Рус.

UDC: 615.37:616-018.74-008.6 **DOI: 10.18413/2313-8971-2016-2-2-25-35**

Ivlitskaya I.L.[1]
Korokin M.V.[2]
Loktionov A.L.[3]

PHARMACOLOGICAL EFFICIENCY OF STATINS AND L-NORVALIN AT AN ENDOTOXIN-INDUCED ENDOTHELIAL DYSFUNCTION

1) Assistance lecturer of the Department of infectious diseases of Kursk State Medical University
3, K. Marksa St., Kursk, 305041, Russia. e-mail: *stilling1@yandex.ru*
2) Doctor of Medical Sciences, Professor of the Department of pharmacology of Medical Institute of Belgorod State National Research University. 85, Pobedy St., Belgorod, 308015, Russia. e-mail: *mkorokin@mail.ru*
3) Doctor of Medical Sciences, Kursk State Medical University
3, K. Marksa St., Kursk, 305041, Russia. e-mail: *ala-loc@yandex.ru*

Abstract. In the animals with endotoxin-induced endothelial dysfunction there were imbalance of contractile response of vessels wall observes when carrying out functional tests, increase of coefficient of endothelial dysfunction, decrease in contractility of a myocardium, activation processes of peroxidation of lipids and decreased activity of antioxidant systems, level of stable metabolites of nitrogen oxide. On extent of decrease in corrective effects on indicators of functional activity of a vessel wall, contractility of a myocardium, oxidatic indicators and level of stable metabolites of nitrogen oxide are studied preparations settle down in the following sequence: L-norvalin and rozuvastatin (coefficient of endothelial dysfunction – 1,2±0,1) → L-norvalin and simvastatin (coefficient of endothelial dysfunction – 1,5±0,1) → rozuvastatin (coefficient of endothelial dysfunction – 1,6±0,1) → L-norvalin (coefficient of endothelial dysfunction – 1,7±0,2) → symvastatin (coefficient of endothelial dysfunction – 2,0±0,2).
Keywords: endotoxin-induced endothelial dysfunction, statins, L-norvalin.

Introduction. Sepsis is the most serious complication of infectious process. The mortality from the sepsis makes not less than 22,2 to 100 000 population and it depends on original resistance of an organism to the infection, age, sex accompanying pathology, system activity including a regulation of homeostasis from functional activity of endotheliocytes [1, 2, 3]. The endothelial dysfunction (ED) is an imbalance between the mediators providing an ideal current of all endothelial dependent workflow. The vascular endothelial is a place for realization of normal and pathological immune and biochemical reactions proceeding with participation of mediators of inflammatory reaction, nitrogen oxide and its metabolites, active radicals of oxygen, etc. at the endotoxin-induced endothelial dysfunction (EIED) [4, 5, 6, 7, 8, 9, 10].

Pharmacological correction EIED must be contained in drugs using directly or indirectly influencing on endothelial functions ,its permeabilities and regulatory properties connecting to it metabolic shifts [11, 12, 13]. It is taken using of statins which are possessed of expressed antiphlogistic action and also they can be used in clinical practice for the main indication connected with the damage of cholesterol metabolism and antioxidant of L-norvalin which must up the level of L-arginin and prevent the endogenous inhibiting of NO-synthase [14, 15, 15].

Research objective – an evaluation of endothelio-and cardioprotective effects of statins and L-norvalin on rats with the endotoxin-induced model of endothelial dysfunction.

OWN RESEARCHES

Object of research. The experiments were on healthy pubertal rats lines Wistar, weighing 180-250 g. In experiences there were the animals who passed a quarantine regimen of a vivarium of Kursk state medical university and they didn't have external symptoms of any diseases. For receiving statistically reliable results of group it was formed 18-20 animals, the researches were at the same times of the days, from 8 to 12 h, according to rules of laboratory practice of the Russian Federation (the order the Ministry of Health of Russian Federation No. 267 of 19.06.2003).

The Modeling EIED was carried out under anesthetic, with keeping of rules of an asepsis and antiseptics: shaved wool, subcutaneously entered 0,1 ml of a fresh suspension of Staphylococcus aureus (the strain 603 which was taken from the museum of department of microbiology, virology and

immunology of KSMU) into concentration $10 \cdot 10^9$/ml. Daily carried out compression, pneumatic massage of the place of an injection within 10 minutes [16, 17]. After carrying out functional assays, for the 7th days from modeling of EIED, under anesthetic it was carried out a thoracotomy and blood sampling of 5 ml from a right ventricle of the reduced heart. Removal of animals from experiment were carried out an overdosage of anesthetics.

Design of research, dosage, ways and frequency rate of administration of drugs. All experimental animals were divided into some groups: the 1st group (15 rats) – intact; the 2nd group (18 rats) – animals with EIED which didn't have the drugs; the 3rd group (19 rats) – animals with EIED which were received symvastatin in a dose of 0,5 mg/kg *per os*; 4th group (20 rats) – animals with EIED which were reseived rozuvastatin in a dose of 0,125 mg/kg *per os*; the 5th group (20 rats) – animals with EIED which were entered L-norvalin in a dose of 10 mg/kg *per os*; the 6th group (18 rats) – animals with EIED which were received a combination of L-norvalin and symvastatin *per os* in the same dosages; the 7th group (20 rats) – animals with EIED were received a combination of L-norvalin and a rozuvastatin *per os* in above-mentioned dosages.

Dosages, ways and frequency administration of drugs were based on results of researches in available literature and the experiments were made earlier in laboratories according to which at such way of introduction the designated medicines render the most effective endothelio- and cardioprotective action [18].

Degree evaluation of endothelium in rats with EIED. Development of endothelial dysfunction in experimental animals was estimated on the settlement coefficient of endothelial dysfunction (CED).

