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Experimental Study of Correction of Endothelial Dysfunction Solutions Ultra-Low Doses of Antibodies.

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ABSTRACT

Modeling deficit of nitric oxide L-NAME administration to rats resulted in the development of hypertension and endothelial dysfunction. Under this model expressed endothelium and cardioprotective properties of ultra-low doses of antibodies to the endothelial NO- synthase, VEGF and c- terminal fragment of the AT1 angiotensin II have been identified. The results of this study allow us to recommend the use of ultra-low doses of antibodies to endothelial NO- synthase, VEGF and c- terminal fragment of the AT1 angiotensin II receptor for the prevention and correction of endothelial dysfunction as the main pathogenetic link cardiovascular disease.

Keywords: VEGF, angiotensin II, Impaza, endothelial dysfunction, nitric oxide, L-NAME

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Introduction

The main reason of hypertension is endothelial dysfunction, especially, violation of the relaxation properties of the endothelium [8, 10].

Vascular endothelial growth factor (VEGF) is a key mediator of angiogenic. Released from endothelial nitric oxide (NO) is a regulator of endothelial function and also the mediator of angiogenesis. NO release from the endothelium increased after the introduction of VEGF [6, 7].

Today mechanism of action and properties endotelioprotektive ultralow doses of antibodies to the endothelial NO- synthase is known, [5], which is important for the prevention and treatment of vascular complications.

Given the results of experimental studies of the phenomenon of modifying action of antibodies in ultralow doses [5, 9], we can assume that potentiated antibodies to endothelial growth factor VEGF and will possess antiischemic endotelioprotektive properties due to the activation of the principle of "feedback" generation VEGF. The purpose of this study was to examine endothelium- and cardio - protective effects of ultra-low doses of antibodies to VEGF, C-terminal fragment of AT1 angiotensin II receptor and impaza as a drug comparison, model L-NAME- induced deficiency of nitric oxide.

METHODS

Experiments were performed on 70 adult male Wistar rats weighing 250-300 g animals and were divided into 6 groups of 10 animals in the group. Group # 1 intact animals; Group # 2 with the introduction of distilled water 9 ml/kg/day . (daily dose of 4.5 ml/kg two times a day for 28 days, 30 minutes after administration of L-NAME); Group # 3 modeling deficit of nitric oxide by intraperitoneal injection of a solution of N- nitro -L- arginin methyl ester (L-NAME) at a dose of 12.5 mg/kg for 28 days. Group 4 modeling deficit of nitric oxide (dose and route of administration similar to L-NAME group # 3), and simultaneously for 28 days with distilled water administration (dose and route of administration, a group similar to # 2.). Group # 5,6,7 with modeling deficiency of nitric oxide (dose and method of administration L-NAME group similar to # 3) and simultaneously within 28 days of the introduction of ultra-low doses of antibodies to VEGF (group 5), ultra-low doses of antibodies to c- terminal fragment of the AT1 angiotensin receptor II (group 6) and impaza (group 7). Ultra-low doses of antibodies to VEGF and ultra-low doses of antibodies to the C-terminal fragment of AT1 angiotensin II, produced by OOO "NPF" Materia Medica Holding", Russia); impaza (ultra-low doses of antibodies to endothelial NO- synthase, production OOO "NPF" Materia Medica Holding", Russia) was administered intraperitoneally at the rate of 9 ml/kg/day. (daily dose of 4.5 ml/kg two times a day for 28 days, 30 minutes after administration of L-NAME). Group Model NO deficiency is a control. Group # 2, 4 - additional control group to assess the severity of the effects of placebo and nocebo.

On day 29 of the experiment anesthetized (chloral hydrate 300 mg/kg) on the first stage of the study catheter was inserted into the left carotid artery were recorded and the systolic and diastolic blood pressure (BP), heart rate. At the second stage of the study were

administered the needle into the left ventricular cavity through the apex of the heart, for the registration of a maximum speed of contraction and relaxation. Measurements were made and processed by the sensor TSD104A and hardware-software complex MP100, production Biopac System, Inc., USA.

