGC32 in human normal and preeclamptic placentas and its role in trophoblast cell invasion and migration. *Placenta.* 36, № 4 (2015): 350-356. [PubMed]

15. Vaisbuch E., Whitty J.E. Circulating angiogenic and antiangiogenic factors in women with eclampsia. *Am. J. Obstet. Gynecol.* 204, № 2 (2011): 152.e1-152.e9. [PubMed]

16. Sánchez-Aranguren L.C., C. E. Prada. Endothelial dysfunction and preeclampsia: role of oxidative stress. *Front. Physiol.* № 5 (2014): 372. [Full text]

17. Denisyuk T.A., Lazareva G.A. Endothelium and cardioprotective effects of HMG-Co-A-reductase in combination with L-arginine in endothelial dysfunction modeling. *Research result: pharmacology and clinical pharmacology.* 2, №1 (2) (2016): 4-8. [Full text]

18. Provotorov V.Y., Korokin M.V. Endothelio- and cardioprotective effects of vitamin B6 and folic acid in modelling methionine-induced hyperhomocysteinemia. *Research result: pharmacology and clinical pharmacology.* 2, №1 (2) (2016): 16-19. [Full text]

19. Yakushev V.I., Filippenko N.G. Studying dose-dependent endothelio- and cardioprotective activity of selective arginase II inhibitor in hyperhomocysteine-induced endothelial dysfunction. *Research result: pharmacology and clinical pharmacology.* 2, №1 (2) (2016): 42-45. [Full text]

20. Gureev V.V. Investigation of the role of ischemic preconditioning in the distant correction of morphological and functional disorders with short episodes of ischemia-reperfusion in the condition of ADMA-like preeclampsia. *Kursk scientific-practical herald "Man and his health.* № 3 (2012): 5-9. (In Russian) [eLIBRARY] [Full text]

21. Gureev V.V., Zhilinkova L.A., Stupakova E.G. Correction of endothelial dysfunction by nicorandil, tetrahydrobiopterin and resveratroly for modeling experimental preeclampsia. *Basic research.* № 1-1 (2015): 58-62. (In Russian) [eLIBRARY] [Full text]

22. Tsukimori K., Komatsu H. Inhibition of nitric oxide synthetase at mid-gestation in rats is associated with increases in arterial pressure, serum tumor necrosis factor-alpha, and placental apoptosis. *Am. J. Hypertens.* 21, № 4 (2008): 477-481. [PubMed]

23. Gilbert J.S., Bauer A.J. Circulating and utero-placental adaptations to chronic placental ischemia in the rat. *Placenta.* 33, № 2 (2012): 100-105. [PubMed]

24. Tam K.B., George E. Endothelin type A receptor antagonist attenuates placental ischemia-induced hypertension and uterine vascular resistance. Am. J. Obstet. *Gynecol.* 204, № 4 (2011): 330.e1-330.e4. [Free PMC]

25. Qadri F., Arens T. Angiotensin-converting enzyme inhibitors and AT1-receptor antagonist restore nitric oxide synthase (NOS) activity and neuronal NOS expression in the adrenal glands of spontaneously hypertensive rats. *Jpn. J. Pharmacol.* 85, № 4 (2001): 365-369. [PubMed]

26. Reznikova L.B. Endotelioprotektornaya activity of GABA derivatives in experimental preeclampsia: dis. ... Cand. honey. Sciences: 14.03.06. – Volgograd, 2013. – 165 p. (In Russian) [Full text]

27. Blaschke T., Serdyuk O. The reproductive function: NO role in ovulation and implantation. *Problems of reproduction.* 18, № 3 (2012): 11-16. (In Russian) [Abstract]