Endothelial protective properties of short-chain peptides that mimic α-helix B of erythropoietin in experimental preeclampsia

Propiedades protectoras endoteliales de péptidos de cadena corta que imitan la α-hélice B de la eritropoyetina en la preeclampsia experimental

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Abstract

Objective. Investigate the endothelioprotective properties of short-chain derivatives of erythropoietin in experimental preeclampsia.

Methodology. The experiment was performed in 100 white female Wistar rats weighing 250-300 g. L-NAME was administered intraperitoneally (25 mg/kg/day) from 14 to 20 days of pregnancy. The studied peptides (50 μg/kg) were administered intraperitoneally once a day from 10 to 20 days of pregnancy. On the 21st day of pregnancy, functional tests and laboratory tests were performed.

Results. Rat pretreatment with the studied peptides reverted pathological changes in experimental ADMA-like preeclampsia. The greatest effect was observed with the peptide with the laboratory code P-αB1. A significant decrease in systolic and diastolic blood pressure was observed, improved microcirculation in the placenta, restoration of the endothelium NO-synthesizing function, and a decrease in proteinuria.

Conclusion. The results of the study are promising for the use of short-chain derivatives of erythropoietin simulating its α-helix B for the correction of morpho-functional changes in experimental preeclampsia and substantiate the desirability of further research in this direction.

Keywords: erythropoietin, preeclampsia, endothelial dysfunction, rats, proteinuria, microcirculation.

Resumen

Objetivo. Investigar las propiedades endotelioprotectoras de los derivados de la eritropoyetina de cadena corta en la preeclampsia experimental.

Metodología. El experimento se realizó en 100 ratas Wistar hembra blancas con un peso de 250-300 g. Se administró L-NAME por vía intraperitoneal (25 mg/kg/día) a los 14 a 20 días de embarazo. Los péptidos estudiados (50 μg/kg) se administraron intraperitonealmente, una vez al día desde el día 10 al 20 de embarazo. El día 21 del embarazo, se realizaron pruebas funcionales y pruebas de laboratorio.

Resultados. El pretratamiento de las ratas con los péptidos estudiados revirtió los cambios patológicos en la preeclampsia experimental similar a ADMA. El mayor efecto se observó con el péptido con el código de laboratorio P-αB1. Se observó una disminución significativa en la presión arterial sistólica y diastólica, una mejor microcirculación en la placenta, la restauración de la función sintetizadora de NO del endotelio y una disminución de la proteinuria.

Conclusión. Los resultados del estudio son prometedores para el uso de los derivados de la eritropoyetina de cadena corta que simulan su α-hélice B para la corrección de los cambios morfo-funcionales en la preeclampsia experimental y corroboran la conveniencia de futuras investigaciones en esta dirección.

Palabras clave: eritropoyetina, preeclampsia, disfunción endotelial, ratas, proteinuria, microcirculación.

Introduction

In search of new drugs for the treatment and prevention of diseases, is an urgent task of modern pharmacology[1-5]. One of these areas is obtaining drugs for the treatment and prevention of preeclampsia (PE)[6-8]. In developed countries, it accounts for about 16-18% of maternal deaths and up to 40% of fetal and newborn deaths[9]. In women with preeclampsia, the complication rate can reach 22%[10]. Preeclampsia (PE) is a complex disease, exclusive to human pregnancy, being the main cause of fetal and maternal morbidity and mortality, preterm birth, intrauterine growth retardation, and perinatal mortality. It is characterized by new-onset hypertension and proteinuria, which usually arises after 20 weeks of gestation, more frequently in the third trimester and reverses in the postpartum period[11].

A large number of studies have been conducted around the world to increase the effectiveness of the treatment and prevention of this disease. Moreover, preeclampsia is increasingly seen in endothelial dysfunction[12]. One of the mechanisms for the development of endothelial dysfunction in preeclampsia is “oxidative stress” as a result of the depletion of the antioxidant system in conditions of tissue ischemia[13]. Developing against this background, endothelial dysfunction leads to impaired microcirculation and tissue hypoxia, and as a result, to the development of multiple organ disorders that make up the clinical manifestations of preeclampsia[14,32]. One of the leading pathophysiological factors in reducing the activity of endothelial NO-synthase (e-NOS) and the development of preeclampsia is placental ischemia. An increase in the activity of NO-synthase can be achieved by reducing the ischemic phenomena of the placenta.

The evidence indicates a positive effects of recombinant erythropoietin and its derivatives in experimental preeclampsia. Among them are the cytoprotective action due to the activation of the heterodimeric receptor for erythropoietin. A simpler and relatively cheaper way may be the use of short-chain derivatives of erythropoietin that mimic its α-helix B[15]. Such derivatives are polypeptides that model the active center of erythropoietin binding to the receptor. With this in mind, these polypeptides retain the ability to bind to the heterodimeric receptor, but concerning erythropoietin, they will have greater penetration into tissues due to their lower mass.

