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Research Article Rus

Correction of morphofunctional disturbances arising when modelling Preeclampsia with resveratrol and nicorandil

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Abstract

Introduction: Preeclampsia is one of the most serious diseases of the second half of pregnancy and is surely amongst the top three causes of maternal mortality. Therefore, the creation of new drugs for preventing and correcting preeclampsia is an urgent task.

Methods: In the experiment, an ADMA-like L-NAME-induced model of preeclampsia was reproduced. To assess the emerging morphofunctional disorders, the following parameters were used: blood pressure, endothelial dysfunction coefficient, microcirculation in the placenta, proteinuria, fluid content in the large omentum, concentration of terminal metabolites in the blood plasma, morphological state of the placenta and kidneys and morphometric parameters of the foetus.

Results and Discussion: Injection of L-NAME into the animals from the 14th to the 20th day of pregnancy causes disorders: an increase in systolic and diastolic blood pressure by 1.4 and 1.5 times, an increase in proteinuria by 3.3 times and an increase in the fluid content in a large omentum from 45.82 ± 1.82% to 54.73 ± 1.96%, which correspond to disorders due to preeclampsia in pregnant women. There was also a disturbance of endothelial function, as evidenced by an increase in the coefficient of endothelial dysfunction (CED) by 2.9 times. The use of resveratrol leads to a pronounced correction in the changes that occur: a decrease in systolic and diastolic arterial pressure by 1.2 and 1.3 times, a decrease in proteinuria by a factor of 1.9 and a decrease in the fluid content in the large omentum to 50.00 ± 1.25%. The use of nicorandil leads to a pronounced correction in the resulting changes: a decrease in the diastolic blood pressure by 1.14 times, a decrease in proteinuria by a factor of 1.7 and a decrease in the fluid content in the large omentum to 50.57 ± 2.08%. CED decreased 1.7 times. When combining their use with amlodipine, the positive effects increased: systolic and diastolic blood pressure decreased 1.13 and 1.24 times and 1.14 and 1.23 times, respectively, proteinuria decreased 2.7 and 2.3 times, the fluid content in the large omentum was reduced to 44.54 ± 1.80% and 46.73 ± 1.30%. CED decreased 1.7 and 2.3 times. The administration of glibenclamide together with resveratrol and nicorandil removes a significant part of their positive effects.

Conclusion: Resveratrol and nicorandil have a significant positive effect in the correction of morphofunctional disorders in animals with ADMA-like preeclampsia. Activation of K^{+}_{ATP} channels plays a significant role in the realisation of their positive effects.

Keywords

resveratrol, nicorandil, preeclampsia, pharmacological preconditioning, K+ ATP channels, rats, ADMA

Introduction

Preeclampsia is a multisystem, highly variable and pregnancy-specific condition that occurs after the 20th week of gestation and is determined by the presence of arterial hypertension and proteinuria. For many years, preeclampsia has been one of the frequent and threatening complications of pregnancy and childbirth, leading to disability of mothers and their children (Gureev et al. 2014, Kolgushkina 2000, Reznikova 2013, Shuvalov et al. 2014, Savelyeva et al. 2011, Federal State Statistics Service).

According to the Ministry of Healthcare of the Russian Federation, over the past decade, hypertensive complications in pregnancy have been ranked third in the list of causes of maternal mortality and, in 2014, accounted for 15.7% of the structure of maternal losses, in 2015 – 10.2% and by the end of 2016, this figure was 11.7% (Shuvalov et al. 2014, Methodical letter 2016).

According to the statistics, the frequency of preeclampsia in pregnant women has increased in recent years and ranges from 7% to 20% (Kolgushkina 2000, Reznikova 2013, Shuvalov et al. 2014, Federal State Statistics Service, Methodical letter 2016).

Perinatal morbidity is 56% and perinatal mortality is 3-4 times higher than that of the population, reaching 12% (Savelyeva et al., 2011).

The etiological aspects of preeclampsia development still provoke heated discussions in the scientific community. There are several theories of preeclampsia development: the immunity theory, the theory of disadaptation, the theory of placental ischemia, the hormonal theory, the theory of toxic effects, the hereditary theory, etc. (Shuvalov et al. 2014, Savelyeva et al. 2011, Sidorova 2003).

With regard to the pathogenesis of preeclampsia, although a large number of uncertainties remain, its main conditions are determined and all the newly discovered information complements pathological events. It is generally accepted that the obligatory component of the pathogenesis of preeclampsia is endothelial dysfunction (Bloshhinskaja 2003, Gureev 2012, Scioscia et al. 2015, Sánchez-Aranguren et al. 2014). Endothelium, or internal cellular lining of vessels, provides selective permeability between intravascular and interstitial space, which is ensured by its specific structure.

A histological study of the placenta in females with preeclampsia revealed incomplete invasion of the cytotrophoblast into the spiral arteries of the mother. The level of maturity of the spiral arteries themselves in cases of preeclampsia does not reach the level of normal pregnancy (Pereira et al. 2015, Verlohren et al. 2010, Ducray et al. 2011). This leads to ischemia of the trophoblast and an increase in the permeability of the foetoplacental barrier (Ducray et al. 2011, van Oppenraaij et al. 2011, Wang et al. 2015).

The response to ischemia is the formation of a large number of vasoactive humoral factors; besides, foetal antigens entering the bloodstream of the mother are likely to cause the accumulation of methylated analogues of L-arginine (ADMA, MMA) in the plasma. The latter causes dysfunction of the endothelium, secondary ischemic lesions and oxidative stress (Sidorova 2003, Bloshhinskaja 2003, Sánchez-Aranguren et al. 2014, Bloshchinskaja 2003). The consequence of the described events is a decrease in the formation of nitric oxide, which has not only a vasodilating effect, but also suppresses apoptosis in the placenta and plays a significant role in the realisation of the effects of many growth factors. Thus, the vicious circle closes: angiogenesis abnormality provokes placenta ischemia and placental ischemia aggravates angiogenesis abnormality.

The described spectrum of pathogenetic mechanisms of pathogenesis, which is far from being complete, shows that this disease is associated with the emergence of several vicious circles, the integral components of which are endothelial dysfunction and ischemia of the placenta.

Earlier in our laboratory, the endoteleoprotective properties of resveratrol and nicorandil (Danilenko 2013, Kochkarov 2009, Kochkarov et al. 2006, Kochkarov 2008) were studied which could activate $K+_{ATP}$ channels (Tanaka et al. 2010, Novaković et al. 2015); therefore we considered them to be the most promising for our studies.

Objective

To experimentally substantiate the prospects of using drugs which activate K^{+}_{ATP} channels: resveratrol and nicorandil in correcting morphofunctional disorders of experimental preeclampsia.