Making an assessment of results of functional test on endothelial dependent vazodilatation (EDVD) was used the area of a rectangular triangle over a curve of recovery of the ABP in response to introduction AH in a dose of 40 mkg/kg. Making an assessment of results of endothelial independent vasodilatation (EIVD) with introduction of NP in a dose of 30 mkg/kg was also used the area of a rectangular triangle over a curve of recovery of the ABP. Larger legs in both triangles were indicators of time of recovery of the ABP in response to intravenous administration AH and NP respectively expressed in seconds. The area of the estimated triangles was expressed in conventional units (conventional unit). CED is the relation of the area of a triangle over a curve of recovery of the ABP in response to introduction of NP (S_{NP}) to the area of a triangle over a curve of recovery of the ABP in response to introduction AH (S_{AH}). CED = S_{NP}/S_{AH}.

Research of contractility of a myocardium at rats in a narcosis on the controlled respiration on open heart. Research of contractility of a myocardium after modeling of pathology was conducted on the rats in a narcosis on the controlled respiration. The cavity of a left ventricle was probed with a needle through an apex of heart and with the help of the sensor and the device for invasive measurement of indicators of a hemodynamics of Biopac (USA) and the computer Asq Knowledge 3.8.1 program were recorded cardiohemodynamics indicators (left ventricular pressure (LVP), the maximum rate of reduction ($+dp/dt_{max}$), the maximum rate of relaxation ($-dp/dt_{max}$), the heart rate (HR) and the intensity of functioning of structures (IFS) (work of rate's heart and pressure developed by a left ventricle (mm of mercury. x beats/min) [21]. For an assessment functionality of a myocardium on animals carried out load assays in the presented sequence:the assay on an adrenoreactivity (intravenous single-step administration of solution of Adrenalinum hydrochloricum 1 • 10 mmol/l, at the rate of 0,1 ml on 100 g); a load resistance (crossclamping of the ascending aorta on 30 sec.); a 3-minute hypoxia with the subsequent reoxygination.

Assessment of oxidatic indicators. Intensity of processes of peroxidation of lipids was estimated according to contents in blood plasma of MDA and AGP. For an assessment of a condition of antioxidatic system was defined the activity of a catalase with using of ready commercial sets. The general anti-oxidizing activity (GAA), determined by the method based on extent of inhibition of oxidation of the twin-80 to GAA. Concentration of stable metabolites of nitrogen oxide (SM_{NO}) was investigated by a spectrophotoscopycal method with the help of Gris's reactant and detection of the formed products at a wavelength of 540 nanometers.

Statistical processing of results of research. Reliability of the changes which are observed on the effect of the studied drugs was determined by methods of descriptive statistics with finding of average values (M), by errors of average (±m) and probability of the possible mistake (r) calculated with use of t-test for groups with various dispersion. Differences were estimated as reliable, at p =0,05.

RESULTS OF RESEARCH

The condition of function of a vascular endothelium and contractility of a myocardium in the conditions of experimental model an

endotoxin-induced endothelial dysfunction. Modeling of EIED authentically didn't exert impact on the SABP and DABP in comparison with intact animals. After introduction AH it was increasing the level of SABP and DABP and the normal level of CCR on the rats with EIED. There were no exchanges on the same rats with EIED after injection of NP. Increasing the areas of recovery of the ABP in assays on EDVD and EIVD on rats with EIED led to rising of CED to 3,7±0,5 in comparison with intact animals (CED = 1,1±0,1). The main contribution to rising of CED was made reliable, more than by 2,5 times, depression of the area of a triangle over a curve of recovery of the ABP in response to intravenous administration AH (table 1).

Table 1

Results of carrying out functional vascular assays on animals with EIED (M±m; n=10)

Groups of animals	Functional tests	SABP, mm of mercury	DABP, mm of mercury	CCR, bits per minute	S of vascular reaction till the carrying out EDVD with AH and EIVD with NP, conventional unit.	CED, convention al unit.
Intact	Initial	129,4±2,2	89,2± 1,1	380±4,3		1,1±0,1
	AH	78,6±4,5	38,7±2,8	362±7,1	1268,0±74,8	
	NP	85,8±3,7	45,5±4,4	364±5,4	1375,3±93,7	
Rats with EIED	Initial	117,6±2,3	85,0±2,1	401±8,6		3,7±0,5*
	AH	88,3±2,8*	53,1±2,4*	382±4,2	489,3±75,5*	
	NP	83,3±4,2	40,2±2,3	387±7,5	1535,6±117,3	

Note: * - at p =0,05 in comparison with intact animals

The specified fact demonstrates the expressed change of endothelia dependent reaction on rats with EIED. The animals with EIED increasing the level of indicators of the maximum speeds of reduction and relaxation of a myocardium on intact rats ($+dp/dt_{max}$ and $- dp/dt_{max}$), at the same time it didn't influence on the LVP, CCR and IFS level were observed. Intravenous injection of solution of adrenaline hydrochloride in a dose of 0,1 ml/100 weight gram in group of animals with EIED was characterized by increase of pressure created by the left ventricle, high-speed parameters of the $+dp/dt_{max}$ and $- dp/dt_{max}$ and IFS. CCR was the same in the group of intact animals. During the first 5 seconds of compression of the ascending aorta on intact animals were observed a sharp increase of pressure developed by the left ventricle, high-speed indicators of contractility reaction of myocardium and IFS. In comparison with intact animals, the rats with EIED had an accelerate relaxation of a myocardium and CCR. LVP and $+dp/dt_{max}$ didn't differ from control values and IFS raised (table 2).

The second phase (25 min) was characterized by gradual weakening of the contractive answer. In this period the level of systolic tension bradycardia in group of intact animals decreased and amplified (LVP decreased to 212,7±10,9, CCR by decreased from 284,9±21,7 beats/min to 306,8±36,1 beats/min). There were higher indicator – dp/dt_{max}, CCR and IFS in experimental group (table 2).