Based on the evidence it could be possible that these peptides could be effective in pregnant women with impaired growth and placenta formation in early pregnancy with outcome in placental ischemia and impaired endothelial function. In support of this assumption, we found that the basic 11-amino acid peptide P-αB (QEQLERALNSS), which mimics the structure of erythropoietin α-helix B has a pronounced endothelial-protective and potentially atheroprotective effect due to its ability to prevent the death of endothelial cells, however it shows prothrombotic activity in rats, suggesting the requirement for necessitates further modifications of this molecule[16]. We look forward as a prospect in the modification of P-αB by attaching peptide motifs with antiplatelet activity. In the framework of this study, the pharmacological activity obtained by modifying the initial peptide with the introduction of the RGD motif (Arg-Gly-Asp) into the structure was studied. In addition, it was investigated the endothelioprotective properties of short-chain derivatives of erythropoietin in experimental preeclampsia induced by the inhibition of nitric oxide (NO) synthesis with N-nitro-L-arginine-methyl ether (L-NAME).

Material and Methods

The experimental study was conducted at the Research Institute of Pharmacology of Living Systems of Belgorod State National Research University. The study was performed in compliance with the requirements of General Requirements for the Competence of Testing and Calibration Laboratories 17025-2009, GOST R ISO 5725-2002 and the Rules of Laboratory Practice, approved by Order of the Ministry of Healthcare and Social Development of the Russian Federation dated August 23rd, 2010 № 708n, in compliance with the European Convention for the Protection of Vertebrates Used for Experiments or Other Scientific Purposes CETS No. 170. All the experiments were approved by the Ethical Committee of Belgorod National Research University.

The experiment was performed in 120 female white Wistar rats weighing 250-300 g. For the formation of groups of pregnant animals with predetermined periods that were kept separately, females (3 animals) were planted males (2 animals) for 24 hours. Then the animals were seated and on the 10th day in the condition of ether sleep, the presence of pregnancy was determined by palpation. In our experiments, pregnancy occurred in 30-40%. ADMA-like agent - a non-selective NO-synthase blocker N-nitro-L-arginine-methyl ether (L-NAME) was administered intraperitoneally at a dose of 25 mg/kg/day for seven days (14-20 days of pregnancy)[15,17].

The studied innovative peptides that mimic the erythropoietin α-helix B were administered intraperitoneally at a dose of 50 μg/kg once a day for 10 days (10-20 days of pregnancy). Intact animals were injected intraperitoneally with saline at a dose of 10 ml/kg for 10 days.

In view of the goal, the following groups of animals were formed:

1. Control (0.9% NaCl).

2. L-NAME 25 mg/kg

3. L-NAME + P-αB (QEQLERALNSS) 50 μg/kg

4. L-NAME + P-αB1 (RGDQEQLERALNSS) 50 μg/kg

5. L-NAME + P-αB2 (QEQLERALNSSRGD) 50 μg/kg

On the 21st day of pregnancy, the laboratory animal was anesthetized by intraperitoneal injection of chloral hydrate at a dose of 300 mg/kg body weight, after which functional tests were performed[17-20].

The degree of endothelial dysfunction in experimental animals was evaluated by the ratio of endothelium-dependent

vasodilation and endothelium-independent vasodilation with subsequent calculation of the coefficient of endothelial dysfunction (QED)[21-25]. The level of NO metabolites (i.e., the total concentration of nitrates and nitrites, NOx) was determined by the colorimetric method according to the development of color in the diazotization reaction of sulfonamide nitrite, which is part of the Griess reagent.

To obtain data on the state of microcirculation in the placenta on the 21st day of pregnancy under anesthesia at 4 points, the microcirculation level was measured at a distance of 1 mm from the edge of the placental disc. To obtain data on the state of microcirculation in the placenta, Biopacsystems equipment was used: MP100 polygraph with laser Doppler flowmetry module (LDF) LDF100C and invasive needle probe TSD144, which was mounted directly on the projection of the placental disc. The LDF results were recorded and processed using the AcqKnowledge software version 3.8.1. Microcirculation values were expressed in perfusion units (PUn)[21,26,27].

The biochemical markers of endothelial dysfunction were indicators of the concentration of stable nitric oxide metabolites (Total NOx). The level of NO metabolites (i.e., the total concentration of nitrates and nitrites, NOx) was determined by the colorimetric method according to the development of color in the diazotization reaction of sulfonamide nitrite, which is part of the Griess reagent.

Descriptive statistics were applied to all data: the data were analyzed for normal distribution. The type of distribution was determined by the Shapiro-Wilk criterion. In the case of a normal distribution, the mean value (M) and the standard error of the mean (m) were calculated. When analyzing intergroup differences, the Student t-test or Mann-Whitney U-test was used, depending on the type of distribution. A values of $p<0.05$ was considered significant.

Results and Discussion

After administration of L-NAME, a significant increase in blood pressure occurred in pregnant rats: systolic blood pressure (SBP) was 194.8±7.88 mmHg, disdtolic blood pressure DBP 149.8±4.73 mm Hg, while in intact animals; systolic and diastolic blood pressure values were 132.3±3.46 and 92.40±3.87 mm Hg, respectively. In pregnant rats treated with L-NAME, the coefficient of endothelial dysfunction increased from 1.20±0.07 to 3.17±0.22, and the rate of microcirculation in the placenta decreased from 465.9±28.79 PUn to 211.8±6.03 PUn ($p<0.05$).