Methods

The experiment was performed on 250 white Wistar female rats weighing 250–300 g. To form the groups of pregnant animals with predetermined periods, the females (3 animals), which had been kept separately, were caged with males (2 animals) for 24 hours. Then the animals were separated and, 10 days later, in the condition of etheric sleep, determined pregnancy by palpation. In our experiments, pregnancy occurred in 30–40%. An ADMA-like agent, a non-selective NO-synthase blocker, N-nitro-L-arginine methyl ester (L-NAME), was administered intraperitoneally at a dose of 25 mg/kg/day for seven days (14-20 days of gestation). According to literature, this moderately prolonged average dose regimen of L-NAME administration results in the blockade of NO-synthase in the endothelium of the placental vessels, which leads to destructive changes in placental tissues, arterial hypertension and proteinuria (Gureev et al. 2014, Gureev 2012, Tsukimori et al. 2008). On the 21st day of pregnancy, under anaesthesia (chloral hydrate 300 mg/kg), a catheter was inserted into the left carotid artery to record haemodynamic parameters and bolus administration of pharmacological agents was performed in the right femoral vein. The haemodynamic parameters: systolic blood pressure (SBP), diastolic arterial pressure (DBP) and heart rate (HR) were measured continuously by means of a sensor and a hardware complex for invasive measurement of haemodynamic parameters

of Biopac (USA) and by the computer programme Asq-Knowledge 3.8.1 (Gureev et al. 2014, Gureev 2012).

The degree of endothelial dysfunction in the experimental animals was assessed by the ratio of indices of endothelium-dependent vasodilation and endothelium-independent vasodilation with subsequent calculation of the coefficient of endothelial dysfunction (CED) (Korokin et al. 2011, Pokrovsky et al. 2006).

The level of NO metabolites (the total concentration of nitrates and nitrites, NO_x) was determined by the colorimetric method for the development of colour in the diazotisation reaction by sulphonamide nitrite, which is part of the reagent (Metelskaya et al. 2004, Oganov et al. 2004).

Measurement of microcirculation in the placenta was carried out with the help of Biopacsystems equipment: MP100 polygraph with LDF100C laser doppler flowmetry module (LDF100C) and invasive needle probe TSD144 [1, 25], which was mounted directly on to the placental disc projection. LDF was recorded and processed using the AcqKnowledge version 3.8.1. The microcirculation values were expressed in perfusion units (PEU).

Collection of urine was carried out within 12 hours with the use of special metabolic cells. Determination of the amount of protein in daily urine was carried out using the pyrogallol red method on a spectrophotometer PE-5400B.

To study the liquid content in the large omentum, it was weighed, followed by drying at 37°C for 24 hours and re-weighing (Reznikova 2013, Ivanova et al. 2012).

For the morphological confirmation of the development of the simulated pathological processes and for the complex evaluation of the efficacy of the preparations, a histological examination (in all series of the experiment) of kidneys and placenta was carried out. The material is fixed in 10% formalin, followed by pouring into paraffin. Sections of the kidneys were taken perpendicular to the main axis of the organ through the pelvis. Histological sections of the placenta were made in a strictly vertical direction through the middle of the placental disc with the capture of all layers of the placenta and the walls of the uterine horn. Microslide studies, photorecording and morphometry were performed on a Leica DM4000B microscope with a video registration and imaging system. For all morphological studies, staining with haematoxylin and eosin was used.

For subsequent statistical processing, the degree of morphological changes was ranked. The foetuses were removed from the uterine cavity and weighed; their growth (craniocaudal size) was measured, followed by calculating the height-weight coefficient (Mironov 2012).

For all the data, descriptive statistics were applied: the data were checked for the normality of the distribution. The type of distribution was determined by the Shapiro-Wilk criterion. In the case of a normal distribution, the mean value (M) and the standard error of the mean (m) were calculated. The intergroup differences were analysed by means of parametric (Student's t-test) or nonparametric (Mann-Whitney test) methods, depending on the type of distribution. The statistical significance of the differences between the morphological changes after their ranking was assessed using the Mann-Whitney analysis of nonparametric data (Glanz 1999, Sydorenko 2003). All the calculations were performed using the Microsoft Excel 7.0 statistical software package.

According to the objective, all the animals were distributed amongst the following groups:

1. Control group (animals with oral administration of NaCl in equivalent doses from 14th to 20th days of pregnancy).
2. Modelling ADMA-like preeclampsia (animals with intraperitoneal administration of L-NAME at a dose of 25 mg/kg once daily from 14th to 20th days of gestation).
3. Modelling ADMA-like preeclampsia + Amlodipine (0.5 mg/kg/day orally).
4. Modelling ADMA-like preeclampsia + Nicorandil (2x10 mg/kg/day orally).
5. Modelling ADMA-like preeclampsia + Resveratrol (2 mg/kg/day orally).
6. Modelling ADMA-like preeclampsia + Nicorandil (2x10 mg/kg/day orally) + Amlodipine (0.5 mg/kg/day orally).
7. Modelling ADMA-like preeclampsia + Resveratrol (2 mg/kg/day orally) + Amlodipine (0.5 mg/kg/day orally).
8. Modelling ADMA-like preeclampsia + Nicorandil (2x10 mg/kg/day orally) + Glibenclamide (50 mg/kg).
9. Modelling ADMA-like preeclampsia + Resveratrol (2 mg/kg/day orally) + Glibenclamide (50 mg/kg).

Results and discussion: Effect of resveratrol and nicorandil on morphofunctional disorders in ADMA-like preeclampsia.

The administration of resveratrol (2 mg/kg/day) to animals with experimental preeclampsia resulted in a statistically significant ($p < 0.05$) decrease in systolic and diastolic blood pressure from 181.3 ± 4.58 and 144.7 ± 4.09 mm Hg to 154.6 ± 7.53 and 110.90 ± 8.71 mm Hg, respectively, but the level of the intact animals was not reached (133.8 ± 2.45 and 95.36 ± 2.82 mm Hg, respectively). The administration of nicorandil (2x10 mg/kg/day) resulted in a statistically significant ($p < 0.05$) decrease in only diastolic blood pressure, blood pressure reaching 174.1 ± 6.14 and 126.9 ± 6.31 mm Hg, respectively.

The ratio of endothelium-dependent vasorelaxation and endothelium-independent vasorelaxation, expressed by CED, was not influenced by resveratrol. Nicorandil statistically significantly reduced CED from 3.36 ± 0.23 to 1.96 ± 0.13 ($p < 0.05$), but not to the level of the intact animals (1.17 ± 0.10).