Modeling of the hypoxia was reached by switching off the medical ventilator in the conditions of an open thorax. By the end of the 5th minute it took place a reoxygenation decrease in indicators of a contractility of the myocardium. In control group of animals when carrying out the test with a reoxygenation increasing the level of LVP, but decreasing the level of CCR were observed. As a result, decrease of IFS took place. In experimental group of rats the pressure developed by the left ventricle, speed of reduction and IFS had appeared higher, than on intact rats (table 2).

So, modeling of EIED the endotheliy causes negative effects in experimental animals not only on contractility function of a myocardium, but also on functional activity that is shown by increase of CED and decrease in reserves of functional activity of a myocardium at statement of the majority of tests. Besides modeling of EIED it can be used for studying endothelio- and cardioprotective effects of various groups of pharmacological preparations.

Table 2

Change of functional activity of a myocardium of animals with EIED receiving statins or L-norvalin (M±m; n=10)

Indicators / Groups of animals		Units of measure	1 Intact	2 EIED	3 EIED + rosuvastatin	4 EIED + symvastatin	5 EIED + L-norvalin
Without functional trials	LVP	mm of mercury	105,3±4,5	110,1±7,1	107,8±4,7	108,0±3,6	103,0±3,0
	$+dp/dt_{max}$	mm of mercury per sec	6570,9±783,4	8838,9±340,7^{*1}	6597,0±372,6^{*2}	7154,3±850,9^{*2}	6822,4±1041,4^{*2}
	$-dp/dt_{max}$	mm of mercury per sec	4142,1±452,6	5206,1±255,7^{*1}	4065,5±320,1^{*2}	5308,3±480,8^{*1}	4768,2±497,0^{*2,4}
	CCR	hart bits per second	361,3±14,7	372,5±10,5	379,3±6,0	373,2±6,3	366,3±12,0
	IFS	mm of mercury x hart bits per sec	38421,1±2849,6	40756,4±2279,9	40905±1970	40251±1341	37867±1956
Test on adrenoreactivity	LVP	mm of mercury	201,5±9,4	240,3±8,7^{*1}	212,3±5,9^{*2}	215,2±7,7^{*2}	214,9±7,7^{*2}
	$+dp/dt_{max}$	mm of mercury per sec	10997,8±777,6	13959,2±735,9^{*1}	9674,9±372,0^{*2}	12876,9±366,2^{*1,3}	10094,6±783,4^{*2,4}
	$-dp/dt_{max}$	mm of mercury per sec	5697,1±650,8	7830,5±520,9^{*1}	4854,3±185,3^{*1,2}	7910,1±504,2^{*1,3}	5497,7±554,7^{*2-4}
	CCR	hart bits per second	374,4±14,5	379,0±9,4	395,7±4,4	381,2±5,6	369,1±8,8
	IFS	mm of mercury x hart bits per sec	76236,2±5813,7	91294,1±4546,3^{*1}	84048±2686^{*2}	82304±3877^{*2}	79014±2326^{*2}
Test with compression of aorta 5 sec.	LVP	mm of mercury	233,2±9,9	220,2±6,5	218,7±5,5	263,1±5,5	233,8±5,6
	$+dp/dt_{max}$	mm of mercury per sec	9227,3±459,3	9555,9±400,2	6541,7±549,9^{*1,2}	10143,2±284,8^{*3}	8589,1±657,2^{*1,2,4}
	$-dp/dt_{max}$	mm of mercury per sec	3674,7±207,5	4241,6±275,4^{*1}	4028,2±289,3^{*1}	4734,8±167,4^{*1,3}	3485,9±260,6^{*2-4}
	CCR	hart bits per second	284,9±21,7	366,8±10,6^{*1}	370,7±9,4^{*1}	331,9±13,9^{*1-3}	334,6±10,6^{*1-3}
	IFS	mm of mercury x hart bits per sec	65459,1±3968,0	80568,6±2804,2^{*1}	80933±2470^{*1}	87336±4119^{*1-3}	78060±2536^{*1,4}
Test with compression of aorta 25 sec.	LVP	mm of mercury	212,7±10,9	179,4±3,9^{*1}	196,3±5,2^{*2}	228,1±5,8^{*2}	216,6±5,9^{*2}
	$+dp/dt_{max}$	mm of mercury per sec	6826,7±458,6	5144,0±238,3^{*1}	5360,5±487,2^{*1}	7945,0±416,2^{*1-3}	8285,2±649,9^{*1-3}
	$-dp/dt_{max}$	mm of mercury per sec	3210,5±261,5	3034,3±237,6	3762,2±244,2^{*1,2}	4281,1±342,2^{*1-3}	2728,4±135,2^{*3,4}
	CCR	hart bits per second	306,8±36,1	259,4±14,4^{*1}	311,3±19,6^{*2}	273,7±11,1^{*2}	266,9±13,2^{*1,3,4}
	IFS	mm of mercury x hart bits per sec	63004,9±5858,3	46520,4±2687,8^{*1}	60903±3863^{*2}	62042±1943^{*2}	57908±3645^{*2}
Reoxygenetion test	LVP	mm of mercury	194,9±9,5	228,5±10,2^{*1}	219,3±10,5^{*1,2}	243,7±9,1^{*1-3}	220,4±6,3^{*1,4}
	$+dp/dt_{max}$	mm of mercury per sec	8488,0±698,1	12221,3±363,4^{*1}	9836,6±338,9^{*1,2}	10965,6±946,3^{*1,2}	8874,7±624,8^{*2-4}
	$-dp/dt_{max}$	mm of mercury per sec	4490,0±320,7	4692,5±219,5	4495,8±653,4	7039,6±495,4^{*1-3}	4755,3±510,9^{*4}
	CCR	hart bits per second	252,3±19,3	269,6±14,9	296,4±20,4^{*1}	231,4±13,4^{*2,3}	240,6±15,6^{*2,3}
	IFS	mm of mercury x hart bits per sec	49670,1±4750,2	62068,7±5006,4^{*1}	64769±5579^{*1}	56293±3561^{*1-3}	52626±2951^{*2-4}

