UDC: 611.018.74:615.22 **DOI: 10.18413/2313-8971-2016-2-1-4-8**

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ENDOTHELIUM AND CARDIOPROTECTIVE EFFECTS OF HMG-CO-A-REDUCTASE IN COMBINATION WITH L-ARGININE IN ENDOTHELIAL DYSFUNCTION MODELING

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Abstract. Using the combined application of L-arginine with HMG-Co-A reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of modeling of sepsis-induced disease through the introduction of strain 603 Staphylococcus aureus shows endotelio- and cardioprotective effects, manifesting itself in preventing the proliferation of endothelial dysfunction coefficient (CED), adrenoreactivity, maintenance of myocardial reserve and the normalization of biochemical markers values (Total NO, eNOS expression, C-reactive protein, IL-6, TNF). In this case, the combined therapy was so effective that the values obtained thereunder did not differ from those obtained from control animals.
Keywords: endothelial dysfunction, HMG-Co-A reductase inhibitor simvastatin, atorvastatin, rosuvastatin, endotoxin.

Introduction: Recently, the abdominal surgery has paid much attention to endotoxin-induced lesions of the cardiovascular system and the development of endothelial dysfunction [1, 2, 3, 4]. At the same time, a clear sequence of events lines up: endotoxin-induced shock with multiorgan pathology, -> release of pro-inflammatory cytokines ->, endothelial dysfunction -> systemic vasculitis ->, increase in vascular and endothelial permeability for lymphocytes ->, hyperlipoproteinemia ->, the beginning of atherosclerosis [1, 2, 3, 4].

It is reasonable to assume that the same algorithm can be adopted for any endotoxin-induced pathology, regardless of its causes, and subjuct to the critically increased levels of pro-inflammatory cytokines [5, 6].

At the same time, despite the oderliness of pathogenic schemes and incorporation of many factors (VEGF, sFlt-1 angiotensin II autoantibody (type 1) (AT1-AA), cytokines (tumor necrosis factor (TNF)-α), endothelin, reactive oxygen species (of ROS), thromboxane, 20-hydroxyeicosatetraenoic acid (20-HETE), increased sensitivity to angiotensin II, etc.), there is an obvious immaturity of pharmacological approaches aimed at correction of endothelial dysfunction in acute systemic inflammation. In this respect, the pharmacological target "ADMA-eNOS" is of great interest [7-13].

We can assume that one of pharmacotherapeutic strategies for correction of endotoxin-induced endothelial dysfunction is the combined use of HMG-Co-A reductase inhibitors [14] and the NO donor – L-arginine [15].

Methods: Experiments were conducted on white male Wistar rats weighing 200-250 g.

Modeling of endotoxin-induced endothelial dysfunction (EIED) was carried out by subcutaneous injection of 0.1 ml of fresh suspension *Staphylococcus aureus* (strain 603) at a concentration of 10 bil microbial bodies per 1 ml.

HMG-Co-A reductase inhibitors in combination with L-arginine 200 mg/kg were administered intragastrically daily for 7 days. All animals were divided into experimental groups (n=10): 1 – intact;

2 – endotoxin-induced endothelial dysfunction (EIED). 3 – EIED + L-arginine 200 mg/kg; 4 – EIED + Simvastatin 8.5 mg/kg; 5 – EIED + Atorvastatin 4.3 mg/kg; 6 – EIED + Rosuvastatin8.5 mg/kg; 7 – EIED + Nanoparticulated rosuvastatin 11.6 mg/kg; 8 – EIED + Simvastatin 8.5 mg/kg + L-arginine 200 mg/kg; 9 – EIED + Atorvastatin 4.3 mg/kg + L-arginine 200 mg/kg; 10 – EIED + Rosuvastatin8.5 mg/kg + L-arginine 200 mg/kg; 11 – EIED + Nanoparticulated rosuvastatin 11.6 mg/kg + L-arginine 200 mg/kg;

On day 8 of the experiment, a catheter was inserted under anesthesia (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 "Biopac" hardware and software system. 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) [7-13, 16].

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) [7-13, 16].

For the evaluation of cardioprotective activity, functional tests on adrenoreactivity [7-13] and the exhaustion of myocardial reserve were performed [7-13].

The dynamics of biochemical markers values (Total NO, eNOS expression, C-reactive protein, IL-6, TNF) in animals with endotoxin-induced endothelial dysfunction was assessed with the standard set of reagents [10].