In the animals with ADMA-like preeclampsia, when resveratrol and nicorandil were administered, an increase in the microcirculation in the placenta was observed from 213.7 ± 14.97 PUn to 452.4 ± 27.16 PUn and 377.9 ± 18.8 PUn, respectively ($p<0$, 05), which did not differ statistically from the level of the intact animals (409.9 ± 30.57 PUn).

In the animals with ADMA-like preeclampsia, a statistically significant ($p<0.05$) decrease in the concentration of final metabolites of NO in blood plasma was observed in comparison with the intact animals from 2.22 ± 0.08 μmol/dl to 1.27 ± 0.04 μmol/dl. Using resveratrol and nicorandil in the animals with ADMA-like preeclampsia, their statistically significant increase was observed to 1.78 ± 0.05μmol/dl and 1.80 ± 0.07 μmol/dl, respectively.

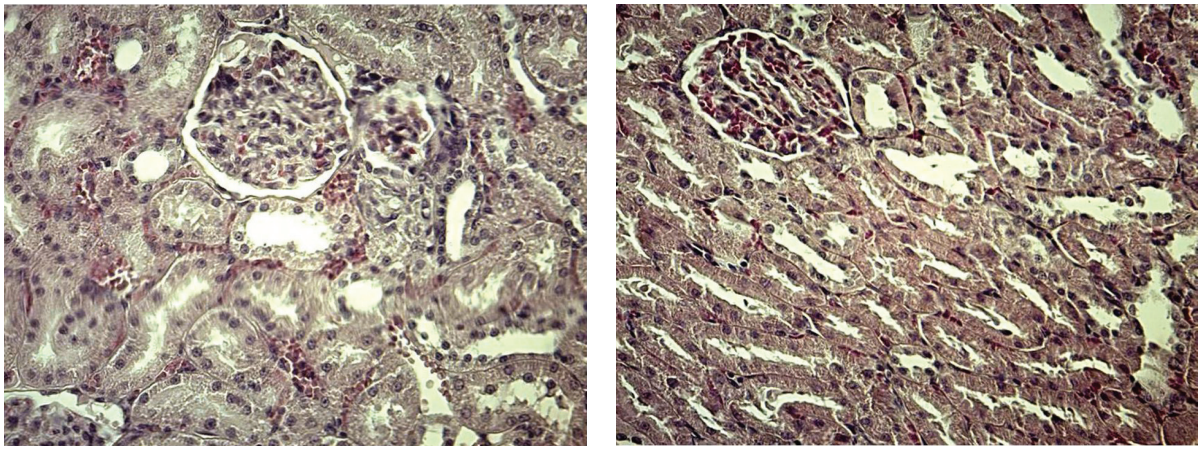
The administration of an ADMA-like agent to the pregnant animals from the 14th to 20th days of gestation resulted in an increase in proteinuria from 0.69 ± 0.70 g/l to 2.30 ± 0.14 g/l ($p<0.05$). The administration of resveratrol and nicorandil to the animals with experimental preeclampsia resulted in a statistically significant decrease in the urinary protein concentration to 1.22 ± 0.11 g/l and 1.39 ± 0.10 g/l, respectively ($p<0.05$).

A study of the fluid content in the large omentum of the animals with ADMA-like preeclampsia revealed its increase in comparison with the intact pregnant animals from 45.82 ± 1.82% to 54.73 ± 1.96% ($p<0.05$). The administration of resveratrol and nicorandil resulted in a decrease in the level of swelling in the tissues of the large omentum to a level statistically indistinguishable in relation to the intact pregnant animals – 50.00 ± 1.25% and 50.57 ± 2.08%, respectively.

A morphological examination of the renal parenchyma in the animals with ADMA-like preeclampsia revealed: anaemia and spasm of renal vessels, thickening of their walls, thickening of the basement membrane of the glomeruli capillaries in the form of a wire loop (Fig. 1(B)). No noticeable reaction of mesangium was observed. Thus, the average number of cells per glomerulus in the group with experimental preeclampsia was 39.60 ± 1.88 (in the normal condition 40.60 ± 2.39).

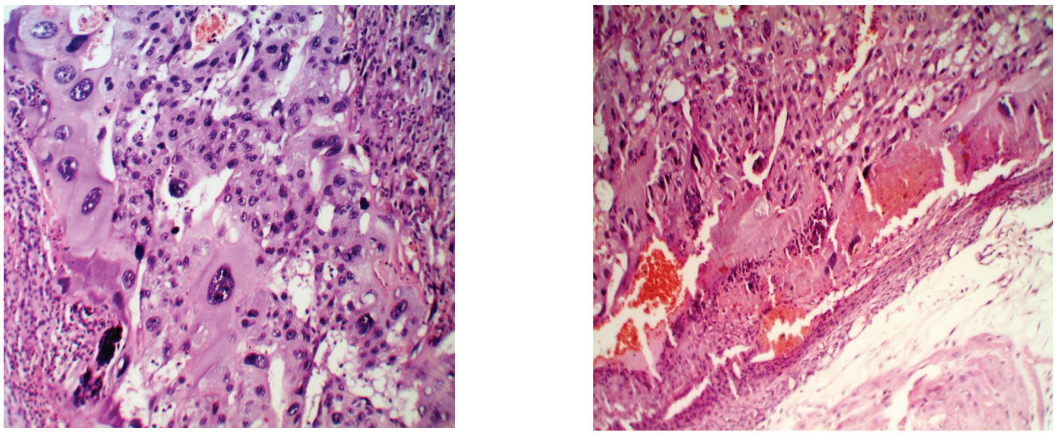
In the placenta of the animals with ADMA-like preeclampsia, massive deposition of fibrin and significant changes in ischemic genesis were observed (Fig. 2(B)).

The integral index of analysis of the morphological study in animals with ADMA-like preeclampsia was 5-6 points ($p<0.05$).



A **B**

Figure 1. Histological picture of the kidney in the modelling of ADMA-like preeclampsia. **Note: (A)** – the glomerule of an intact animal. (**B**) the glomerule of an animal with ADMA-like preeclampsia. ×200.



A **B**

Figure 2. Histological picture of the placenta in the modelling of ADMA-like preeclampsia. **Note: (A)** type of intact placenta. (**B**) ADMA-like preeclampsia. Vacuolar degeneration of giant cell trophoblast; dystrophic changes in the decidual layer, foci of necrosis at the border of the giant cell trophoblast and decidual tissue. ×200.

