Research Journal of Pharmaceutical, Biological and Chemical Sciences

Simulation of Endothelial Dysfunction Associated With Hypestrogen-Induced Nitric Oxide Deficit.

Vladimir Ischacovich Kochkarov*, Olga Viktorovna Molchanova,
Mikhail Vladimirovich Pokrovskii, Tatyana Grigorievna Pokrovskaia, Vladimir Ivanovich Jakushev, and Oleg Sergeevich Gudyrev.

Federal Autonomous Educational Establishment for Higher Vocational Education, Belgorod State National Research University, 85, Pobedy St., Belgorod, 308015, Russia.

ABSTRACT

In the result of the performed research, there was elaborated technique of formation of endothelial dysfunction model in menopause in animals, and there were studied changes of endothelium-dependent and endothelium-independent vascular responses at this model.

Keywords: ovariectomy, L-NAME, endothelial dysfunction, nitric oxide, hypestrogenism.

*Corresponding author

INTRODUCTION

Endothelium which total weight is 2 kg is a cell layer of about 7 km length; it regulates the process of vasodilation, vasoconstriction, synthesis and inhibition of factors of proliferation, fibrinolysis and thrombocyte aggregation, of local inflammation by producing pro- and anti-inflammatory cytokines; thus, it is of great importance in the processes of homeostasis, hemostasis and inflammation [1]. By covering all the vessels not depending on their organ location, endothelium produces many vasoactive substances; one of the most important among these substances is nitric oxide (NO). Synthesis decreasing, increased bio-degradation of NO are the basis of endothelial dysfunction (ED) being the predictor of cardio-vascular diseases such as arterial hypertension, chronic cardiac insufficiency, atherosclerosis, coronary heart disease [2, 3].

Hence, one of the contemporary pharmacology current tasks is search of effective means able to correct endothelial dysfunction. Estimation of endothelium protective properties of different agents is possible by their investigation at various experimental models [4]. "Metabolic syndrome X", homocysteine-induced, and sepsis-induced models belong to these "ADMA-eNOS-associated" models. One variant is simulation of homocysteine-induced nitric oxide deficit and its combination with L-NAME. Organism changes occurring within menopause or hypestrogenism connected with surgical ovariectomy became a background for possibility of this model creation [5].

According to literature data, in the middle age, arterial hypertension dissemination in men is higher than in women, but in 60 years old, this dependency is contrary, and late in life, this disease is more widely spread among the women [6]. Estrogen deficit while menopause favors appearance of various risk factors composing "menopause metabolic syndrome" making, in the current time, conception of arterial hypertension (AH) pathogenesis in women in menopause [7].

Influence of estrogen deficit on arterial hypertension (AH) levels and frequency of cardiovascular complications can be mediated by various mechanisms. As is well known, that with aging, arteries non-crushing properties worsen, but these changes can occur due to menopause [8]. Estrogens influence on renin-angiotensin system – they prevent angiotensin-1 transformation to angiotensin-2 (AT2), and decrease AT-2 receptors sensitivity [9]. Renin activity in women plasma is lower than in men's one, but it increases with menopause [10]; at this, sympathic activity also increases within this period [11]. Investigation has determined higher noradrenaline secretion in women with AH in comparison with women with arterial pressure normal level; at this, at menopause, this difference was more detected [12].

In women, endothelial dysfunction considerably increases at perimenopausal period, what testifies about hormonal component participation in these disorders pathogenesis [13, 14]. Estrogens induce vasodilation by influencing on nitric oxide (NO) synthesis; they also initiate calcium channels opening in cell membranes of vessels smooth muscles cells. Menopause onset and following decreasing of estrogens level unfavorably tells on mechanisms vasodilation and AT decreasing [15].

Regards to the above mentioned, our investigation task was elaboration of model hypestrogen-L-NAME-induced nitric oxide deficit and evaluation of endothelium-dependent and endothelium-independent vascular reactions to this model.