Note: here and in the subsequent tables the asterisk has noted reliable differences of arithmetic averages at p = 0,05; figures near an asterisk indicate a column in relation to which this difference is reliable. Reductions: LVP – the left ventricular pressure, $+dp/dt_{max}$, - dp/dt_{max} - the maximum speeds of reduction and relaxation of a myocardium, CCR – the heart rate, IFS - intensity of functioning of structures.

Using of statins and L-norvalin in correction of violations of functions vascular an endotheliy and contractility function of a myocardium at animals with experimental model endotoxin-induced endothelial dysfunction. The animals with EIED receiving statins and L-norvalin, initial vascular reactions didn't differ from those group of the intact animals or rats who weren't received preparations. When carrying out test on EDVD with AH only in group of the animals receiving L-norvalin it was observed reliable in comparison with group of animals from EDED which weren't receiving preparations, decrease the SABP. When carrying out test from NP on EIVD contractility reactions of a vascular wall of rats from EIED receiving statins and L-norvalin were the same, as in groups with intact animals and when modeling EIED without introduction of preparations. The described changes of vascular reactions on the rats receiving rozuvastatin, symvastatin and L-norvalin (lack of the expressed increase the SABP and DABP at introduction AH), as a result, have led to increase, but not to the corresponding values in group of intact animals, the area of a triangle over a restoration curve ABP in test on EDVD. In turn, these changes, in comparison with model EIED became the reason of statistically essential decrease in CED: the introduction of a rozuvastatin – to 1,6±0,1, a symvastitine – to 2,0±0,2 and L-norvalin – to 1,7±0,4 (table 3).

Table 3

Results of carrying out functional vascular tests on animals with EIED and its correction by means of a rozuvastatin, a symvastatin and L-norvalin (M±m; n=10)

Groups of animals	Functional tests	SABP, mm of mercury	DABP, mm of mercury	CCR, bits per minute	S of vascular reaction till the carrying out EDVD with AH and EIVD with NP, conventional unit.	CED, conventional unit.
Intact	Initial	129,4±2,2	89,2± 1,1	380±4,3		1,1±0,1
	AH	78,6±4,5	38,7±2,8	362±7,1	1268,0±74,8	
	NP	85,8±3,7	45,5±4,4	364±5,4	1375,3±93,7	
Rats with EIED	Initial	117,6±2,3	85,0±2,1	401±8,6		3,7±0,5*
	AH	88,3±2,8*	53,1±2,4*	382±4,2	489,3±75,5*	
	NP	83,3±4,2	40,2±2,3	387±7,5	1535,6±117,3	
Rats with EIED + rozuvastatin	Initial	129,8±2,9	89,1±2,6	372±12,3		1,6±0,1**
	AH	85,0±1,9	46,3±2,0	365±12,8	864,3±60,4*	
	NP	82,0±3,2	44,9±1,7	382±7,5	1375,8±112,2	
Rats with EIED + symvastatin	Initial	121,4±2,8	85,0±2,6	368±8,5		2,0±0,2**
	AH	83,5±2,6	43,3±2,1	375±7,3	727,0±43,1*	
	NP	75,3±3,0	43,8±2,2	399±10,2	1439,7±138,3	
Rats with EIED + L-norvalin	Initial	125,5±2,3	85,9±1,8	391±10,9		1,7±0,4**
	AH	73,0±3,7**	45,5±1,4	379±11	836,8±72,7*	
	NP	88,4±1,6	50,0±3,3	376±9,5	1286,0±150,4	

Note: * - at p =0,05 in comparison with intact animals; ** - at p =0,05 in comparison with the EIED model.

The rats with EIED caused by introduction of staphylococcal endotoxin were observed statistically essential increase of high-speed parameters – $+dp/dt_{max}$ and - dp/dt_{max}. Introduction of rozuvastatin or L-norvalin normalized both broken indicator and symvastatin normalized $+dp/dt_{max}$, but it didn't exert impact on - dp/dt_{max} (table 2).

When carrying out test on an adrenoreactivity on animals with EIED, receiving rozuvastatin, observed normalization of LVP, $+dp/dt_{max}$ and IFS, but decrease - dp/dt_{max}. The group of the animals receiving sumvastatin was normalized LVP and IFS, however in comparison with the rats who weren't receiving preparations, changes of $+dp/dt_{max}$ and - dp/dt_{max} didn't occur. The maximum corrective effects were observed in the group receiving L-norvalin as all studied parameters of contractility function of a myocardium didn't differ from group of healthy animals (table 2).