The administration of resveratrol and nicorandil to the animals with ADMA-like preeclampsia led to significant positive morphological changes in the kidneys and placenta. In the kidneys, less significant changes were recorded (Fig. 3(A)). There was no noticeable mesangium reaction (42.20 ± 1.91 cells when administering resveratrol and 39.40 ± 1.53 cells when administering nicorandil). In the placenta, moderately significant fibrous deposits, less significant dystrophic changes in the giant cell trophoblast and decidual layer and no necrosis foci were observed. The integral index of the analysis of the morphological study in the animals with the correction of ADMA-like preeclampsia by resveratrol and nicorandil was 3 points ($p < 0.05$).

The study of the features of foetal development in the animals with ADMA-like preeclampsia revealed a moderately significant foetal hypotrophy, which manifested itself in a decrease in mass and length (growth). It should be noted that the mass of the foetuses was most affected. With the administration of resveratrol, only the mass of the foetus decreased. With the administration of nicorandil, neither the weight nor height indices differed from those of intact foetuses (Table 1).

Thus, the results of the series of experiments indicate a pronounced protective activity of resveratrol and nicorandil in the correction of morphofunctional disorders occurring in the animals when modelling ADMA-like preeclampsia.

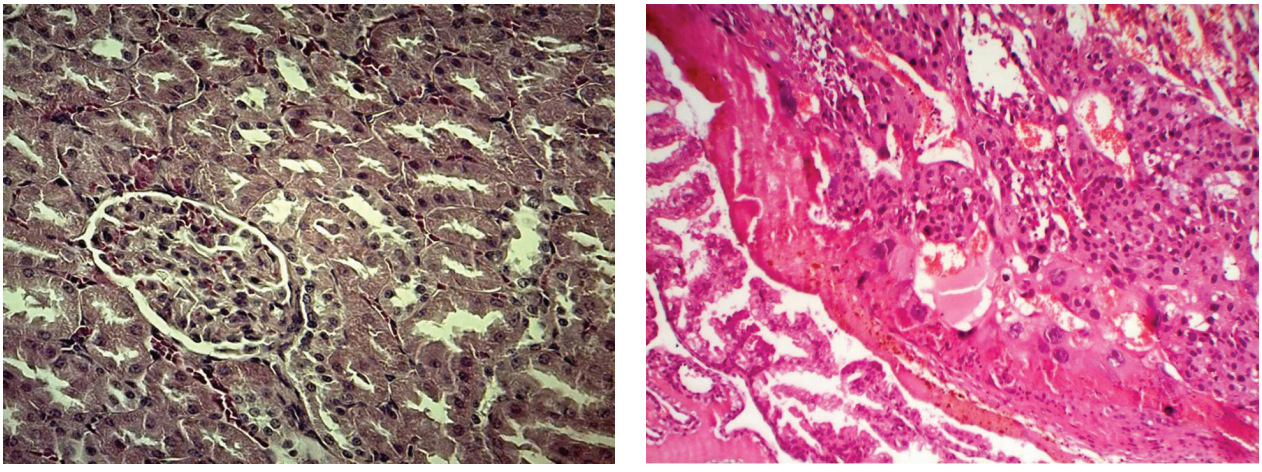
Effect of resveratrol and nicorandil when combined with amlodipine on morphofunctional disorders in ADMA-like preeclampsia

The administration of nicorandil in combination with amlodipine to the animals with experimental ADMA-like preeclampsia resulted in a statistically significant ($p < 0.05$) decrease in systolic and diastolic blood pressure (Table 2). It should be noted that in monotherapy, statistically neither amlodipine nor nicorandil significantly reduced systolic pressure. Therefore, a decrease in systolic pressure with their combined use can be regarded as a potentiation of their action. Addition of amlodipine to resveratrol did not lead to an increase in its hypotensive effect.

The administration of resveratrol and nicorandil in combination with amlodipine to the animals with ADMA-like preeclampsia led to a more significant decrease in CED, compared to the groups of animals where these pharmacological agents were used as monotherapy (Table 2).

The administration of resveratrol and nicorandil in combination with amlodipine to the animals with ADMA-like preeclampsia led to an increase in the microcirculation in the placenta ($p < 0.05$) (Fig. 4(A)).

In the biochemical study of the final metabolite concentration of NO in the blood plasma, their statistically significant increase ($p < 0.05$) was recorded (Fig. 4(B)). The most significant effect was observed when amlodipine was combined with nicorandil. In the animals of this



A **B**

Figure 3. Morphological picture of kidneys and placenta in animals with correction of ADMA-like preeclampsia by resveratrol. **Note:** (**A**) kidney; (**B**) placenta. ×200.

Table 1. Effect of Resveratrol and Nicorandil on the Height-weight Indices of Foetal Development in ADMA-like Preeclampsia (M ± m; n = 10).

Group Index	Pregnant intact	Pregnant + L-NAME	Pregnant + L-NAME + Resveratrol	Pregnant + L-NAME + Nicorandil
	1.68±0.09^{y}	1.38±0.07*	1.45±0.06*	1.53±0.06
Foetus weight, g	23.79±0.42^{y}	21.82±0.34*	22.59±0.61*	22.91±0.61
Foetus height, mm	14.01±0.28^{y}	15.81±0.43*	15.58±0.35*	14.97±0.43

Notes: * - $p<0.05$ in comparison with intact pregnant females; ^{y} - $p < 0.05$ in comparison with L-NAME group.

group, the microcirculation rate in the placenta was at the level of intact animals.

The administration of resveratrol and nicorandil in combination with amlodipine to the animals with experimental preeclampsia led to a statistically significant decrease in urinary protein concentrations ($p<0.05$) (Fig. 4(C)).

The combined administration of amlodipine with resveratrol or nicorandil to the animals with ADMA-like preeclampsia resulted in reduced swelling of the large omentum to a level statistically distinct when compared with "untreated" animals (Figure 4(D)).

The combined use of these drugs in the animals with ADMA-like preeclampsia led to significant positive morphological changes in the kidneys and placenta. In the kidneys, there was a moderate vasospasm. No symptoms of the wire loop were observed (Fig. 5). There was no noticeable mesangium reaction (38.10 ± 2.23 cells and 40.50 ± 2.33 cells, respectively) (in the normal condition 40.60 ± 2.39 cells).

Table 2. Results of Correction of ADMA-like Preeclampsia with Resveratrol and Nicorandil in Combination with Amlodipine (M ± m; N = 10).