In addition to measuring blood pressure (BP) performed a series of functional tests with subsequent evaluation of parameter changes in systolic and diastolic blood pressure (SBP and DBP): endothelium -dependent vasodilation in response to an intravenous solution of acetylcholine (AH) at a dose of 40 mg/kg at the rate of 0,1 ml per 100 g body weight of the animal, as well as the endothelium independent vasodilation in response to intravenous injection of a solution of sodium nitroprusside (NP) in a dose of 30 mg/kg of 0.1 ml per 100 g body weight of the animal [1, 2].

Endothelial dysfunction in experimental animals was assessed by the estimated coefficient of endothelial dysfunction (CED) by the formula = CED SAD^{NP}/SAD^{AH} where SAD^{NP} - area of the triangle above the recovery curve of blood pressure , and lower leg of the points are the point of maximum decrease in blood pressure and blood pressure exit point on the plateau during the functional test with the introduction of nitroprusside , SAD^{AH} - area of the triangle above the recovery curve AD in the test of acetylcholine , and for the smaller of the other two sides are taking the difference between the end point of cardiac bradikadia component and recovery point BP. This indicator reflects the change in reactivity of the vascular bed in the modeling deficiency of nitric oxide and to evaluate the degree of correction of endothelial dysfunction [3, 4].

To assess the functional capacity of the myocardium in animals was carried out stress tests: a test for adrenergic responsiveness (intravenous epinephrine hydrochloride $1*10^5$ mol/l, at a dose of 0.1 ml / 100 g) [1, 2], the load resistance (clamping of the ascending aorta at 30 c).

For statistical evaluation of the data obtained using the methods of parametric statistics. The significance of differences between groups was determined using the Student t-test for dependent samples. Considered reliable differences between the compared values with a significance level of 95% ($p < 0.05$).

The main part

NO- synthase blockade with L-NAME administration caused the development of severe hypertension. In groups of animals treated with ultra-low doses of antibodies to VEGF, ultralow doses of antibodies to the c- terminal fragment of the AT1 angiotensin receptor II, impaza and distilled water showed no decline in the initial values of blood pressure (Table 1).

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Table 1: BP readings and CED correction in the simulation and L-NAME - deficit -induced NO (M ± m, n = 10).

Group	BP, mm Hg		HR, beats/min.	CED
	systolic	diastolic		
Intact	139,2 ±5,4	104,2 ± 4,7	340±13	1,2 ± 0,1
Distilled water	137,7 ±3,7^{+i}	101,9 ± 4,3^{+i}	373±18	1,1±0,1^{+i}
L-NAME (12,5 mg/kg) for 28 days	204,8 ± 10**	164,2 ± 5,9**	371±17*	3,5 ± 0,5**
L-NAME + distilled water	204,0 ± 8,9**	163,8 ± 4,8**	381±15*	3,5 ± 0,5**
L-NAME + ultra-low doses of antibodies to VEGF	200,6±8,2**	162,8±6,1**	375±10*	1,7 ± 0,1^{+}
L-NAME + ultra-low doses of antibodies to the C-terminal fragment of AT1 angiotensin receptor II	200.5 ± 9.2**	164.4 ± 6.9**	394±10**	1.5 ± 0.2^{+}
L-NAME + Impaza	201,1 ± 6,7**	163,1 ± 6**	377±8*	1,8 ± 0,2^{+}

Note: p < 0.05 compared with: * intact animals; + control; i impaza. p < 0.001 compared to: ** intact animals; + + Control; ii impaza.

With administration of L-NAME in the control group occurred endothelium to increase the ratio of endothelium -dependent vasodilation, thus CED was 3,5 ± 0,5, while the intact rats, it was - 1,2 ± 0,1. In the group of animals treated with ultra-low doses of antibodies to VEGF and ultralow doses of antibodies to the C-terminal fragment of angiotensin AT1 receptor II, CED was - 1,7 ± 0,1 and 1.5 ± 0.2 respectively. Impaza also provided endotelioprotektive action of CED in this group was - 1,8 ± 0,2.

Table 2: Effect of antibody dose ultra VEGF, a C-terminal fragment of the receptor AT1 angiotensin II, impaza and functionality of the myocardium during loading tests on background modeling L-NAME-induced endothelial dysfunction (M ± m, n = 10).

