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ENDOTHELIO- AND CARDIOPROTECTIVE EFFECTS OF VITAMIN B6 AND FOLIC ACID IN MODELLING METHIONINE-INDUCED HYPERHOMOCYSTEINEMIA

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Abstract. The endotelio- and cardioprotective effects of vitamin B_6 (2 mg/kg) and folic acid (0.2 mg/kg) upon modeling of methionine-induced hyperhomocysteinemia via methionine intragastric administration at a dose of 3 g/kg were studied. It was shown that the combined use of vitamin B_6 and folic acid allows on the background of a significant reduction in homocysteine concentrations normalizing the endothelial dysfunction coefficient and the parameters of maximum left ventricular pressure in response to intravenous administration of adrenaline.
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Keywords: endothelial dysfunction, folic acid, vitamin B_6, hyperhomocysteinemia.

Introduction. By now, a number of factors of different nature has been determined [1], contributing to the development and progression of cardiovascular disease such as dyslipidemia, hypertension, overweight, smoking, physical inactivity, and diabetes. In addition, a group of so-called "new" risks can be distinguished, which primarily includes an increase in homocysteine levels in the blood. More than 80 clinical and epidemiological studies have confirmed that hyperhomocysteinemia is a significant, independent risk factors for early development and rapid progression of atherosclerosis, and thrombosis of the coronary, cerebral and peripheral arteries, and may be a predictor of death [2]. The obtained reliable evidences have served as the basis for creating homocysteic theory of pathogenesis of atherosclerosis development [3]. The first report on the role of homocysteine as a possible risk factor for cardio-vascular diseases goes back to 1964. S.H.Mudd, T.Gerritsen, H.A.Waismann et al. demonstrated that high concentration of homocysteine in the blood and, consequently, in urine, resulting in homocystinuria is a consequence of deficiency of the cystathionine-beta-synthase enzyme.

Subject to the above, the objective of the study was to investigate the endotelio- and cardioprotective effects of vitamin B_6 and folic acid upon modeling of methionine-induced hyperhomocysteinemia.

Materials and methods. Experiments were conducted on white male Wistar rats weighing 200-250 g. Hyperhomocysteinemia was simulated by intragastrical administration of methionine (JSC "Synthesis") at a dose of 3 g/kg/day for 7 days. Solution for intragastric administration of methionine was prepared ex tempore with polysorbate TWEEN-80 and 1% starch solution. Data obtained by intragastric administration of an equivalent amount of polysorbate of 10% TWEEN-80 solution were used as control. Folic acid (Valenta Pharmaceuticals, OJSC) was administered intragastrically at a dose of 0.2 mg/kg/day for 7 days. Vitamin B_6 (Veropharm)

was administered intraperitoneally at a dose of 2 mg/kg/day for 7 days.

Animals were divided into groups (n=10): I – daily, once a day, for 7 days, intragastric administration of 10% Tween-80 solution at a dose of 1 ml/kg (control, n=10 animals); II - daily, once a day, intragastric administration of methionine at a dose of 3 g/kg for 7 days (n=10 animals); III - administration of folic acid on the background of methionine (0.2 mg/kg, intraperitoneally) and vitamin B_6 (2 mg/kg, i.p.) once a day for 7 days.

On day 8 of the experiment, a catheter was inserted under anesthetisia (chloral hydrate 300 mg/kg) into the left carotid artery to record blood pressure (BP); bolus administration of pharmacological agents was into the femoral vein. Hemodynamic parameters: systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured continuously with the use of a sensor and the computer program "Biopac". In addition to blood pressure measurements a series of functional tests was carried out in the following sequence: 1. Test for endothelium-dependent vascular relaxation (intravenous solution of acetylcholine (ACh) at a dose of 40 mg/kg at the rate of 0.1 ml per 100 g). 2. Test for endothelium-independent vascular relaxation (intravenous solution of sodium nitroprusside (NP) at a dose of 30 mg/kg at the rate of 0.1 ml per 100 g) [4, 5, 6, 7, 8].