Group	SBP mm Hg	DBP mm Hg	CED relative units
Intact	133.8±2.45^{y}	95.36±2.82^{y}	1.17±0.10^{y}
L-NAME	181.3±4.58*	144.7±4.09*	3.36±0.23*
L-NAME + Amlodipine	167.0±7.67*	122.9±7.49^{y}	2.24±0.19^{y}
L-NAME +Resveratrol	154.6±7.53^{y}	110.90±8.71^{y}	3.36±0.41*
L-NAME +Nichorondil	174.1±6.14*	126.9±6.31^{y}	1.96±0.13^{y}
L-NAME +Resveratrol+Amlodipine	159.9±4.36^{y}	116.5±4.41^{y}	2.02±0.19^{y}
L-NAME +Nicorondil+Amlodipine	158.5±3.84^{y}	117.2±3.70^{y}	1.49±0.15^{y}

Notes: SBP, DBP - systolic and diastolic blood pressure (mmHg); CED - coefficient of endothelial dysfunction (r.u.); * - $p<0.05$ in comparison with the group of intact animals; ^{y} - $p<0.05$ in comparison with the L-NAME group.

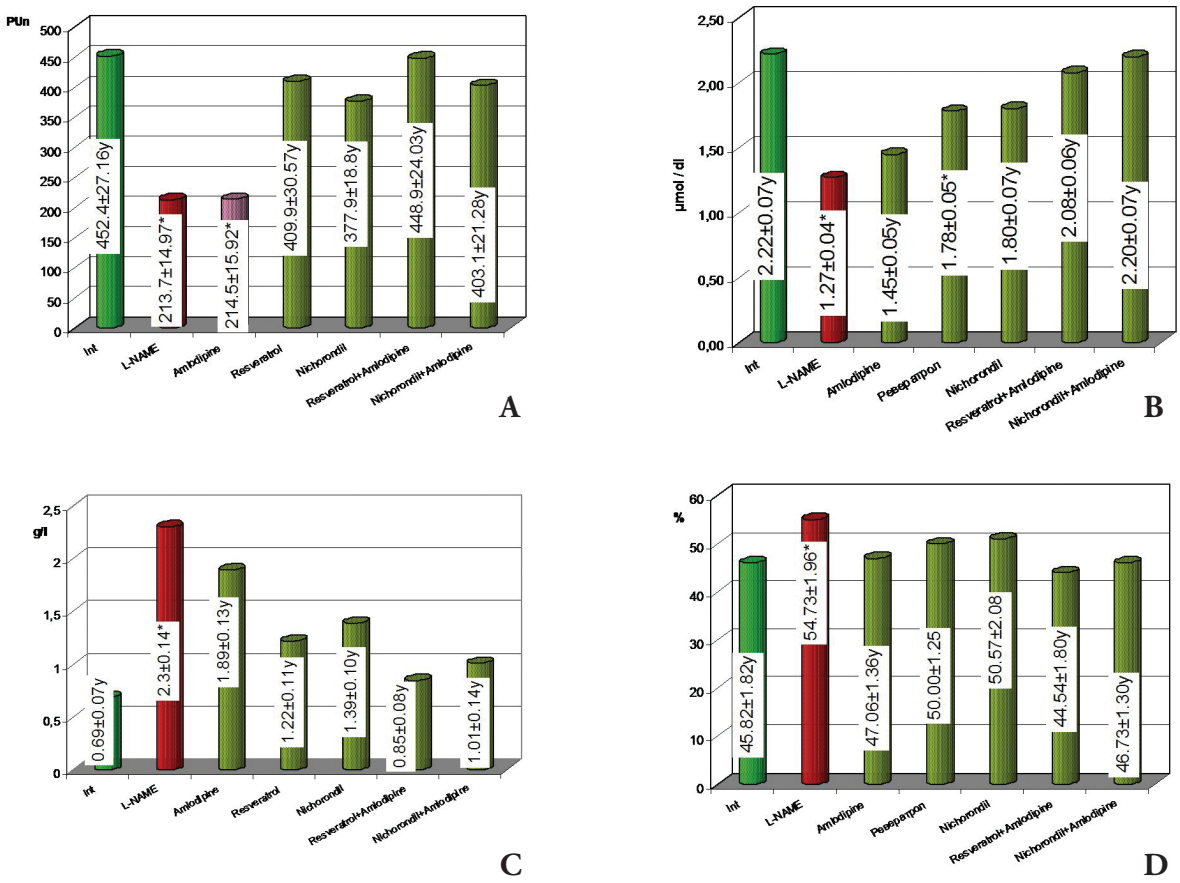


Figure 4. Effect of resveratrol and nicorandil in combination with amlodipine on microcirculation in the placenta, plasma NO_x content, proteinuria and swelling of the large omentum with ADMA-like preeclampsia. **Notes:** * - $p<0.05$ in comparison with the group of intact animals; ^{y} - $p<0.05$ in comparison with the L-NAME group.

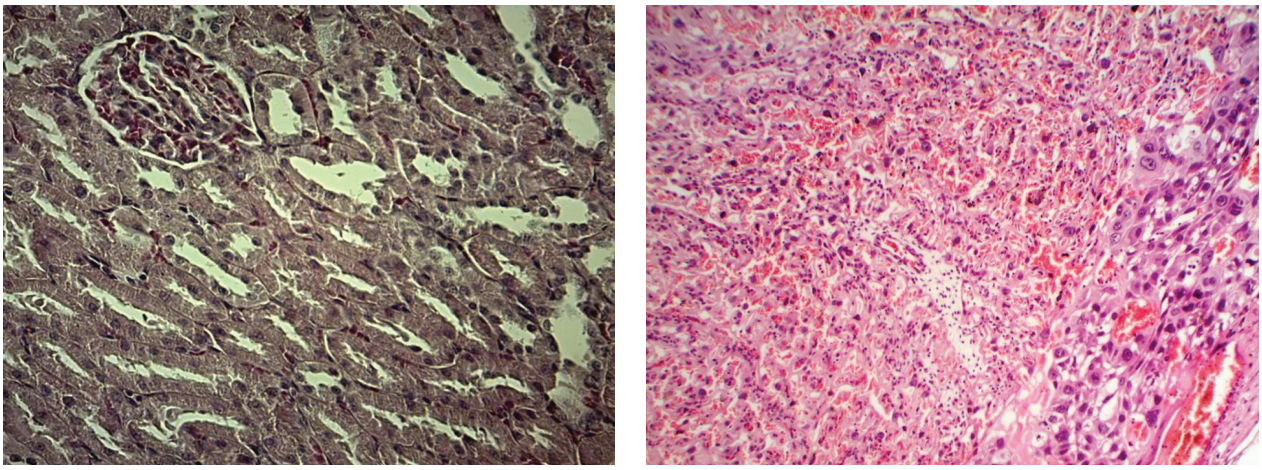
In the placenta, weakly expressed fibrous deposits were observed; dystrophic changes of the giant cell trophoblast and decidual layer were not significantly expressed; there were no necrosis foci (Fig. 5). The integral index of the analysis of the morphological study in the animals with the correction of ADMA-like preeclampsia by resveratrol and nicorandil in combination with amlodipine is given in Table 3.