Group	Adrenergic responsiveness, mm Hg	Exhaustion of myocardial reserve, %
Intact	199,8±9,9	93,9%
Distilled water	199,2±8,3^{++}	89,6%$^{++i}$
L-NAME (12,5 mg/kg) for 28 days	281,1±6,5**	67,5**
L-NAME + distilled water	280,2±6,9**	68%**
L-NAME + ultra-low doses of antibodies to VEGF	243,9±9,9^{+}	97,1 %$^{++}$
L-NAME + ultra-low doses of antibodies to the C-terminal fragment of AT1 angiotensin receptor II	237.9±9.1^{+}	102.1^{++}
L-NAME + Impaza	239,4±13,8^{+}	105,6^{++}

Note: p < 0.05 compared with: * intact animals; + control; i impaza. p < 0.001 compared to: ** intact animals; + + Control; ii impaza.

Significant differences between values CED intact animals and animals with the administration of distilled water was not. As well there were no significant differences between the value of CED in animals with disease and correction of this pathology with distilled water.

The results indicate the correction of endothelial dysfunction midget doses of antibodies to VEGF, a C-terminal fragment of AT1 angiotensin II receptor impaza, in the absence of a significant impact on the development of hypertension.

Carrying out the test on adrenergic responsiveness in the control group of animals left ventricular systolic pressure was significantly higher (281,1 ± 6,5 mm Hg.) Than in the intact animals (199,8 ± 9,9 mm Hg.). A solution of ultralow doses of antibodies to VEGF and c- terminal fragment of angiotensin II AT1 receptor, systolic left ventricular pressure decreased to 243,9 ± 9,9 mm Hg. and 237.9 ± 9.1 Impaza also prevented adrenergic responsiveness (Table 2).

In the trial the load resistance index was calculated exhaustion myocardial reserve, equal to the ratio of left ventricular systolic pressure increase by 5 with aortic cross-clamping of the left ventricular systolic to the increase of pressure on the 25 with aortic clamping, expressed as a percentage.

In the group of intact animals, the figure was 93.9% in the control group - 67.5%. The animals treated with ultra-low doses of antibodies to VEGF, this would not only significantly different from the control group, but higher than the values in the intact group 97.1 %.

In animals with the introduction impaza, this figure also exceeds the value of intact groups and groups with leading ultra-low doses of antibodies to VEGF and c- terminal fragment of the AT1 angiotensin receptor II. However, it should be noted that this figure does not significantly differ from the group administered with ultra-low doses of antibodies to VEGF and c- terminal fragment of the AT1 angiotensin receptor II, was 105.6 %.

Left ventricular systolic pressure in the test on adrenergic responsiveness and exhaustion indicator of myocardial reserve in the group with the introduction of distilled water were at the level of the group values intact animals and did not differ significantly from the values in the group. Introduction of distilled water on the background of L-NAME, during this sample also has no effect on left ventricular systolic pressure figures and exhaustion indicator of myocardial reserve.

Thus , the study of the functional state of the myocardium during stress tests revealed a distinct cardioprotective effects of ultra-low doses of antibodies to VEGF, to the C-terminal fragment of AT1 angiotensin II receptor and the reference drug Impaza expressed in reducing adrenergic responsiveness, left ventricular systolic pressure drop in the test load resistance compared with the control animals. In the groups with administration of additional distilled water is not detected cardioprotective effects.

CONCLUSION

Summarizing these experimental data, we can conclude that the solution of ultra-low doses of antibodies to VEGF, a solution of ultra-low doses of antibodies to the C-terminal fragment of AT1 receptor for angiotensin II and the reference drug Impaza equally possess endothelial and cardioprotective properties. In groups of animals with the introduction of distilled water is not revealed its impact on the performance of the functional state of the

cardiovascular system, which may indicate unexpressed placebo effects and nocebo in this series of experiments in rats.

CONCLUSIONS

The results of this study allow us to recommend the use of ultra-low doses of antibody solution to VEGF, a solution of ultra-low doses of antibodies to the C-terminal fragment of AT1 angiotensin II receptor for the prevention and correction of endothelial dysfunction as the main pathogenetic link cardiovascular disease. It is promising to study these solutions in combination with classical antihypertensive drugs.

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