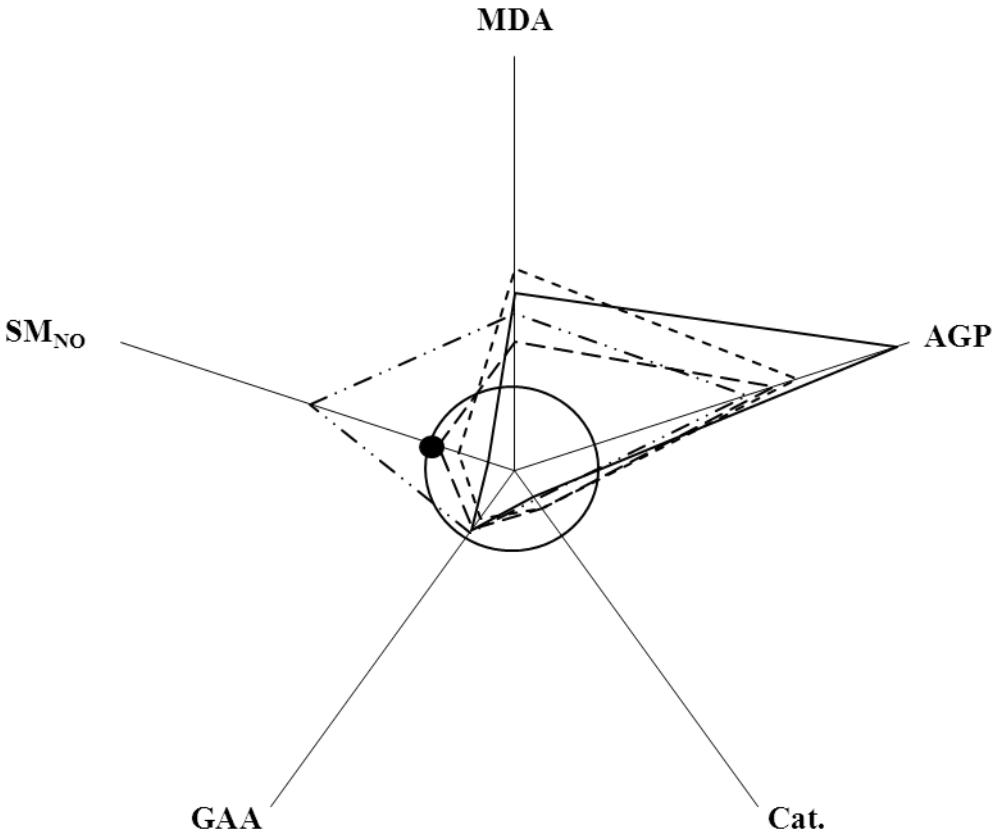
When carrying out test with compression of aorta on the 5th sec. on rats with EIED, receiving rozuvastatin, decrease observed $+dp/dt_{max}$, and indicators - dp/dt_{max}, CCR and IFS – remained raised. $+dp/dt_{max}$ remained with the animals receiving sunvastatin at the normal level, but considerably raised - dp/dt_{max}, CCR and IFS. After introduction the experimental animals with EIED L-norvalin there were normalization of - dp/dt_{max}, decrease $+dp/dt_{max}$, but CCR and IFS were raised (table 2).

On the 25th sec. at statement of test with compression of aorta in group of animals with EIED, receiving rozuvastatin, normalized LVP, CCR, IFS, raised - dp/dt_{max} and the reduced $+dp/dt_{max}$ level didn't change. The rats receiving sumvastatin LVP, CCR and

IFS were normalized, but high-speed parameters of contractility function of a myocardium increased (+dp/dt$_{max}$ and - dp/dt$_{max}$). Introduction of L-norvalin to rats with EIED caused normalization of LVP, IFS, increase of +dp/dt$_{max}$ and decrease CCR (table 2).

In test on a reoxygenetion at animals with EIED receiving rozuvastatin decreased the level +dp/dt$_{max}$ was observed, but CCR and IFS were raised. In group of the animals receiving symvastatin CCR was normalized, LVP, high-speed parameters of contractility function raised (+dp/dt$_{max}$ and - dp/dt$_{max}$), it was exchanges of IFS, but not to indicators of the control group. Against application of L-norvalin for rats with EIED, observed the normalization of +dp/dt$_{max}$ levels, - dp/dt$_{max}$, CCR and IFS which at the same time are slightly raised LVP (table 2).

The rats with EIED were observed the rise of details in intermediate and final products of peroxidation of lipids (MDA, AGP) in blood plasma, decrease of the activity of a catalase, the general anti-oxidizing activity (GAA) of blood plasma and concentration of SM$_{NO}$ (figure 1).



Designations: circle radius – indicators of intact animals;
——— - indicators of rats with EIED;
- - - - - - - indicators of rats with EIED, receiving symvastatin;
– – – – – indicators of rats with EIED, receiving rozuvastatin;
– ·· – ·· – indicators of rats with EIED, receiving L-norvalin;
● - normalized indicators.

Figure 1. Change of oxidatic indicators and level of stable metabolites of nitrogen oxide at rats with EIED against application of statins and L-norvalin

Introduction to rats with EIED of a simvastatin in a dose of 0,5 mg/kg reduced the maintenance of AGP, corrected activity of a catalase, the SM$_{NO}$ level, but it didn't control increase the level of MDA and decrease the level of GAA. The animals with EIED receiving rozuvastatin in a dose of 0,125 mg/kg, concentration of

SM_{NO} was normalized, corrective effects were noted on indicators of products of peroxidation of lipids, activity of a catalase and GAA. Introduction of L-norvalin in a dose of 10 mg/kg to rats with EIED corrected the level of MDA and AGP, but the most maximum level of SM_{NO} and probably at the expense of its corrective effect of GAA was distinctive feature of this connection (figure 1).

By quantity the number of corrected indicators it is possible to claim that rozuvastatin and L-norvalin were slightly more effective than a simvastatin, however the last rendered corrective effects concerning separate indicators of vascular reactions and contractility function of a myocardium on which didn't render significant effects rozuvastatin and L-norvalin. In this regard it is possible to assume that combinations of statin and L-norvalin will possess bigger corrective efficiency of influence on the broken parameters.