A study of the features of foetal development in the animals with ADMA-like preeclampsia revealed no foetal hypotrophy when administering resveratrol and nicorandil in combination with amlodipine (Table 4).

Thus, the results of a series of experiments indicate a significant protective activity of resveratrol and nicorandil when combined with amlodipine to correct morphofunctional disorders occurring in the animals when modelling ADMA-like preeclampsia. It should be noted that the combinations used have a more significant correction of morphofunctional disorders in comparison with the drugs used in monotherapy.

Role of K^+_{ATP} channels in the positive effects of resveratrol and nicorandil in ADMA-like preeclampsia.

The administration of resveratrol and nicorandil in combination with an inhibitor of K^+_{ATP} channels to the animals with experimental ADMA-like preeclampsia led to



A **B**

Figure 5. Morphological picture of kidneys and placenta in animals with correction of ADMA-like preeclampsia by nicorandil in combination with amlodipine. **Note**: (**A**) kidney; (**B**) placenta. ×200.

Table 3. Effect of Resveratrol and Nicorandil in Combination with Amlodipine on Comprehensive Assessment of Pathomorphological Changes in Kidneys and Placenta in ADMA-like Preeclampsia (M ± m; n = 10).

Series of experiments	Comprehensive assessment in points
Pregnant intact	0–1^{y}
Pregnant + L-NAME	5–6*
Pregnant + L-NAME + Amlodipine	4^{y}
Pregnant + L-NAME + Resveratrol	3^{y}
Pregnant + L-NAME + Nicorandil	3^{y}
Pregnant + L-NAME + Resveratrol+ Amlodipine	2^{y}
Pregnant + L-NAME + Nicorandil+ Amlodipine	1-2^{y}

Notes: * - p<0.05 in comparison with intact pregnant females; ^{y}- p<0.05 in comparison with L-NAME group.

Table 4. Effect of Resveratrol and Nicorandil in Combination with Amlodipine on Height-weight Indicators of Foetal Development in ADMA-like Preeclampsia (M ± m; n = 10).

Group Indicator	Foetus weight, g	Foetus height, mm	Height / weight, mm/g
Pregnant intact	1.68±0.09^{y}	23.79±0.42^{y}	14.01±0.28^{y}
Pregnant + L-NAME	1.38±0.07*	21.82±0.34*	15.81±0.43*
Pregnant + L-NAME + Amlodipine	1.40±0.09*	22.09±0.40*	15.78±0.51*
Pregnant + L-NAME + Resveratrol	1.45±0.06*	22.59±0.61	15.58±0.35*
Pregnant + L-NAME + Nicorandil	1.53±0.06	22.91±0.61	14.97±0.43
Pregnant + L-NAME + Resveratrol+ Amlodipine	1.60±0.07^{y}	23.19±0.53	14.49±0.48
Pregnant + L-NAME + Nicorandil+ Amlodipine	1.59±0.05^{y}	22.92±0.53	14.42±0.53

Notes: * - p<0.05 in comparison with intact pregnant females; ^{y} - p<0.05 in comparison with L-NAME group.

a decrease in the intensity of their positive effects. Thus, an increase in systolic and diastolic arterial pressure was observed in comparison with the groups of the animals in which resveratrol and nicorandil were used in monotherapy (Table 5). It should be noted that the diastolic pressure in the group of the animals with combined administration of nicorandil and glibenclamide did not statistically differ from that in the group of the "untreated" animals.

The administration of nicorandil in combination with glibenclamide to the animals with ADMA-like preeclampsia led to an increase in CED, compared to the groups of the animals where nicorandil was used as a monotherapy (Table 5), but this coefficient continued to differ statistically significantly from that in the group of the "untreated" animals ($p < 0.05$).

Adding glibenclamide to nicorandil and resveratrol when correcting ADMA-like preeclampsia led to reduced microcirculation in the placenta to a level statistically distinct from that in the animals with correction by these drugs in the monotherapy (Fig. 6(A)) ($p < 0.05$), while adding resveratrol almost completely removed its positive effect on microcirculation.

In a biochemical study of the concentration of terminal metabolites of NO in the blood plasma, a statistically significant decrease in glibenclamide when using resveratrol and nicorandil to correct morphofunctional disorders in the animals with ADMA-like preeclampsia was revealed (Fig. 6(B)). Nevertheless, this indicator was at a level statistically different from that in the group of the "untreated" animals ($p < 0.05$).

Adding glibenclamide to resveratrol and nicorandil caused an increase in proteinuria compared to the groups of the animals in which these pharmacological agents were used in the monotherapy (Fig. 6(C)) ($p<0.05$).

The administration of resveratrol and nicorandil in combination with glibenclamide led to an increased swelling of the tissues of the large omentum to a level statistically indistinguishable when compared to that of the "untreated" animals (Fig. 6(D)).

The administration of resveratrol and nicorandil in combination with glibenclamide in the animals with ADMA-like preeclampsia aggravated the morphological pattern in the kidneys and placenta. In the kidneys, there was a pronounced spasm of the vessels. There were symptoms of a wire loop. There was no noticeable mesangium reaction (40.20 ± 1.68 cells and 38.60 ± 1.63 cells, respectively) (in the normal condition 40.60 ± 2.39 cells).

In the placenta, there were observed fibrous deposits; the dystrophic changes of the giant cell trophoblast and the decidual layer were not significantly expressed; there were isolated foci of necrosis. The integral index of the analysis of the morphological study in the animals with correction of ADMA-like preeclampsia by resveratrol and nicorandil affected by glibenclamide is given in Table 6.

Investigation of peculiarities of foetal development in the animals with ADMA-like preeclampsia revealed foetal hypotrophy when administering resveratrol and nicorandil in combination with glibenclamide (Table 7). The mass of the foetus was most affected.

Thus, the results of the series of experiments conducted indicate a significant decrease in the positive effects of resveratrol and nicorandil by glibenclamide. Since glibenclamide is a blocker of K^+_{ATP} channels, the series of experiments makes it possible to assert the significant role of K^+_{ATP} in revealing protective effects of resveratrol and nicorandil when correcting morphofunctional disorders in ADMA-like preeclampsia. Incomplete cancellation of their positive effects is due to the fact that

Table 5. Results of Correction of ADMA-like Preeclampsia with Resveratrol and Nicorandil Affected by Glibenclamide ($M \pm m$; $n = 10$).

