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Endothelial and Cardio Protective Effects of Tetrahydrobiopterin, L-Norvaline, L-Arginine and their Combinations by Simulation of Hyperhomocysteine Induced Endothelial Dysfunction.

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ABSTRACT

The analysis of the endothelial and cardio protective effects of Tetrahydrobiopterin, L-norvaline, L-arginine and their combinations by simulation of Hyperhomocysteine induced endothelial dysfunction has been performed. Implementation of the combined approach to correction of the pathological conditions related to or followed by endothelial dysfunction allows achieving the statistically significant impact on the most indicators of performance of the vascular endothelium and the cardiovascular system in whole.

Keywords: endothelial dysfunction, Tetrahydrobiopterin, L-arginine, L-norvaline, Hyperhomocysteinemia.

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INTRODUCTION

B 1969 Kilmer S. McCully assumed the presence of correlation between the increased level of homocysteine in the blood serum and arterial diseases, by studying a rare genetic disease expressed in Homocystinuria (high concentration of homocysteine in urine). Without treatment such children usually died at an early age of the myocardial infarction (IM) or cerebral strokes and by autopsy McCully found out that their vessels were damaged and thickened like changes in the elderly patients with cardiovascular diseases [1, 2]. The negative effects of Homocysteine include its direct damaging effect on the arterial endothelium with development of the endothelial dysfunction, ability to stimulate thrombi formation by activating the coagulation system and thrombocyte aggregation, increase in the mitotic activity of the vascular smooth muscle cells [3].

As of today it is known that there is a possibility to correct hyperhomocysteinemia by means of additional administration of the folic acid [4]. Taking into account the role of Hyperhomocysteinemia in the development of endothelial dysfunction and recent appearance of the concepts of endogenous inhibition and separation of NO-synthase from the substrate in the present study the possibility of the pharmacologic correction of Hyperhomocysteine induced endothelial dysfunction with the of Tetrahydrobiopterin, L-norvaline, L-arginine and their combinations has been considered [5, 6].

PROCEDURE

The experiments were performed on the white male Wistar rats weighing 200-250 g. The solution for intragastric administration of methionine in the dose of 3 g/kg was prepared ex tempore with the use of the polysorbate TWEEN-80 and 1% starch solution.

The animals were divided into groups (n=10): 1 – control; 1 – daily, once a day, intragastric administration of methionine in the dose of 3 g/kg during 7 days; 3 – methionine 3 g/kg + Tetrahydrobiopterin (BH4) 10 mg/kg ; 4 – methionine 3 g/kg + L-arginine 200 mg/kg; 5 – methionine 3 g/kg + L-norvaline 10 мг/кг; 6 – methionine + BH4 (10 mg/kg) + L-arginine (200 mg/kg); 7 – methionine + L-arginine (200 mg/kg)+ L-norvaline (10 mg/kg).

On the 8th day after the beginning of the experiment under anesthesia (chloral hydrate 300 mg/kg) the catheter was inserted into the left carotid artery in order to record the arterial blood pressure values (ABP), bolus administration of the pharmacological agents was performed through the femoral vein. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured continuously with the use of the hardware and software complex "Biopac" (USA). Then a series of functional tests was performed: 1. Intravenous administration of the acetylcholine solution (ACH) in the dose of 40 μg/kg. 2. Intravenous administration of sodium nitroprusside solution (NP) in the dose of 30 μg/kg [7, 8, 9].

The degree of endothelial dysfunction in the experimental animals as well as degree of its correction by the investigated preparations were evaluated according to the rated endothelial dysfunction coefficient (CED) [7, 8, 10].

In order to assess the functional capabilities of myocardium of animals under controlled respiration the left ventricle cavity was catheterized and the loading tests were performed in the following sequence: 1. Test for adrenoreactivity (intravenous single-stage administration of the solution of adrenaline hydrochloride 1.10-5 mole/L at 0,1 ml per 100 g) [11]. By performing this test the evaluation of the maximum build-up of the left-ventricular pressure (LVP) in response to administration of adrenaline was carried out. 2. Resistance test (compression of the ascending aorta for 30 seconds [11]. After performance of this test the indicator of the myocardial reserve depletion (expressed as a percentage) was calculated which equals to the ratio between the LVP build-up as of the 5th second of the aorta compression and LVP build-up as of the 25 second of the aorta compression.

The biochemical markers of endothelial dysfunction were the values of concentration of the stable metabolites of the nitrogen oxide (NOx), concentration of homocysteine (HCy) in the blood serum of the experimental animals [12].

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FINDINGS OF THE STUDY

The impact of the investigated substances on the reference values of the blood pressure in the anesthetized rats with simulation of the Hyperhomocysteine induced endothelial dysfunction is presented in the Table 1. It was found out that the investigated preparations did not affect the hemodynamics indicators and the SBP and DBP values did not significantly differ from those in the control group in all experiment series (Table 1, SBP, DBP).

Processing of the experimental data obtained allowed establishing that BH4 in the dose of 10 mg/kg, L-norvaline in the dose of 10 mg/kg and L-arginine in the dose of 200 mg/kg as a mono-therapy prevented increase in the endothelial dysfunction coefficient (Table 1, CED). The combined administration of Tetrahydrobiopterin and L-arginine as well as L-norvaline and L-arginine resulted in the maximum decrease in the endothelial dysfunction coefficient the values of which in the mentioned groups approached to those in the control series of experiments (Table 1, CED).