METHOD

Investigation is performed at white rat females of 200-250 g weight, Wistar line, 3.5 months old. To simulate endothelial dysfunction, rats were anesthetized with chloral hydrate (300 mg/kg) and were made bilateral ovariectomy. For this, under aspetic conditions, one opened the anterior abdominal wall, tied silpingian and peritonial ovaries portion by using non-traumatic suture material, then one performed the resection of the latest. Then, the maim was repaired by layers.

The experiment consisted of three groups of animals: intact, ovariectomy, animals group receiving L-NAME associated with ovariectomy.

According to the experiment design, at the 43rd day (6 weeks after operation) inhibitor NO-synthase N-nitro-L-arginine methyl ether (L-NAME, Sigma) was injected abdominally once a day in a dose 25 mg/kg in

volume of 1 ml/kg within 7 days (n = 10 animals). Animals of intact group (n = 10 animals) and in the group with ovariectomy without L-NAME (n = 10 animals) were injected with normal saline solution NaCl in the same volume. At the 8th day, the animal under anesthesia was taken to the experiment by evaluating arterial pressure and arterial pressure reactions to endothelium-dependent (acetylcholine) and endothelium-independent (nitroprusside) vasodilation.

Investigation of hemodynamics factors in the groups of animals was performed under anesthesia (300 mg/kg chloral hydrate and 150 mg/kg Zoletil, abdominaly) by introducing catheter to arteria carotis. Pharmacological agents were injected bolusly to right femoral vein.

Hemodynamics factors: systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and cardiac rate (CC) were measured constantly by TSD104A sensor and MP150 hardware and software system manufactured by Biopac System, Inc., USA).

There were performed vascular batches for endothelium-dependent vasodilation (EDV) – intravenous injection of acetylcholine (ACH) in dose of 40 mg/kg and endothelium-independent vasodilation (EIV) – intravenous injection of sodium nitroprusside (SN) in dose of 30 mg/kg. To estimate developing degree of endothelial dysfunction in experimental animals and its correction by agents under study, we have calculated endothelial dysfunction factor (EDF) [16].

As biochemical markers of endothelial dysfunction, we determined eNOS level in the rat's aorta and level of total nitrite in the rats' blood serum [17]. At statistical processing of data, we calculated the average value, standard deviation value. Differences were considered as authentic at p<0.05.

Investigation results

Arterial pressure in intact animals was the following: systolic (SAP) – 128.1±6.0 mm Hg, diastolic (DAP) – 95.7±4.0 mm Hg. Disturbance simulation by creation of hypestrogen-induced NO deficit resulted in arterial hypertension (AH) (SAP – 160.0±6.2, DAP – 124.9±5.5 mm Hg). On the group of animals with ovariectomy receiving blocking agent of NO-synthase, analogue of L-arginine – L-NAME associated with hypestrogen-induced nitric oxide deficit, factors were the following: SAP – 169.2±7.3 mm Hg and DAP – 123.2±7.5 mm Hg. Results of vascular batches for endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitroprusside) relaxation of vessels and EDF increasing from c 0.8±0.11 in intact animals to 1.9±0.3 and 4.6±0.6 (p<0.05) in groups ovariectomy and ovariectomy+L-NAME testify about disturbance of interrelations of vasodilation and vasoconstrictive mechanisms of vascular tone regulation.

Table 1: Dynamics of factors of systolic (SAP), diastolic (DAP) arterial pressure, endothelial dysfunction factor in the experimental groups of animals (M±m, n=10).

Group of animals	SAP, mm Hg	DAP, mm Hg	EDF, relative units
Intact	128.1±6.0	95.7±4.0	0.8±0.1
Ovariectomy	160.0±6.2	124.9±5.5	2.1±0.2
Ovariectomy+L-NAME	169.9±7.3*	123.2±7.5*	4.6±0.7*

Note: EDF – endothelial dysfunction factor; * - p < 0.05 compared to intact animals group; ** - p < 0.05 compared to L-NAME group.