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: Monotherapy with NO donor L-arginine (200 mg/kg) daily intraperitoneally on the background of EIED modeling normalized EDC and insignificantly affected the BP values (Table 1). The HMG-Co-A reductase inhibitors simvastatin (8.5 mg/kg), atorvastatin (4.3 mg/kg), rosuvastatin (8.5 mg/kg) and nanoparticulated rosuvastatin (11.6 mg/kg) at the most effective doses improved significantly EDC and had no effect on blood pressure (Table 1).

The combined use of L-arginine with HMG-Co-A reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin showed the most pronounced endothelium-protective effect, and the hemodynamic parameters such as systolic and diastolic blood pressure and EDC were not statistically different from those in intact animals (Table 1).

At the same time, there was positive dynamics of contractility indicators during exercise testing in animals with EIED (Table 2). For example, we detected the prevention of increase in adrenoreactivity and reduction in myocardial reserve both when using L-arginine and under its concomitant use with HMG-Co-A reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin. In this case, as well as for EDC, the combined use of drugs has resulted in values not differing from those in intact animals (Table 2).

The most pronounced endothelium- and cardioprotective effect of combined use of the NO donor L-arginine with HMG-Co-A reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin was on values of biochemical markers in EIED animals (Table 3).

The protective effect of combined use of the NO donor L-arginine with HMG-Co-A reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin was most significant on the level of C-reactive protein and values of pro-inflamatory cytokines IL-6 and TNF, which values did not differ from those in intact animals (Table 3).

Conclusion: using the combined application of L-arginine with HMG-Co-A reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of modeling of sepsis-induced disease through the introduction of strain 603 Staphylococcusaureus shows endotelio- and cardioprotective effects, manifesting itself in preventing the proliferation of endothelial dysfunction coefficient (CED), adrenoreactivity, maintenance of myocardial reserve and the normalization of biochemical markers values (Total NO, eNOS expression, C-reactive protein, IL-6, TNF). In this case, the combined therapy was so effective that the values obtained thereunder did not differ from those obtained from control animals.

Table 1

Influence of combined use of L-arginine with an HMG-CoA reductase inhibitor simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the dynamics of hemodynamic parameters in animals with endotoxin-induced endothelial dysfunction (M ± m, n = 10)

Groups of animals	SBP	DBP	EDC
Intact	129.4±2.2	89.2± 1.1	1.1 ± 0.1
Endotoxin-induced endothelial dysfunction (EIED) (n=10)	117.6±2.3*	85.0±2.1	3.7±0.5*
EIED + L-arginine 200 mg/kg (n=10)	118.5±2.1	76.3±2.1	2.1±0.3*#
EIED + Simvastatin 8.5 mg/kg (n=10)	127.3±2.8	87.1±1.9	2.3±0.5*#
EIED + Atorvastatin 4.3 mg/kg (n=10)	130.0±3.3	85.8±2.2	2.1±0.3*#
EIED + Rosuvastatin 8.5 mg/kg (n=1)	135.0±3.8	83.1±2.1	1.7±0.5*#
EIED + Nanoparticulated rosuvastatin 11.6 mg/kg (n=10)	129.6±4.3	84.9±2.0	1.5±0.2*#
EIED + L-arginine 200 mg/kg + Simvastatin 8.5 mg/kg (n=10)	142.4±3.1	94.2±2.2	1.6±0.3*#
EIED + L-arginine 200 mg/kg + Atorvastatin 4.3 mg/kg (n=10)	137.8±3.4	92.5±2.3	1.5±0.3*#
EIED + L-arginine 200 mg/kg + Rosuvastatin 8.5 mg/kg (n=10)	136.9±3.3	91.1±2.1	1.7±0.4*#
EIED + L-arginine 200 mg/kg + Nanoparticulated rosuvastatin 11.6 mg/kg (n=10)	137.4±3.6	94.3±2.3	1.5±0.2*#

Note: SBP – systolic blood pressure (mm Hg), DBP - diastolic blood pressure (mm Hg), EDC – endothelial dysfunction coefficient (rel. unit), * – significant difference with the intact group ($p<0.05$); # – significant difference with the group of animals with endotoxin-induced endothelial dysfunction (EIED) ($p<0.05$).