The degree of endothelial dysfunction in experimental animal, as well as the degree of its correction with the studied medications was assessed by the estimated coefficient of endothelial dysfunction (EDC). The coefficient was calculated by the formula: EDC = BPS_{NP}/ BPS_{AC}, where BPS_{NP} is an area of the triangle above the blood pressure recovery curve, where points of the smaller leg are the point of maximum blood pressure drop and the point of BP level egress to the plateau during the functional test with the administration of sodium nitroprusside, BPS_{AC} - is an area of the triangle above the blood pressure recovery curve during the test with acetylcholine, where a smaller leg shall be the difference between the end point of bradycardiac cardiac component and a BP recovery point [6, 9, 10, 11, 12].

The development of hyperhomocysteinemia and its correction with the studied medications were assessed by the content of homocysteine in the blood serum of experimental animals. Homocysteine concentration was measured by immunoturbidimetric method with the use of a set by Pliva-Lachema Diagnostika s.r.o.

To assess the myocardial functionality in animals under controlled respiration, the cavity of the left ventricle was catheterized and stress tests were performed in the following sequence:

1. Test for adrenoreactivity (a one-time intravenous administration of epinephrine hydrochloride solution $1 \cdot 10^{-5}$ mol/L at the rate of 0.1 ml per 100 g) [9, 10, 11]. During this test, the maximum increase in LVP in response to adrenaline administration was assessed.

2. Resistance load (ascending aorta compression for 30 seconds) [1, 3]. After this test, index of myocardial reserve exhaustion was calculated (expressed as a percentage) equal to the ratio of increase in the LVP on the 5th second of compression to the increase in the LVP on the 25th second of compression.

The significance of changes in absolute parameters was determined by the difference method of variation statistics with finding the average values of the shifts (M), the arithmetic mean (±m) and the probability of possible error (p) by using the Student tables. Differences were evaluated as significant at $p<0.05$. Statistical calculations were performed with Microsoft Excel 7.0.

Results

According to the study design, a hyperhomocysteinemia-induced endothelial dysfunction was simulated by daily intragastric administration of methionine at a dose of 3 g/kg for 7 days.

Intragastric administration of the stated dose of methionine resulted in a significant increase in the endothelial dysfunction coefficient up to 3.3 ± 0.3, while the EDC in the control group was 0.9 ± 0.2. Systolic and diastolic blood pressure values remained within physiological limits in all series of the experiments (Table 1).

Simultaneous administration of methionine, vitamin B_6 and folic acid led to a significant reduction in EDC up to 1.7 ± 0.1 (Table 1).

Table 1

Effect of vitamin B_6 and folic acid on functional and biochemical parameters of rats in modeling the endothelial dysfunction by intraperitoneal administration of nitro-L-arginine methyl ester (L-NAME) at a dose of 25 mg/kg (M ±m, n=10)

Groups of animals	SBP, mm Hg	DBP, mm Hg	EDC, c.u.
Control 10% TVIN-80	129.2 ±4.3	82.4 ± 5.9	0.9±0.2
Methionine 3 g/kg	118.9±10.1	76.6±7.2	3.3±0.3*
Methionine + folic acid + vitamin B_6	121.4±5.6	82.6±6.1*	1.7±0.1**

Note: SBP – systolic blood pressure; DBP – diastolic blood pressure; EDC – endothelial dysfunction coefficient; *– at $p<0.05$ as compared to control animals; **– at $p<0.05$ as compared to group receiving Methionine.

The results of the study of homocysteine concentration in the blood serum of experimental animals are shown in Table 2. Intragastric administration of methionine resulted in significant increase in the concentration of homocysteine, while co-administration of vitamin B_6 and folic acid allowed significantly reducing this coefficient and making it closer to the values of the control group (Table 2).