Investigation of biochemical markers in series of experimental animals have confirmed increasing of NO deficit while using L-NAME associated with hypestrogen state.

Table 2: L-nitro-arginine methyl ether (L-NAME) in dose of 25 mg/kg abdominal influence (M±m, n=10) associated with hypestrogen-induced Nitrogen deficit.

Group of animals	eNOS, % from control	NO$_x$ total, Mc Mole
Intact	80.1±3.8	122.8±9.6
Ovariectomy	32.4±4.3	75.1±7.3
Ovariectomy+L-NAME	23.6±3.4*	53.4±6.1*

Note: * - at p < 0.05 compared to intact animals group; ** - at p < 0.05 compare to L-NAME-NO deficit group.

Thus, expression factor eNOS in the model ovariectomy + L-NAME has authentically much more decreased than in the group of animals not having received L-NAME. Nitrite-ions (NO$_X$ total) concentration has also considerably decreased in the group with L-NAME injection associated with pathology simulation.

CONCLUSIONS

Thus, use of L-nitro-arginine methyl ether (L-NAME) in dose of 25 mg/kg abdominally associated with hypestrogen-induced nitrogen deficit resulted in development of more prominent features of endothelial dysfunction than at the model of hypestrogen-indeuced nitrogen deficit; it was expressed endothelial dysfunction factor (EDF)increasing, NOx nitrite-ions contents decreasing. At this, there was no any substantial difference between arterial pressure factors and AH developing. Thus, this model can be used while further experimental investigations as for search of possible ways for endothelial dysfunction correction.

REFERENCES

[1]   Saveliev VS. Surgery, Consilium medicum Annex 2008;1: 3-11.
[2]   Belousov YB, Namsaraev Zh N. Pharmeteka 2000;6: 62-72.
[3]   Martynov AI, Avetyan NG, Akatova EV. et al. Cardiol 2005;45(10): 101-104.
[4]   Fortepiani LA, Zhang H, Racusen L, et al. Hypertension 2003;41: 460-463.
[5]   Casiglia C, Tikhonoff P, Mormina P, et al. J Hyperten 2002;20(Suppl 2): 17-22.
[6]   Burt VL, Whelton P, Roccella EJ, et al. Hyperten 1995;25: 305-313.
[7]   August P, Oparil S. J Clin Endocrinol Metab 1999;8:1862-1866.
[8]   Staessen JA, Celis H, Fagard R. J Hum Hypertens 1998;12(9): 587-592.
[9]   Hall JE, Guyton AC, Brands MJ. 1995. Control of Sodium Excretion and Arterial Pressure by Intrarenal Mechanisms and the Reninan-Giotensin System. In: Laragh JH, Brenner BM, Eds. Hypertension: Pathophysiology, Diagnosis, and Management, 2nd edn. New York: Raven Press, 1451-1475.
[10]   Hall JE, Brands MJ, Henegar JR. J Am Soc Nephrol 1995;10:258-265.
[11]   Seals DR, Esler MD. J Physiol 2000; 528 (Pt3): 407-417.
[12]   Weinberger MH, Fineberg NS. Hyperten 1991;18: 67.
[13]   De Nuccio I, Salvati G, Genovesi G, et al. Minerva Endocrinol 1999;24:77-81.
[14]   Anttila L, Penttila AT, Matinlauri I, et al. Gynecol Endocrinol 1998; 12: 327-331.
[15]   Erman A, Chen-Gal B, van Dijk DJ, et al. J Endocrinol Metab 1996;81:1104-1107.
[16]   Pokrovskii MV, Kochkarov VI, Pokrovskaia TG. et al. Kuban Sci Med Bull 2006;10: 72-77.
[17]   Metelskaia VA. Clin Lab Diagn 2004;9: 86.