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STUDYING DOSE-DEPENDENT ENDOTHELIO- AND CARDIOPROTECTIVE ACTIVITY OF SELECTIVE ARGINASE II INHIBITOR IN HYPERHOMOCYSTEINE-INDUCED ENDOTHELIAL DYSFUNCTION

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Abstract. This paper deals with the study of endothelio- and cardioprotective activity of arginase II selective inhibitor, the substance under the code ZB49-0010C in the model of hyperhomocysteine-induced endothelial dysfunction. The results of the studies prove the presence in the arginase II selective inhibitor, the substance under the code ZB49-0010C, dose-dependent endothelioprotective and cardioprotective activity, which expression increases with the increase in dose and is maximum at a dose of 10 mg/kg.
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Keywords: endothelial dysfunction, a selective inhibitor of arginase II, nitric oxide, methionine, homocysteine.

Nowadays, mortality from diseases of the cardiovascular system is a leader in the developed countries of the world. The main predictor of this group of diseases is endothelial dysfunction, which manifests itself in the impaired relaxation, antiagregation and other properties of the vascular endothelium [1, 2, 3, 4]. The key component of the pathogenesis of endothelial dysfunction (ED) is a deficit of endogenous nitric oxide. Nitric oxide (NO) is a leading humoral vascular relaxing factor produced by the endothelium [5, 6, 7]. In human and animal body, nitric oxide is synthesized from the amino acid L-arginine by the enzyme of endothelial NO-synthase (e-NOS). At the same time, arginase II catalyzes the transition of L-arginine to L-ornithine, which results in reduced content of a substrate for nitric oxide synthesis. That is why the selective blockade of arginase II will enhance the content of L-arginine, which will increase the production of nitric oxide [8, 9]. Hyperhomocysteinemia has just recently been referred to the potential risk factors of cardiovascular diseases. Hyperhomocysteinemia may either be an independent factor for the development of endothelial dysfunction or strengthen an already existing endothelial injury [1, 10]. It is supposed that the effects of homocysteine are associated with its propensity to form disulfide bonds and ability of forming free radicals reacting with NO and reducing its biological activity. Moreover, NO synthesis is possibly disrupted [1, 10]. In this regard, the objective of our study was to investigate the endothelioprotective and cardioprotective activity of the selective inhibitor of arginase II in a wide range of doses upon simulation of hyperhomocysteine-induced endothelial dysfunction.

Materials and methods

Experiments were performed in male albino Wistar rats weighing 200-250 g. For simulation of hyperhomocysteine-induced endothelial dysfunction, the methionine solution was administered intragastrically at a dose of 3 mg/kg/day for 7 days. Methionine solution for intragastric administration

was prepared with the use of stabilizator Tween 80 and 1% starch mucilage [11, 12]. The experiment involved the following groups of animals: 1) intact – intragastric administration of a solubilizer Tween 80 at a dose of 10 ml/kg for 7 days (n=10); 2) control - methionine intragastric administration at a dose of 3 g/kg once a day for 7 days (n=10); 3) selective inhibitor of arginase II, ZB49-0010C substance intragastrically at a dose of 1 mg/kg once a day for 7 consecutive days; 4) selective inhibitor of arginase II, ZB49-0010C substance intragastrically at a dose of 5 mg/kg once a day for 7 consecutive days; 5) selective inhibitor of arginase II, ZB49-0010C substance intragastrically at a dose of 10 mg/g once a day for 7 consecutive days. A selective inhibitor of arginase II, ZB49-0010C substance was administered for 7 days, 30 minutes prior to methionine (n=10).

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 TSD104A sensor and the MP150 hardware and software system (BiopacSystem, Inc., USA). In addition to blood pressure measurements, a series of functional tests was performed with subsequent evaluation of changes in hemodynamic parameters (SBP, DBP, HR) in response to endothelium-dependent vasodilatation (EDVD) and endothelium-independent vasodilatation (EIVD) [13].

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) [11, 12, 13, 14].

To assess the myocardial functionality in animals under controlled respiration, the cavity of the left ventricle was catheterized and stress tests for adrenoreactivity and resistance load were performed [13, 15].

The level of stable NO metabolites (i.e., total concentration of nitrite and nitrate, Total NOx) was measured colorimetrically in rat plasma, and homocysteine concentration was determined with the biochemical analyzer Olympus AU 640.

Statistical processing of the results of the study was performed by conventional methods, by calculating the average values (M) and the arithmetic mean error (±m). The significance of differences between the average values was determined by unpaired Student's t-criterion.

Results

Simulation of hyperhomocysteine-induced ED via intragastric administration of methionine at a dose of 3 g/kg resulted in a significant increase of EDC and homocysteine concentration in the blood plasma, as well as Total NOx reduction. While these indicators in intact animals were within the physiological norm. The effect of a selective inhibitor of arginase II, ZB49-0010C substance, on the initial values of EDC, homocysteine concentration and Total NOx in blood plasma in anesthetized rats upon modeling of hyperhomocysteine-induced ED is shown in Table 1.