Table 1: Impact of Tetrahydrobiopterin, L-arginine, L-norvaline and their combinations on the functional and biochemical indicators by simulation of endothelial dysfunction

Group of animals	SBP, mm Hg	DBP, mm Hg	CED, relative units	Adrenoreactivity, mm Hg	Resistance test, %	HCy, µmole/L	NOx, µmole
Control 10%TWEEN80 1ml/kg	129,2 ±4,3	82,4 ± 5,9	0,9±0,2	189,7±9,1	85,4±3,1	8,6±0,4	121,2±10,4
Methionine 3 g/kg once a day for 7 days	118,9±10,1	76,6±7,2	3,3±0,3*	238,1±9,2*	69,8±2,9*	53,5±6,2**	72,9±4,1*
Methionine + BH4 10 mg/kg	126,2±8,9	75,9±3,1	1,6±0,2**	204,9±5,1**	79,5±3,1**	27,8±3,1**	102,9±9,7**
Methionine + L-Norvaline 10 mg/kg	129,4±2,8	72,6±5,4	1,4±0,1**	192,4±5,7**	84,7±4,0**	43,2±4,7	91,3±6,0**
Methionine + L-arginine 200 mg/kg	121,2±10,3	83,7±6,3	1,7±0,2**	193,3±6,3**	83,5±2,7**	44,2±3,9	103,1±7,2**
Methionine + BH4 + L-arginine	119,3±4,5	68,9±8,9	1,1±0,1**	192,9±5,9**	84,1±3,8**	18,2±2,9**	118,9±6,4**
Methionine + L-Norvaline + L-arginine	122,6±7,5	74,3±4,1	1,2±0,2**	190,9±5,7**	88,7±5,1**	20,1±5,7**	116,7±9,8**

Remarks: SBP, DBP – systolic and diastolic blood pressure; CED – endothelial dysfunction coefficient.

Adrenoreactivity – blood pressure in the cavity of the left ventricular of the heart in response to the intravenous administration of adrenaline; NOx – nitrite ions concentration; HCy – homocysteine concentration; *- $p < 0,05$ as compared to the group of intact animals; ^{y}- $p < 0,05$ as compared to the control group of animals.

By performing the test for adrenoreactivity there has been detected the statistically significant decrease in the maximal LVP in all series of experiments with L-norvaline, L-arginine, Tetrahydrobiopterin and their combinations. The minimal value of the LVP detected by the administration of the combination of L-norvaline (10 mg/kg) and L-arginine (200 mg/kg) (Table 1, Adrenoreactivity).

By performing the resistance test there have been observed the results comparable to those obtained by performing the test for adrenoreactivity (Table 1, Resistance loading). The most expressed prevention if the myocardial reserve depletion was detected in the group of animals receiving the combination of L-arginine with L-norvaline (88,7±5,1%) where this indicator appeared to be higher than in the control group of animals (85,4±3,1%)

The administration of Tetrahydrobiopterin in the dose of 10 mg/kg as well as combination of Tetrahydrobiopterin with L-arginine, L-arginine with L-norvaline resulted in the statistically significant decrease in the homocysteine concentration in the relevant groups of experimental animals (Table 1, HCy). The homocysteine concentration made, correspondingly: 27,8±3,1 μMole/L, 18,2±2,9 μMole/L and 20,1±5,7 μMole/L. However, the homocysteine concentration in the blood serum of the animals in the mentioned groups remained 2,5 times higher than such values of the control group of animals (8,6±0,4 μMole/L).

By the analysis of concentration of stable metabolites of the nitrogen oxide it was found out that all the preparations and their combinations exercise a statistically significant impact on the increase in concentration of the nitrogen oxide stable metabolites (Table 1, NOx).

The concentration of the stable metabolites of the nitrogen oxides (NOx) under influence of the combinations of L-arginine with BH4 and L-norvaline increased and did statistically significant differ from the indicators in the control group of animals.

Thus, the results of our studies have proved that the presence of BH4 is necessary but not sufficient for restoration of the nitrogen oxide metabolism and activity of the endothelial NO-synthase (eNOS) [13]. Taking the above into account, the mechanisms of implementation of the obtained effects of L-arginine seem to be due to the fact that L-arginine boosts the NO synthesis in the endothelial cells through the competitive replacement of inhibitors from the connection with the eNOS enzyme [14]. The mechanism of the L-norvaline action is explained by its structural similarity to ornithine that is being one of the metabolic products of the urea cycle. The mediated action of L-norvaline on the arginase activity is related to the inhibition of ornithine transcarbamylase that catalyzes the change from ornithine to citrulline during the urea cycle.

CONCLUSIONS

The implementation of strategies of the pharmacological correction of endothelial dysfunction aimed at prevention of the eNOS disconnection, boosting its activity, impact on the nitrogen oxide metabolism and its bioavailability in particular allow achieving the positive dynamics by analysis of the vascular endothelium performance and cardio protective effect of preparations. The implementation of the combined approach to correction of the pathological conditions related to or followed by endothelial dysfunction allows achieving the statistically significant impact on the most indicators of performance of the vascular endothelium and the cardiovascular system in whole.

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