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Assessment of Correction of the Endothelial Dysfunction by the Very-Low-Doses of Antibodies to VEGF by the Example of the 28-Days Model of the L-Name Deficiency in the Nitrogen Oxide.

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ABSTRACT

The simulation of deficiency in the nitrogen oxide by means of administration of L-NAME (intraperitoneal introduction of N-nitro-L-arginine methyl ether (L-NAME) in the dose of 12,5 mg/kg) to the rats resulted in the development of arterial hypertension and endothelial dysfunction. By the example of this model there have been established the intensive endothelial and cardio protective properties of the very-low-doses of antibodies to VEGF.

Keywords: VEGF, impaza, endothelium, nitride oxide, L-NAME.

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INTRODUCTION

The antibodies in the very-low doses (potentiating antibodies) to the endogenous regulator (antigen) do not inhibit the molecule activity; on the contrary, modify it [1, 2]. The vascular endothelium growth factor (VEGF) is the key mediator of angiogenesis. The release of NO from the endothelium is increased after introduction of VEGF [3]. Taking into account that one of the directions in the development of methods of the arterial hypertension pharmacological correction is searching for the directed pharmacodynamic impact on the correction of endothelial dysfunction [4, 5], the study of the very-low doses of antibodies to VEGF is a promising area.

The objective of the present study is the analysis of endothelial and cardio protective effects of the of the very-low doses of antibodies to VEGF and impaza as a comparator drug by the example of the 28-days model of the L-NAME induced deficiency in the nitrogen oxide.

PROCEDURE

The experiments were performed on the 60 mature Wistar male rats. The animals were divided into 6 groups, 10 animals per a group. The group I intact animals; group II with administration of the distilled water 9 ml/kg/days (daily in the dose of 4,5 ml/kg twice a day during 28 days 30 minutes after the administration of L-NAME); Group III with simulation of the nitrogen oxide deficiency by intraperitoneal injection of the solution of N-nitro-L-arginine methyl ether (L-NAME) in the dose of 12,5 mg/kg during 28 days. Group IV with simulation of the nitrogen oxide deficiency (doses and route of administration of L-NAME similar to the group III) and the concurrent injection of the distilled water during 28 days (doses and route of administration similar to the group II). Group V, VI with simulation of the nitrogen oxide deficiency (doses and route of administration of L-NAME similar to the group №3) and concurrent administration of the very-low doses of antibodies to VEGF (group V) and impaza (group VI) during 28 days. The very-low doses of antibodies to VEGF (produced by SPC "Materia Medica Holding" LLC, Russia) and impaza (very-low doses of antibodies to the endothelial NO-synthase (produced by SPC "Materia Medica Holding" LLC, Russia) were administered intragastrically at 9 ml/kg/day (daily in the dose of 4,5 ml/kg twice a day during 28 days 30 minutes after the administration of L-NAME). The group with the NO deficiency was the control one. Groups II, IV – additional control groups for evaluation of intensity of the placebo and nocebo effects.

On the 29th day of experiment under anesthesia (chloral hydrate 300 mg/kg) at the first stage a catheter was introduced into the left carotid artery and the systolic and diastolic blood pressure (BP) as well as the heart rate were measured. At the second stage of the experiment a needle was introduced in the left ventricular cavity through the apex of the heart for measurement of the maximal contraction and relaxation rate. The measurements were carried out and processed with the use of the TSD104A sensor and the hardware and software complex MP100 (produced by Biopac System, Inc., USA).

Along with the blood pressure (BP) measurement a series of the functional tests with further evaluation of changes of the systolic and diastolic blood pressure (SBP and DBP) parameters was performed: endothelium-dependent vasodilation in response to the intravenous injection of the acetylcholine (AC) solution in the dose of 40 μg/kg at the rate of 0,1 ml per 100 g of the animal body weight as well as endothelium-independent vasodilation in response to the intravenous injection of the sodium nitroprusside (NP) solution in the dose of 30 μg/kg at the rate of 0,1 ml per 100 g of the animal body weight.

The degree of endothelial dysfunction in the experimental animals was assessed on the basis of the endothelial dysfunction rated coefficient (KED) according to the formula: KED = $САД_{НП}$/ $САД_{АХ}$ where $САД_{НП}$ – the area of the triangle above the BP build-up curve whereas the points of the lesser leg are represented by the point of the maximum drop in the BP and the point of the BP reaching the plateau level by performance of the functional test with administration of nitroprusside, $САД_{АХ}$ – the area of the triangle above the BP build-up curve by performing the test with acetylcholine whereas the lesser leg is taken as the difference between the point of ending of the bradycardic component and the point of the BP build-up. This indicator reflects the change in the vasculature activity by simulation of the nitrogen oxide deficiency and allows evaluating the degree of the endothelial dysfunction correction [6].