Group	SBP mm Hg	DBP mm Hg	CED relative units
Intact	133.8 ± 2.45^{y}	95.36 ± 2.82^{y}	1.17 ± 0.10^{y}
L-NAME	$181.3 \pm 4.58^{*}$	$144.7 \pm 4.09^{*}$	$3.36 \pm 0.23^{*}$
L-NAME + Resveratrol	$154.6 \pm 7.53^{y*}$	110.90 ± 8.71^{y}	$3.36 \pm 0.41^{*}$
L-NAME + Nicorandil	$174.1 \pm 6.14^{*}$	126.9 ± 6.31^{y}	1.96 ± 0.13^{y}
L-NAME + Resveratrol + Glibenclamide	158.1 ± 5.16^{y}	120.8 ± 3.16^{y}	$2.95 \pm 0.22^{*}$
L-NAME + Nicorandil + Glibenclamide	$186.0 \pm 7.46^{*}$	$134.5 \pm 8.92^{*}$	$2.55 \pm 0.0.17^{y*}$

Notes: SBP, DBP – systolic and diastolic blood pressure (mmHg); CED – coefficient of endothelial dysfunction (r.u.); * - $p<0.05$ in comparison with the group of intact animals; ^{y} - $p<0.05$ in comparison with the L-NAME group.

Table 6. Effect of Resveratrol and Nicorandil Affected by Glibenclamide on the Comprehensive Assessment of Pathomorphological Changes in Kidneys and Placenta in ADMA-like Preeclampsia ($M \pm m$; $n = 10$).

Series of experiments	Comprehensive assessment in points
Pregnant intact	$0–1^{y}$
Pregnant + L-NAME	$5–6^{*}$
Pregnant + L-NAME + Resveratrol	3^{y}
Pregnant + L-NAME + Nicorandil	3^{y}
Pregnant + L-NAME + Resveratrol + Glibenclamide	4^{y}
Pregnant + L-NAME + Nicorandil + Glibenclamide	$4–5^{y}$

Notes: * - $p<0.05$ in comparison with pregnant intact females; ^{y}- $p<0.05$ in comparison with L-NAME group.

resveratrol and nicorandil have a pronounced biological activity in their implementation in which K^+_{ATP} channels do not participate.

Conclusion

The administration of a nonselective inhibitor of NOS - L-NAME resulted in an increased systolic and diastolic pressure, a disrupted relationship between endothelium-dependent and endothelium-independent vasodilating reactions, a reduced microcirculation in the placenta, a decreased concentration of terminal metabolites of NO in the blood plasma, increased proteinuria, an increased fluid content in the large omentum, disorders in the placenta of ischemic origin and foetal hypotrophy. The described symptom complex of morphofunctional changes quite accurately represents the clinical picture of syndromes arising during preeclampsia. Thus, ADMA-like preeclampsia fully matches the model chosen for our experiment.

The administration of resveratrol led to a statistically significant decrease in systolic and diastolic arterial pressure, an increase in microcirculation in the placenta, a decrease in the protein content in the urine, swelling of the

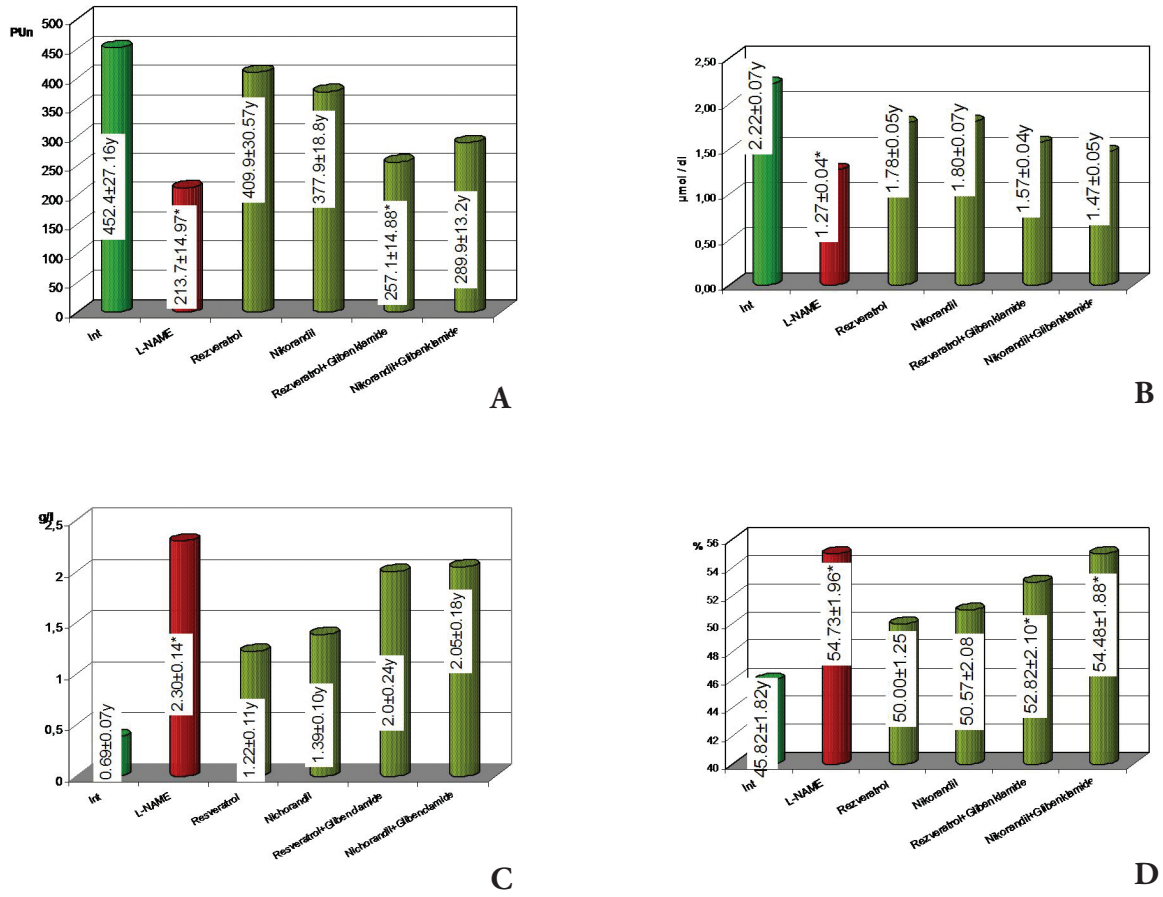


Figure 6. Effect of resveratrol and nicorandil in combination with glibenclamide on microcirculation in the placenta, proteinuria, plasma NO_x concentration and swelling of the large omentum with ADMA-like preeclampsia. **Notes:** * - p<0.05 in comparison with the group of intact animals; ʸ- p<0.05 in comparison with L-NAME group.