Table 1

Indicators of endothelial dysfunction coefficient, Total NOx and homocysteine concentration in rat blood plasma upon modeling and correction of homocysteine-induced endothelial dysfunction by arginase II selective inhibitor (substance code ZB49-0010C) (n=10, M±m)

Groups of animals	EDC, (c.u.)	Total NOx in blood plasma (μM)	Homocysteine concentration in blood plasma (μM)
Intact (TWIN 80, 10 ml/kg)	0.9 ± 0.1	119.0±2.6	8.3±0.3
Methionine (3 g/kg)	3.3 ± 0.3*	68.9±4.3*	51.0±2.0*
Methionine (3 g/kg)+ ZB49-0010C (1 mg/kg)	2.1±0.3**	79.6±2.6**	44.7±3.2**
Methionine (3 g/kg)+ ZB49-0010C (5 mg/kg)	1.8±0.2**	93.3±3.5**	36.6±2.9**
Methionine (3 g/kg)+ ZB49-0010C (10 mg/kg)	1.6±0.2**	113.8±3.7**	28.7±1.5**

Note: EDC – endothelial dysfunction coefficient; Total NOx – the concentration of stable metabolites of nitric oxide in the blood plasma; * – significant difference as compared to the group of intact animals ($p<0.05$); ** – significant difference as compared to the group of animals treated with L-NAME ($p<0.05$).

It was found that daily administration of a selective inhibitor of arginase II, ZB49-0010C substance, upon modeling of hyperhomocysteine-induced ED, prevents the increase of EDC and the level of homocysteine in the blood plasma, as well as reduction of Total NOx. At the same time, these effects were dose-dependent, so, at a dose of 10 mg/kg a selective inhibitor of arginase II, substances under the code ZB49-0010C, the most strongly preventied an increase in EDC and the level of homocysteine in the blood plasma and reduction of Total NOx upon modeling of hyperhomocysteinemia-induced ED, whereas at a dose of 1 mg/kg the impact on these indicators was insignificant, and at a dose of 5 mg/kg the efficiency was intermediate between dose 1 and 10 mg/kg (Table 1).

To evaluate the effect of a selective inhibitor of arginase II, substances under the code ZB49-0010C, on functional contractility indices of left ventricular myocardium upon modeling of hyperhomocysteinemia-induced ED, the tests for adrenoreactivity and resistance load were conducted, the results of which are presented in Table 2.

Table 2

Effect of arginase II selective inhibitor (substances under the code ZB49-0010C) on functional contractility indices of left ventricular myocardium during stress tests upon modeling of hyperhomocysteinemia-induced ED (M±m, n=10)

Group of animals	Adrenoreactivity (mm Hg)	Myocardial reserve level (%)
Intact (TWIN 80, 10 ml/kg)	188.9±8.5	89.3 ± 4.2
Methionine (3 g/kg)	239±3.8*	66.5 ± 4.4*
Methionine (3 g/kg)+ ZB49-0010C (1 mg/kg)	205.6±7.7**	79.2 ± 3.1 **
Methionine (3 g/kg)+ ZB49-0010C (5 mg/kg)	204.2±4.8**	83.3 ± 5.0 **
Methionine (3 g/kg)+ ZB49-0010C (10 mg/kg)	196.7±3.3**	89.5±4.9**

Note: * – significant difference with the intact group ($p<0.05$); ** – significant difference with the control group ($p<0.05$).

Test for adrenoreactivity was characterized by a pronounced increase in absolute values of LVP in the group of animals treated with methionine as compared to the group of intact animals. At the same time, daily administration of arginase II selective inhibitor, substance code ZB49-0010C, upon modeling of hyperhomocysteine-induced nitric oxide deficiency prevented increase in adrenoreactivity, with the most pronounced effect at a dose of 10 mg/kg.

Test for resistance load established that arginase II selective inhibitor, substances code ZB49-0010C, prevented the decrease of myocardial contractility of the left ventricle from the 5th to the 25th second of the the ascending aorta compression. The effect was dose-dependent, since at a dose of 10 mg/kg there was the most pronounced prevention of decrease of myocardial reserve, whereas at a dose of 1 mg/kg the effect on myocardium contractile activity was minimal.

Discussion

Thus, the conducted studies have shown that a selective inhibitor of arginase II, substance under the code ZB49-0010C, has a pronounced dose-dependent endothelioprotective activity, which is expressed in the prevention of impaired relationship between endothelium-dependent and endothelium-independent vasodilation and improvement of NO-synthesizing endothelial function upon modeling of hyperhomocysteine-induced ED. The said effects are most pronounced at a dose of 10 mg/kg. The study of the effect of a selective inhibitor of arginase II on myocardial contractility showed its pronounced dose-dependent cardioprotective effect, which manifests itself in a decrease in the sensitivity of the myocardium to adrenaline and maintenance of myocardial reserve during the test for resistance load. The said effects are also most pronounced at a dose of 10 mg/kg. These effects are primarily connected with increase in L-arginine bioavailability as the main substrate for nitric oxide synthesis, by blocking its biotransformation with arginase II.

The obtained data are fully aligned with the results obtained by other authors [10, 14, 16, 17], and indicate the prospects of creating the arginase II-inhibiting drugs. The selectivity of the studied drug makes it more advantageous due to lack of impact on the urea metabolism [8, 9].

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