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For assessment of the functional capabilities of myocardium in the animals the stress tests for adrenoreactivity (intravenous single-stage administration of the solution of adrenaline hydrochloride $1 \cdot 10^{-5}$Mole/L at 0,1 ml per 100 g) and resistance loading (compression of the ascending aorta for 30 seconds) were performed [7].

For the statistical evaluation of the data obtained the parametric statistics methods were used. The significance of differences was determined with the use of the Student's t-test for dependent samples. The differences between the comparable values with the significance level of 95% ($p<0,05$) were considered to be significant.

MAIN PART

The blockage of NO-synthase by administration of L-NAME resulted in the development of the pronounced arterial hypertension. In the groups of animals receiving the very-low doses of antibodies to VEGF, impaza and distilled water no decrease in the reference BP values was observed (Table 1).

Against the administration of L-NAME in the control group the increase in the ratio of the endothelium-independent to the endothelium-dependent vasodilation was observed whereas the KED made 3,5±0,5, in the intact rats this coefficient was equal to 1,2±0,1. In the groups of animals receiving the very-low doses of antibodies to VEGF the KED made 1,7± 0,1. Impaza also had an endothelium-protective effect, the KED in this group made 1,8±0,2.

The results obtained confirm the correction of the endothelial dysfunction by the very-low doses of antibodies to VEGF and impaza without exerting a significant impact on the development of arterial hypertension [8, 9, 10].

Table 1: Values of the BP and KED by simulation and correction of the L-NAME – induced NO deficiency (M±m, n=10)

Group	BP, mm Hg		Heart rate, bpm	KED
	systolic	diastolic		
Intact	139,2 ±5,4	104,2 ± 4,7	340±13	1,2 ± 0,1
Distilled water	137,7 ±3,7$^{+и}$	101,9 ± 4,3$^{+и}$	373±18	1,1±0,1$^{+и}$
L-NAME (12,5 mg/kg) 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 + very-low doses of antibodies to VEGF	200,6±8,2**	162,8±6,1**	375±10*	1,7 ± 0,1^{+}
L-NAME + impaza	201,1 ± 6,7**	163,1 ± 6**	377±8*	1,8 ± 0,2^{+}

Remark: $p<0,05$ as compared to: * intact animals; + control, and impaza. $p<0,001$ as compared to: ** intact animals; ++ control, and impaza.

By performing the adrenoreactivity test in the control group the systolic left ventricular pressure appeared to be significantly higher (281,1±6,5 mm Hg) than in the group of intact animals (199,8±9,9 mm Hg). The solution of the very-low doses pf antibodies to VEGF reduced the systolic left ventricular pressure to 243,9±9,9 mm Hg. Impaza also prevented the adrenoreactivity (Table 2).

Table 2: Impact of the very-low doses of antibodies to VEGF and impaza on the functional capabilities of myocardium by performance of the stress tests against the simulation of the L-NAME-induced endothelial dysfunction (M±m, n=10)

Group	Adrenoreactivity, mm Hg	Exhaustion of the myocardial reserve
Intact	199,8±9,9	93,9%
Distilled water	199,2±8,3^{++}	89,6%$^{++и}$
L-NAME (12,5 mg/kg) 28 days	281,1±6,5**	67,5**
L-NAME+distilled water	280,2±6,9**	68%**
L-NAME + very-low doses of antibodies to VEGF	243,9±9,9^{+}	97,1 %$^{++}$
L-NAME + Impaza	239,4±13,8^{+}	105,6^{++}

Remark: $p<0,05$ as compared to: * intact animals; + control and impaza. $p<0,001$ as compared to: ** intact animals; ++ control and impaza.

During the resistance loading test the value of exhaustion of the myocardial reserve was calculated which equals to the ratio of the build-up of the systolic left ventricular pressure as of the 5th second of the aorta compression to that as of the 25th second expressed as a percentage. In the group of intact animals this value made 93,9%, in the control group – 67,5%, in the animals receiving the very-low doses of antibodies to VEGF and impaza - 97,1% and 105,6 %, respectively.

Thus, the results of analysis of the functional status of myocardium during performance of the stress tests revealed the definite cardio protective effect of the very-low doses of antibodies to VEGF and the comparator drug impaza expressed in the decreased adrenoreactivity, drop in the systolic left ventricular pressure by performance of the resistance loading test as compared to the control animals. In the groups with additional administration of the distilled water no cardio protective effect was observed.

SUMMARY

By summarizing the experimental data the following conclusion may be drawn: the solution of the very-low doses of antibodies to VEGF and the comparator drug impaza feature endothelial and cardio protective properties.

In the groups of animals receiving the distilled water no effect on the functional status of the cardiovascular system was observed which may point to the poor pronouncement of the placebo and nocebo effects in these series of experiments in the rats.

CONCLUSIONS

The results of the study conducted allow recommending the solution of the very-low doses of antibodies to VEGF for prevention and correction of the endothelial dysfunction as the main pathogenic link of the cardiovascular diseases. The investigation of the specified drug in combination with the main antihypertensive agents is very promising.

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