Table 2

Effect of vitamin B_6 and folic acid on homocysteine concentration in the blood serum of experimental animals (M±m, n=10)

Groups of animals	Control	Methionine	Methionine + folic acid + vitamin B_6
Homocysteine concentration (mkmol/l)	8.6±1.4	53.5±8.1*	24.3±4.6**

Note: *- at p<0.05 as compared to control animals; **- at p<0.05 as compared to group receiving Methionine.

During test for adrenoreactivity, the group of animals, which received intragastrically vitamin B6 and folic acid on the background of methionine, showed a decrease in the absolute values of left ventricular pressure, which indicates the prevention of hyperhomocystein-induced increase of adrenoreactivity (Table 3).

During test for resistance load, neither vitamin B6 nor folic acid prevented the drop of contractility in the period from 5 to 25 second of aortic compression. For example, index of myocardial reserve exhaustion on the 25th second of the test was 85.4±3.1% in the control group. The same in the methionine-treated animals was 69.8±3.4%. Results in the group of animals treated with vitamin B6 and folic acid were equal to 72.4±4.1% (Table 3).

Table 3

Effect of vitamin B6 and folic acid on the contractile parameters of the left ventricle of rats during stress testing (M±m, n=10)

Group of animals	drenoreactivity LVP, mm Hg)	ortic compression %)
Control	189.7±9.1	85.4±3.1
Methionine	238.1±3.4*	69.8±3.4*
Methionine + folic acid + vitamin B_6	217.9±4.8**	72.4±4.1*

Note: *- at p<0.05 as compared to control animals; **- at p<0.05 as compared to group receiving Methionine.

Discussion. Possible role of homocysteine (HC) in the development of cardiovascular diseases (CVD) started to be investigated after K. McCully demonstrated in 1969 a predisposition to atherothrombotic events in patients with severe hyperhomocysteinemia (HHC) (HC> 100 μmol/l). The mechanism of development of atherosclerotic vascular lesions with hyperhomocysteinemia remains unclear. Experimental findings suggest that the products of homocysteine autoxidation proceeding with the formation of reactive oxygen species induce the formation of atherosclerotic plaques by damaging endothelium, destroying the integrity of the vascular wall and stimulating the proliferation of medial smooth muscle cells [2, 3]. Homocysteine also impairs the normal NO production by endothelial cells, reduces the bioavailability of NO by decreasing its synthesis. Increased lipid peroxidation with homocysteine involvement leads both to a reduction in NO production by the NO-synthase enzyme, and to NO direct degradation.

The literature provides evidences that the use of vitamin B6 and folic acid can effectively reduce homocysteine levels [2]. In our study, the use of therapeutic doses of vitamin B6 and folic acid allowed normalizing the ratio of endothelium-dependent and endothelium-independent vasodilation with a significant decrease in the endothelial dysfunction coefficient. During stress testing, intragastric administration of vitamin B6 and folic acid on the background of methionine allowed reducing the adrenoreactivity in the experiment with an open heart, however, has not resulted in the prevention of myocardial reserve exhaustion. Positive dynamics of functional performance was accompanied by a significant decrease in the concentration of homocysteine.

These facts confirm the important role of vitamin B6 and folic acid in the implementation of the protective effect in case of hyperhomocysteinemy, however, raise the question of the possibility of their combined application with conventional drugs used to treat cardiovascular disease (ATE inhibitors, AT-I receptor blockers, etc.).

Conclusions.

1. Combined application of vitamin B6 at a dose of 2 mg/kg and folic acid at a dose of 0.2 mg/kg has endothelioprotective effect shown in the model of methionine-induced hyperhomocysteinemia.

2. Combined application of vitamin B6 at a dose of 2 mg/kg and folic acid at a dose of 0.2 mg/kg on the background of simulated methionine-induced hyperhomocysteinemia reduces the maximum pressure in the cavity of the left ventricle during test for adrenoreactivity and has no effect on the myocardial reserve exhaustion during the test for resistance load.

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