The combined application of statins and L-norvalin in correction of violations of functional activity an endothelium and a myocardium on animals with endotoxin induced endothelial dysfunction. Combined using of L-norvalin and rozuvastatin on rats with EIED raised indicators the SABP and DABP in test on EDVD, and in group of the animals receiving a combination of L-norvalin and simvastatin, there was an introduction of NP reliable decrease level of DABP in comparison with EIED rats. In calculation areas of the triangles over restoration curves of ABP in tests with AH and NP, in group of the animals receiving a combination of L-norvalin and rozuvastatin there was revealed statistically significant, in comparison with EIED group, increase in value of area of a triangle over restoration curve ABP in test with AH, close to that at intact animals and statistically significant decrease in this indicator at introduction of NP. The rats from EIED receiving a combination of L-norvalin and simvastatin, corrective changes were noted only concerning area of a triangle over a restoration curve ABP when carrying out test on EDVD, when carrying out test on EIVD area of a triangle over a restoration curve ABP didn't differ from that at intact animals or rats from EIED which weren't receive preparations. So, the combined application of L-norvalin and statins has allowed to achieve more corrective effects on CED, in particular from the animals receiving L-norvalin and simvastatin, CED decreased in comparison with the EIED model to 1,5±0,1, and in the group receiving L-norvalin and rozuvastatin – to 1,2±0,1 (table 4).

Table 4

Results of carrying out functional vascular tests on animals with EIED and its correction by combinations of rozuvastatin, simvastatin and L-norvalin (M±m; n=10)

Groups of animals	Functional tests	SABP, mm of mercury	DABP, mm of mercury	CCR, bits per minute	S of vascular reaction till the carrying out EDVD with AH and EIVD with NP, conventional unit.	CED, conventional unit.
Intact	Initial	129,4±2,2	89,2± 1,1	380±4,3		1,1±0,1
	AH	78,6±4,5	38,7±2,8	362±7,1	1268,0±74,8	
	NP	85,8±3,7	45,5±4,4	364±5,4	1375,3±93,7	
Rats with EIED	Initial	117,6±2,3	85,0±2,1	401±8,6		3,7±0,5*
	AH	88,3±2,8*	53,1±2,4*	382±4,2	489,3±75,5*	
	NP	83,3±4,2	40,2±2,3	387±7,5	1535,6±117,3	
Rats with EIED + rozuvastatin + L-norvaliv	Initial	118,1±2,3	75,6±4,5	400±7,1		1,2±0,2**
	AH	78,4±2,5	36,6±1,6	390±6,8	1124,2±63,7**	
	NP	101,1±4,1**	57,8±2,8**	362±5,8	1011,8±94,6**	
Rats with EIED + symvastatin + L-norvalin	Initial	128,1±2,1	83,1±2,6	376±7,1		1,5±0,1**
	AH	83,2±3,4	40,3±2,7	380±6,8	1090,0±72,9**	
	NP	80,4±2,9	35,9±1,9**	364±5,8	1563,6±135,2	

Note: * - at p =0,05 in comparison with intact animals; ** - at p =0,05 in comparison with the EIED model.

Introduction of L-norvalin with rozuvastatin or simvastatin normalized $+dp/dt_{max}$ and $- dp/dt_{max}$ a myocardium of animals with EIED. However introduction of a combination L-norvalin and simvastatin raised CCR and IFS. When carrying out test on an adrenoreactivity on rats with EIED against application of combinations L-norvalin with rozuvastatin or simvastatin LVP, $+dp/dt_{max}$ and IFS were normalized, decreased the level of $- dp/dt_{max}$ (table 5).

In test with compression of aorta on loading L-norvalin with combination of rozuvastatin or simvastatin on the 5th sec. normalized $- dp/dt_{max}$, CCR, IFS, but it reduced the reduction speed. On the 25th sec. of test version it was a normalization of all broken parameters of contractility function of myocardium animals with EIED (table 5).

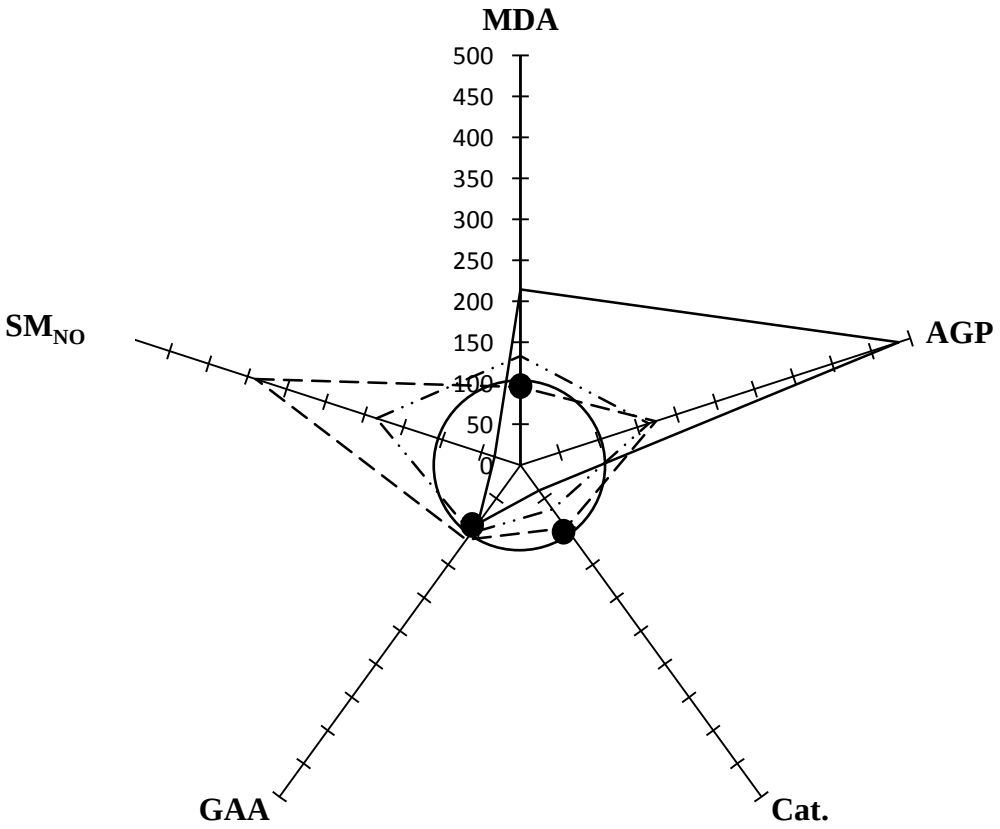
Table 5

Correction of functional activity of myocardium at animals with EIED, combinations of statins and L-norvalin (M±m; n=10)