Administration of peptide (P-αB) (50 μg/kg), mimiking the α-helix B of erythropoietin, during the 10 to 20 day of pregnancy, there was a significant ($p<0.05$) decrease in systolic and diastolic blood pressure to 142.8±1.98 and 90.40±5.21 mm Hg, compared with L-NAME pregnant rats (Table 1). Meanwhile, the coefficient of endothelial dysfunction decreased to 2.0±0.06, and the microcirculation index increased to 343.2 ± 5.98 PUn ($p <0.05$).

Table 1. The effect of innovative peptides that mimic the α-helix B of erythropoietin on blood pressure, CED and microcirculation in the placenta with ADMA-like preeclampsia

	SBP, mm hg	DBP, mm hg	CED, cond. un.	Microcirculation, PUn
Control saline	132.3±3.46*	92,4±3,87*	1.20 ± 0.07*	465.9±28.79*
L-NAME	194.8 ± 7.88#	149.8 ± 4.73#	3.17 ± 0.22#	211.8±6.03#
P-αB (50 μg/kg)	142.8±1.98*#	90.4±5.21*	2.0±0.06*#	343.2±5.98*#
P-αB1 (50μg/kg)	143.1±5.18*	100.6±3.80*	1.80±0.15*#	378.1±9.45*#
P-αB2 (50μg/kg)	172.8±5.06*#	135.2±3.54*#	2.25±0.16*#	351.2±10.04*#

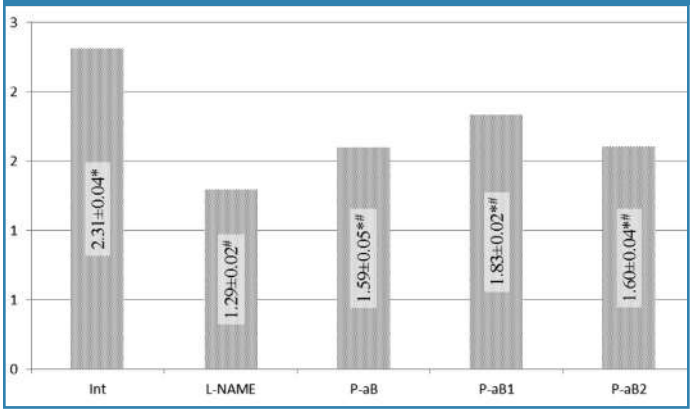
SBP, DBP - systolic and diastolic blood pressure (mmHg) CED - coefficient of endothelial dysfunction (cond. un.); PUn - perfusion units; #p<0.05 compared with a group of intact animals; *p<0.05 compared with the L-NAME group.

Administration of the studied innovative peptide P-αB1, that mimics the α-helix B of erythropoietin, presented a greatest endothelioprotective effect. The coefficient of endothelial dysfunction decreased to 1.80±0.15, and microcirculation rose to 378.1±9.45 PUn, however the values did not reach basal levels (Table 1). It should be noted that peptides P-αB1 administration reduced blood pressure values to those control animals.

Administration of the peptide P-αB2, also reverted, in less extend, the hemodynamic parameters in experimental preeclampsia induced by L-NAME (Table 1).

The study of the NO-synthesizing function of the endothelium was carried out based on the determination of nitrite - NOx ions in blood plasma. During experimental preeclampsia there was a significant decrease in the content of final plasma NOx metabolites (Figure 1). Intraperitoneally administration of P-αB during the day 10 to 20 of pregnancy, reverted partially plasma NOx metabolites levles ($p<0.05$) to 1.59±0.05 μmol/dL. In addition, this eefect was also observed after the administration of P-αB1 (1.83±0.02 μmol/dL) and P-αB2.

Figure 1. The effect of innovative peptides that mimic the erythropoietin α-helix B on the content of final NOx metabolites in blood plasma with ADMA-like pre-eclampsia #p<0.05 in comparison with a group of intact animals; *p<0.05 in comparison with the L-NAME group.



In response to placental ischemia, a large number of humoral factors are released that provoke the development of endothelial dysfunction[28,30,33]. From the foregoing, it was logical to assume that pharmacological agents with anti-ischemic and cytoprotective effects can indirectly reduce endothelial dysfunction with preeclampsia. However, it should be noted that preeclampsia often develops and is more severe in women with previous endothelial dysfunction. Therefore, for our experiment, we chose an ADMA-like model, which also has an ischemic component[31]. Activation of the heterodimeric receptor to erythropoietin increases tissue resistance to ischemia. This leads to a decrease in the formation of humoral factors causing endothelial dysfunction.

Conclusion

Administration of short-chain derivatives of erythropoietin simulating its α-helix B in rats with experimental preeclampsia induced by NO synthesis inhibition, showed a revertion in the parameters evaluated, being P-αB1 with the amino acid sequence: RGDQEQLERALNSS the most promising. The results suggest that is required further research on the search for drugs for the treatment and prevention of preeclampsia among erythropoietin derivatives.

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