Table 7. Effect of Resveratrol and Nicorandil in Combination with Glibenclamide on the Height-weight Foetal Development Indicators in ADMA-like Preeclampsia (M ± m; n = 10).

Group Indicator	Foetus weight, g	Foetus height, mm	Height / weight, mm/g
Pregnant intact	1.68±0.09ʸ	23.79±0.42ʸ	14.01±0.28ʸ
Pregnant +L-NAME	1.38±0.07*	21.82±0.34*	15.81±0.43*
Pregnant + L-NAME + Resveratrol	1.45±0.06*	22.59±0.61*	15.58±0.35*
Pregnant +L-NAME + Nicorandil	1.53±0.06*	22.91±0.61*	14.97±0.43*
Pregnant + L-NAME + Resveratrol + Glibenclamide	1.44±0.09*	22.05±0.67*	15.31±0.54*
Pregnant + L-NAME + Nicorandil + Glibenclamide	1.47±0.07*	21.88±0.48*	14.88±0.61*

Notes: * - p<0.05 in comparison with pregnant intact females; ʸ- p<0.05 in comparison with L-NAME group.

large omentum and an increase in the NO-synthesising function of the endothelium. The morphological examination of the kidneys and placenta revealed moderately pronounced pathological changes of a functional nature.

One of the mechanisms for preventing the development of morphofunctional disorders in ADMA-like preeclampsia by resveratol is its pronounced endothelial-protective properties (Kochkarov 2009). Another positive aspect is an antisense activity in resveratrol (Camont et al. 2010, Camont et al. 2012). By reducing the number of free radicals, it thereby increases the bioavailability of the NO formed. An equally important endotheletoprotective mechanism is an ability of resveratrol to increase the activity of NO-synthase, promoting greater formation of NO and a subsequent increase in the level of cGMP that leads to vasodilation (Leikert et al. 2002, Wallerath et al. 2002). Under the conditions of oxidative stress, resveratrol restores the activity of DDAH, which is an enzyme metabolising ADMA (Frombaum et al. 2011). This is of great importance, since an increase in ADMA inhibits eNOS and contributes to an impaired endothelial function (Bivalacqua et al. 2001, White et al. 2006).

The ability of resveratrol to induce a dose-dependent relaxation in isolated strips of the pregnant uterus myometrium, which is realised through K^+_{ATP} channels (Rose et al. 2014), plays an important role, since relaxation of the myometrium helps improve microcirculation in it.

Against the background of the significant complex protective effect of resveratrol on the development of morphofunctional disturbances arising when modelling ADMA-like preeclampsia, the lack of its positive effect on the coefficient of endothelial dysfunction was somewhat unexpected for the authors, especially as in the model of ADMA-like pathology in males, there was a significant decrease in CED (Kochkarov 2009). We believe that this fact can be explained by an increased gestogenic background in pregnant females, with which resveratrol, being a phytoestrogen, enters into some antagonistic relationships.

The administration of nicorondil led to a statistically significant decrease in diastolic pressure and CED, an increase in the microcirculation in the placenta, a decrease in the protein content in the urine and swelling of the large omentum and an increase in the NO-synthesising function of the endothelium. The morphological examination of the kidneys and placenta revealed moderately pronounced pathological changes of a functional nature.

The pronounced protective effects of nicorandil are linked to its ability to activate K^+_{ATP} channels and to be a donor of nitric oxide (Tanaka et al. 2010, Gayet et al. 2011, Holzmann 1983). Activation of K^+_{ATP} channels leads to hyperpolarisation of the membrane, reduction of Ca^{2+} current and relaxation of small arterial vessels. Vasodilation is also facilitated by the formation of NO by activating guanylate cyclase (Tanaka et al. 2010, Wei et al. 2016). In addition, the positive effects of nicorandil are enhanced by its having a preconditioning effect (Belousov 2012), endothelium-protective properties (Malysheva et al. 2011) and its ability to reduce apoptosis (Staroseltseva 2012).

A certain role in the positive effects of nicorandil can be played by its having an anti-inflammatory activity (Li et al. 2015, Serizawa et al. 2014, Chao et al. 2016). In addition, the ability of nicorandil to relax isolated strips of myomentry of the pregnant uterus, which is realised through K^+_{ATP} channels, can improve microcirculation in the placenta (Matsui et al. 2015, Hong et al. 2016).

Thus, the use of drugs possessing preconditioning properties for correcting morphofunctional disorders in ADMA-like preeclampsia leads to pronounced positive effects, which indicates the correctness of the chosen approach.

The administration of the tested pharmacological agents in combination with a prolonged form of the antagonist of the Ca^{2+}-channels of the dihydropyridine series of amlodipine led to a more significant correction of morphofunctional disorders in ADMA-like preeclampsia. This can be explained by the fact that amlodipine has its own endothelium-protective activity, which is fulfilled through the mechanisms different from those in the tested drugs. There are published data on the improvement of endothelium-dependent vasodilation due to an increased level of NO (Parimala et al. 2006) and an increased level of endothelial NO synthase (Parimala et al. 2006, Korokin et al. 2011, Xu et al. 2016). Besides, Ca^{2+} antagonists have an angioprotective effect due to a decrease in Ca^{2+} current through L-channels.

Thus, administration of drugs with different mechanisms of action to animals with ADMA-like preeclampsia leads to more significant protective effects compared to those in the groups in which these drugs were used in the monotherapy. It becomes clear that the use of new pharmacological agents for treating preeclampsia is most appropriate with the drugs within the standard therapy.

The available information on the ability of resveratrol and nicorandil to activate K^+_{ATP} channels logically predetermined the positive effects of their use in correcting morphofunctional disorders in ADMA-like preeclampsia. It is clear that the improvement of microcirculation due to a reduced tone of the myointrium and the preconditioning effect indirectly improves the endothelial function by reducing placental ischemia. However, the drugs under study have a whole spectrum of biological activities that can also bring about positive effects. Therefore, a further series of our experiments were aimed at elucidating the role of K^+_{ATP} channels in the mechanism of protective effects of resveratrol and nicorandil in ADMA-like preeclampsia.

To determine the role of K^+_{ATP} channels in revealing the positive effects of the tested pharmacological agents, we used a blocker of these channels – glibenclamide.

The administration of glibenclamide in combination with resveratrol and nicorandil significantly reduced the effectiveness of these drugs, but not completely. Thus, the results of the series of experiments make it possible to conclude that K^+_{ATP} channels play a significant role in revealing the positive effects of resveratrol and nicorandil in correcting morphofunctional disorders in ADMA-like preeclampsia, but do not completely determine them.

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