Table 2

Influence of combined use of L-arginine with an HMG-CoA reductase inhibitor simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the dynamics of contractility parameters during exercise testing in animals with endotoxin-induced endothelial dysfunction (EIED) (M ± m, n = 10)

Group of animals	Adrenoreactivity (mm Hg)	Myocardial reserve exhaustion (%)
Intact	201.5±9.4	112.7±10.9
Endotoxin-induced endothelial dysfunction (EIED) (n=10)	240.3±8.7*	79.4±3.9*
EIED + L-arginine 200 mg/kg (n=10)	211.4±7.8*	92.4±5.7*
EIED + Simvastatin 8.5 mg/kg (n=10)	232.0±8.9*	87.4±3.7*
EIED + Atorvastatin 4.3 mg/kg (n=10)	222.1±8.5*#	97.0±4.9*
EIED + Rosuvastatin 8.5 mg/kg (n=1)	221.0±8.4*#	109.4±5.7*#
EIED + Nanoparticulated rosuvastatin 11.6 mg/kg (n=10)	219.1±8.7*#	99.9±6.3*#
EIED + L-arginine 200 mg/kg + Simvastatin 8.5 mg/kg (n=10)	204.8±6.9*#	109.7±4.8*#
EIED + L-arginine 200 mg/kg + Atorvastatin 4.3 mg/kg (n=10)	201.0±5.3*#	107.6±3.5*#
EIED + L-arginine 200 mg/kg + Rosuvastatin 8.5 mg/kg (n=10)	193.8±5.8*#	108.4±4.8*#
EIED + L-arginine 200 mg/kg + Nanoparticulated rosuvastatin 11.6 mg/kg (n=10)	196.7±5.7*#	110.5±5.6*#

Note: * – significant difference with the intact group ($p<0.05$); # – significant difference with the group of animals with endotoxin-induced endothelial dysfunction (EIED) ($p<0.05$).

Table 3

Influence of combined use of L-arginine with an HMG-CoA reductase inhibitor simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the dynamics of biochemical markers values (Total NO, eNOS expression, C-reactive protein, IL-6, TNF) in animals with endotoxin-induced endothelial dysfunction (EIED) (M ± m, n = 10)

Group of animals	NOx	ENOS Expression	CRP level	IL-6	TNF
Intact	116.8±10.3	5.4±0.21	0.05±0.01	0.43±0.17	8.42±2.51
Endotoxin-induced endothelial dysfunction (EIED) (n=10)	182.3±12.4*	0.04±0.01*	0.38±0.01*	6.87±1.93*	17.83±3.79*
EIED + L-arginine 200 mg/kg (n=10)	132.7±11.3*#	2.14±0.22*#	0.17±0.02*#	2.23±1.67*#	10.23±2.08*#
EIED + Simvastatin 8.5 mg/kg (n=10)	122.9±8.4*#	1.93±0.12*#	0.08±0.01*#	1.03±0.62*#	10.76±1.70*#
EIED + Atorvastatin 4.3 mg/kg (n=10)	130.0±10.9*#	2.07±0.21*#	0.09±0.01*#	1.27±0.33*#	9.89±1.79*#
EIED + Rosuvastatin 8.5 mg/kg (n=1)	122.1±9.9*#	3.04±0.35*#	0.11±0.01*#	1.17±0.33*#	10.80±1.99*#
EIED + Nanoparticulated rosuvastatin 11.6 mg/kg (n=10)	132.1±10.3*#	4.01±0.56*#	0.18±0.01*#	1.48±0.24*#	9.56±1.87*#
EIED + L-arginine 200 mg/kg + Simvastatin 8.5 mg/kg (n=10)	123.5±9.7*#	4.12±0.65*#	0.08±0.02*#	0.92±0.12*#	9.12±1.12*#
EIED + L-arginine 200 mg/kg + Atorvastatin 4.3 mg/kg (n=10)	121.7±9.5*#	4.23±0.69*#	0.07±0.02*#	0.90±0.13*#	8.79±0.91*#
EIED + L-arginine 200 mg/kg + Rosuvastatin 8.5 mg/kg (n=10)	119.5±9.3*#	4.47±0.72*#	0.06±0.02*#	0.78±0.11*#	8.42±0.87*#
EIED + L-arginine 200 mg/kg + Nanoparticulated rosuvastatin 11.6 mg/kg (n=10)	117.8±10.0*#	4.92±0.86*#	0.06±0.02*#	0.63±0.10*#	7.56±0.79*#

Note: NOx - end metabolites NO (μmol/l); eNOS expression (%); CRP level - level of C-reactive protein (mg/l); IL-6 – Interleukin 6 (pg/ml); TNF – tumor necrosis factor (pg/ml); * – significant difference with the intact group ($p<0.05$); # – significant difference with the group of animals with endotoxin-induced endothelial dysfunction (EIED) ($p<0.05$).

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