Indicators \ Groups of animals		Units of measure	1	2	3	4
			Intact	EIED	EIED + rosuvastatin + L-norvalin	EIED + symvastatin + L-norvalin
Without functional trials	LVP	mm of mercury	105,3±4,5	110,1±7,1	105,3±3,8	110,9±4,3
	+dp/dt$_{max}$	mm of mercury per sec	6570,9±783,4	8838,9±340,7^{*1}	6262,2±552,7^{*2}	5714,6±603,9^{*2}
	-dp/dt$_{max}$	mm of mercury per sec	4142,1±452,6	5206,1±255,7^{*1}	3824,5±381,4^{*2}	4016,9±361,0^{*2}
	CCR	hart bits per second	361,3±14,7	372,5±10,5	375,4±7,0	393,5±5,3^{*1-3}
	IFS	mm of mercury x hart bits per sec	38421,1±2849,6	40756,4±2279,9	39500±1557	43716±2050^{*1-3}
Test on adrenoreactivity	LVP	mm of mercury	201,5±9,4	240,3±8,7^{*1}	200,1±9,9^{*2}	210,1±5,2^{*2}
	+dp/dt$_{max}$	mm of mercury per sec	10997,8±777,6	13959,2±735,9^{*1}	10770,0±401,6^{*2}	11646,8±700,9^{*2}
	-dp/dt$_{max}$	mm of mercury per sec	5697,1±650,8	7830,5±520,9^{*1}	6051,7±345,8^{*1,2}	6456,2±434,5^{*1,2}
	CCR	hart bits per second	374,4±14,5	379,0±9,4	376,4±8,3	379,9±9,4
	IFS	mm of mercury x hart bits per sec	76236,2±5813,7	91294,1±4546,3^{*1}	75000±3416,0^{*2}	79884±2947,0^{*2}
Test with compression of aorta 5 sec.	LVP	mm of mercury	233,2±9,9	220,2±6,5	217,5±6,8	219,4±11,1
	+dp/dt$_{max}$	mm of mercury per sec	9227,3±459,3	9555,9±400,2	7153,0±688,3^{*1,2}	7890,1±780,7^{*1,2}
	-dp/dt$_{max}$	mm of mercury per sec	3674,7±207,5	4241,6±275,4^{*1}	3708,8±215,2^{*2}	3949,0±488,4^{*2}
	CCR	hart bits per second	284,9±21,7	366,8±10,6^{*1}	315,0±11,4^{*2}	320,0±16,7^{*2}
	IFS	mm of mercury x hart bits per sec	65459,1±3968,0	80568,6±2804,2^{*1}	68806±3781,0^{*2}	70116±5322,0^{*2}
Test with compression of aorta 25 sec.	LVP	mm of mercury	212,7±10,9	179,4±3,9^{*1}	202,8±7,2^{*2}	200,0±8,7^{*2}
	+dp/dt$_{max}$	mm of mercury per sec	6826,7±458,6	5144,0±238,3^{*1}	6534,3±347,8^{*2}	6639,1±529,4^{*2}
	-dp/dt$_{max}$	mm of mercury per sec	3210,5±261,5	3034,3±237,6	3146,8±230,4	3425,4±344,4
	CCR	hart bits per second	306,8±36,1	259,4±14,4^{*1}	309,4±10,6^{*2}	279,1±21,5^{*2}
	IFS	mm of mercury x hart bits per sec	63004,9±5858,3	46520,4±2687,8^{*1}	62196±1577,0^{*2}	56322±5373,0^{*2}
Reoxygeneti on test	LVP	mm of mercury	194,9±9,5	228,5±10,2^{*1}	196,5±5,2^{*2}	213,7±7,2^{*2}
	+dp/dt$_{max}$	mm of mercury per sec	8488,0±698,1	12221,3±363,4^{*1}	9150,5±383,3^{*1,2}	9937,2±500,6^{*1,2}
	-dp/dt$_{max}$	mm of mercury per sec	4490,0±320,7	4692,5±219,5	5047,6±496,1	4550,6±390,7
	CCR	hart bits per second	252,3±19,3	269,6±14,9	286,7±16,3	276,1±20,1
	IFS	mm of mercury x hart bits per sec	49670,1±4750,2	62068,7±5006,4^{*1}	56149±3179^{*1,2}	58749±4200^{*1,2}

When carrying out test with a reoxygenetion on animals was normalized with EIED against application of L-norvalin and rozuvastatin or simvastatin LVP, indicators of +dp/dt$_{max}$ and IFS decreased, but not to control values. reliable changes - dp/dt$_{max}$ and CCR didn't take place (table 5).

In group of animals from EIED receiving a combination of L-norvalin and simvastatin GAA was normalized, the level of MDA and AGP decreased, the activity of catalase corrected and the maintenance of SM_{NO} increased. L-norvaline combination with rozuvastatin normalized MDAL, activity of catalase, reduced the AGP level and increased concentration of SM_{NO}, GAA (figure 2).



Designations: circle radius – indicators of intact animals;
_____ - indicators of rats with EIED;
_..._.. - indicators of rats with EIED, receiving symvastatin + L-norvalin;
_ _ _ _ indicators of rats with EIED, receiving rozuvastatin + L-norvalin;
● - normalized indicators.

