Effect of L-Arginine, Vitamin B_6 and Folic Acid on Parameters of Endothelial Dysfunction and Microcirculation in the Placenta in Modeling of L-NAME-Induced NO Deficiency

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L-arginine (200 mg/kg), vitamin B_6 (2 mg/kg), and folic acid (0.2 mg/kg) exert a protective effect on endothelial function in L-NAME-induced NO deficiency in male and pregnant female Wistar rats. Combined administration of these agents effectively prevented the development of endothelial dysfunction and L-NAME-induced preeclampsia.

Key Words: *endothelial dysfunction; gestosis; preeclampsia; vitamin B_6; folic acid*

Extensive investigations resulted in the concept of the endothelium as a target for prevention and treatment of many pathological states. Among a variety of bioactive substances produced by the endothelium, the most important is NO. Normally functioning endothelium is characterized by continuous basal NO production by endothelial NO-synthase (eNOS) from L-arginine, which is necessary for the maintenance of normal basal vascular tone. At the same time, NO has angioprotective properties: it inhibits proliferation of vascular smooth muscle and monocytes, thus preventing pathological reorganization of the vascular wall (remodeling) and progression of atherosclerosis [2,3].

Despite the fact that a number of factors participate in the pathogenesis of preeclampsia, most recent attention was given to endothelial dysfunction [7]. Placental ischemia leads to the release of numerous placental factors modulating blood flow and regulation of the vascular bed [5,6]. It was found that the concentration of asymmetric dimethilarginine in the maternal plasma is much higher in women with preeclampsia [10] and elevated concentration of this suspence is a predictor of preeclampsia [8-10].

Here we studied effects of L-arginine, vitamin B_6, folic acid, and their combinations on parameters of endothelial dysfunction and microcirculation during modeling of L-NAME-induced NO deficiency in male and pregnant female Wistar rats.

MATERIALS AND METHODS

Two series of experiments were carried out. In series I, 50 pregnant female Wistar rats (5 groups of 10 animals in each) were used (days 14-15 of pregnancy at the beginning of the experiment, body weight 220-260 g). In series II, 50 male Wistar rats (5 groups of 10 animals in each) weighing 180-220 g were used.

In all experimental series, NO deficiency was modeled by eNOS blockade with L-NAME (25 mg/kg/day for 7 days intraperitoneally). L-arginine (EUROBIOPHARM GmgH) in a dose of 200 mg/kg was administered intraperitoneally once a day for 7 days. Vitamin B_6 (Verofarm) was administered in a dose of 2 mg/kg intraperitoneally in combination with folic acid (Valenta Farmatsevtika OAO) in a dose of 0.2 mg/kg intragastrically 1once a day for 7 days. Combination of

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L-arginine, vitamin B_6, and folic acid was administered according to the above schemes for 7 days.

On day 8 of the experiment, catheter for blood pressure (BP) recording was introduced into the left carotid artery under chloral hydrate anesthesia (300 mg/kg). Bolus injection of pharmacological agents was performed into the femoral vein. Systolic BP (SBP), diastolic BP (DBP), and heart rate were measured continuously using a Biopac hardware-software complex. In addition to BP measurements, a series of functional tests was carried out in the following sequence: test for endothelium-dependent relaxation of vessels (intravenous injection of acetylcholine solution in a dose of 40 μg/kg); test for endothelium-independent relaxation of blood vessels (intravenous injection of sodium nitroprusside solution in a dose of 30 μg/kg) [2-4].

The degree of endothelial dysfunction in experimental animals, as well as the degree of its correction with studied medications, was evaluated by the derived coefficient of endothelial dysfunction (CED). This coefficient was calculated by the formula:

$$CED=SBP_{SN}/SBP_{ACH},$$

where SBP_{SN} is the area of the triangle above the BP recovery curve and the smaller side lies between the point of maximum BP drop and the point of BP exit on the plateau during the functional test with sodium nitroprusside; SBP_{ACH} is the area of the triangle above BP recovery curve during the test with acetylcholine, and the smaller cathetus is the difference between the end of bradycardic component and BP recovery point [3,4]. Concentrations of stable NO metabolites served as biochemical markers of endothelial dysfunction [4].

The study of placental microcirculation was performed using Biopac systems equipment: MP100 polygraph with LDF100C laser Doppler flowmetry unit and TSD144 sensor. The results were recorded with the program AcqKnowledge 3.8.1. Values of microcirculation were expressed in perfusion units (PU) [1]. For collection of daily urine and subsequent evaluation of proteinuria, the animals were placed into metabolic cages.

Significance of absolute changes in the parameters was determined using methods of variation statistics by calculating the arithmetic mean (M), error of the mean ($\pm m$) and the probability of possible error (p) by Student's tables. The differences were considered significant at $p<0.05$. Statistical calculations were performed using Microsoft Excel 7.0.

RESULTS

Blockade of eNOS caused by 7-day administration of L-NAME to pregnant rats increased CED in intact pregnant animals from 1.28±0.23 to 3.06±0.32 ($p<0.05$). In addition, SBP, DBP, and protein content in daily urine significantly increased (Table 1). Administration of L-NAME significantly reduced placental microcirculation to 237.50±38.18 PU *vs.* 425.90±39.55 PU in intact animals ($p<0.05$; Table 1).

Long-term (8 days) daily intragastric administration of L-arginine (200 mg/kg) against the background of L-NAME-induced preeclampsia resulted in significant reduction in CED and BP. Microcirculation in the placenta in animals treated with L-arginine significantly increased. At the same time, proteinuria was found to decrease to the levels observed in intact animals (Table 1). Daily intragastric administration of vitamin B_6+folic acid combination proved to be less effective (Table 1).

Combined use of L-arginine, vitamin B_6, and folic acid resulted in further reduction of CED, BP, placenta microcirculation, and proteinuria (Table 1).

Analyzing obtained results, we can conclude that L-arginine (200 mg/kg) and its combination with vitamin B_6 and folic acid can prevent the development of ADMA-like L-NAME-induced preeclampsia in the

TABLE 1. Effect of L-arginine, Vitamin B_6, and Folic Acid on the Development of Symptoms during Modeling of L-NAME-Induced Preeclampsia in Rats ($M\pm m$)

Group	SBP, mm Hg	DBP, mm Hg	CED, arb. units	Placental microcirculation, PU	Proteinuria, g/liter
Intact	125.0±6.3	82.0±5.8	1.28±0.23	425.90±39.55	0.90±0.10
L-NAME	183.1±9.4*	136.7±7.4*	3.06±0.32*	210.0±21.8*	1.88±0.19*
L-NAME+L-arginine	151.2±5.3$^+$	112.8±6.8$^+$	1.50±0.25$^+$	342.50±29.33$^+$	0.98±0.11$^+$
L-NAME+B_6+folic acid	180.9±5.0	133.2±5.8	2.1±0.1$^+$	316.4±17.4$^+$	1.26±0.08$^+$
L-NAME+B_6+ folic acid+L-arginine	149.4±5.1$^+$	101.6±5.4$^+$	1.40±0.31$^+$	401.4±31.2$^+$	0.93±0.13$^+$

Note. Here and in Table 2: $p<0.05$ in comparison with: *intact group, $^+$L-NAME group.

TABLE 2. Effect of L-Arginine, Vitamin B_6, and Folic Acid on Functional Properties during Modeling of L-NAME-Induced Endothelial Dysfunction (*M*±*m*)

Group	SBP, mm Hg	DBP, mm Hg	CED, arb. units	NO_x total, μmol
Intact	137.7±3.7	101.9±4.3	1.1±0.1	114.1±10.5
L-NAME	190.3±6.7*	145.0±3.9*	5.4±0.6*	61.2±8.5*
L-NAME+L-arginine	173.3±8.3*	137.3±9.4*	2.5±0.05+	115.1±9.7+
L-NAME+B_6+folic acid	182.4±4.1*	131.2±6.4+	2.7±0.4+	78.4±9.1
L-NAME+B_6+ folic acid+L-arginine	165.1±4.9+	128.1±6.9+	2.1±0.3+	109.1±8.3+

Note. NO_x total: concentration of nitrite ions in blood serum.

experiment. This is accompanied by improvement in both placental microcirculation and correction of hypertension and endothelial dysfunction.

In experiments on male rats, L-arginine (200 mg/kg) was found to exert a protective effect in the model of L-NAME-induced endothelial dysfunction, which manifested in decreased BP, normalization of CED, and increased concentrations of stable NO metabolites (Table 2). At the same time, BP did not reach the target level and did not differ significantly from that in control animals treated with L-NAME. Administration of vitamin B_6 and folic acid against the background of experimental L-NAME-induced NO deficiency leads to a significant decrease in CED. No significant differences in parameters of BP and concentrations of stable NO metabolites were detected in this group of animals (Table 2).

A positive pharmacodynamic interaction was revealed during combined use of L-arginine, vitamin B_6, and folic acid manifesting in a significant decrease in BP, maximal reduction of CED, and increase in the concentration of stable NO metabolites (Table 2).

These results suggest that L-arginine (200 mg/kg) and combination of vitamin B_6 (2 mg/ g) and folic acid (0.2 mg/kg) as monotherapy exert a protective effect on endothelium in L-NAME-induced NO deficiency in male and pregnant female Wistar rats. During combined use, a positive pharmacodynamic interaction was revealed manifesting in normalization of CED, concentration of stable NO metabolites, level of proteinuria, and placental microcirculation.

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