Figure 2. Change of oxidatic indicators and level of stable metabolites of nitrogen oxide on rats with EIED against the combined application of statins and LL-norvalin

As a matter of record it is possible to claim that L-norvalin combinations to statines were more effective, than separate application of preparations in correction of functional activity of endothelium and myocardium, metabolic indicators of animals with EIED. At the same time the combination of L-norvalin and a rozuvastatin was more effective.

The estimated mechanism of development of endothelial dysfunction at inflammatory process which has made a work basis, following: the monocytes activated under the influence of bacterial endotoxin migrate in a subendotelialny layer, allocate cytokine, active radicals of oxygen, activate endoteliocite which in turn allocate adhesive molecules, hem attractant (attract neutrophils and monocytes in the inflammation center), cytokine. All these processes in total strengthen processes of an inflammation, aggregation of blood cells, violation of processes of microcirculation. Besides, there is an activation the process of peroxidation which products

damage endoteliotcite. In this situation synthesized L-arginin NO at interaction with active radicals of oxygen is left on education by peroxynitrite of the ion possessing most expressed cytotoxic activity. Besides, the peroxidation, active radicals of oxygen, cause oxidation of lipoprotein of lower density which together with peroxide products are taken fabric macrophages, are transformed to foamy cages that causes remodeling of vessels. At the macromorphological level the vazokonstriktion, microthrombosis in internals (kidneys, a liver, heart) is result of such pathogenetic mechanisms, as becomes the main reason for a lethal outcome at EIED [20, 21].

NO which is formed during activation of enzymatic systems of endoteliotsite in the conditions of infectious process contacts radicals of oxygen, forming the peroxynitrites aggravating a current of septic shock and ischemia of bodies as this connection suppresses work of a cycle of Krebs and activity of a ribonukleotidreduktaza inhibits formation of energy in mitochondrions. In turn, bacterial endotoxin intensifies synthesis of the major proapoptotichesky factor – a squirrel r53, figuratively called «the guard of a genome». On the other hand, the induction of eNOS under the influence causes in the endothelial cages morphological and biochemical changes, typical for apoptosis, at the same time dependence of activity of apoptosis on concentration radical superoxide of oxygen and nitrogen oxide is proved. The balanced proportion of these substances in a cage which is an important factor of regulation of apoptosis possesses the most expressed antiapoptotichesky action [22].

Estimated points of application of preparations in the development mechanism sepsis - the induced endothelial dysfunction the following: statins possess the expressed anti-inflammatory activity, thereby modulate activity of macrophages, endoteliocite, reduce aggregation of blood cells, concentration of a substratum for processes the peroxidation, thus under the influence of statin expressiveness of inflammatory reaction in a wall of vessels decreases, functional activity of macrophages, endothelium is normalized. L-norvalin, in turn, blocks the arginaza turning L-arginin in ornitin that is provides increase of concentration of a substratum (L-arginin) for a NO, and, as a result, raises effective concentration. At the expense of it there is a vazodilatation and microcirculation in internals improves [23, 24]. Similar results were obtained on a similar model using statins meldonium and selective inhibitor of arginase 2 [25, 26, 27].

References

1. Bogacheva V.V., Gardovska D.X. Sepsis markers. XXI Century. Therapeutic archive. Vol. 86, №3 (2014): 111-4. [eLIBRARY]
2. Lazanovich V.A., Markelova E.V., Smirnov G.A., Smolina T.P. Clinical significance of Toll2, Toll4, CD14, AND HLA- DR expression on the monocytes in patients with sepsis. Medical Immunology. Vol. 17, №3. (2015): 221-8. [eLIBRARY] [Full text]
3. Loktionova I.L., Pokrovsky M.V., Ragulina V.A. [et al.], The status of vascular endothelial function in infectious diseases of various etiologies. Belgorod State University Scientific bulletin. Medicine Pharmacy. Vol. 17/1, № 4 (123) (2012): 20–31. [eLIBRARY] [Full text]
4. Konoplya A.I., Lazarenko V.A., Loctionov A.L. Interrelation of the immunometabolic and the erythrocyte dependent violations with etiology of acute pancreatitis. Kursk: Publishing KSMU (2013): 162. [eLIBRARY]
5. Tsepeleva S.A., Pokrovsky M.V., Pokrovskaya T.G. [et al.] Cardio- and endothelioprotective effects of arginase inhibitor l-norvalin at modelling l-name indused deficiency of nitric oxide. Kuban Research Medical Bulletin. №4. (2011): 185–8. [eLIBRARY] [Full text]
6. Pokrovsky M.V., Alehin S.A., Lopatin D.V. L-norvalin and ischemical preconditioning action mechanisms analysis at liver ischemia/reperfusion. I.P. Pavlov Russian Medical Biological Herald. № 1. (2013): 56-9. [eLIBRARY] [Full text]
7. Pokrovsky M.V., Pokrovskaya T.G., Gureev V.V. Correction of endothelial dysfunction by L-Arginine under Experimental Pre-Eclampsia Conditions. Russian Journal of Experimental and Clinical Pharmacology. Vol.75. №2. (2012): 14-16. [eLIBRARY] [Full text]
8. Bedilo N.V., Vorobyova N.A., Ismailova N.V. Evaluetion of haemostasis and endothelial dysfunction in patient with community-acquired pneumonia. Russian journal of Anesthesiology and Reanimatology. № 1. (2014): 33-7. [eLIBRARY] [Full text]
9. Goldenberg N.M., Steinberg B.E., Slutsky A.S. [et al.] Broken barriers: a new take on sepsis pathogenesis. Sci. Transl. Med. Vol. 3. №88. (2011): P. 88ps25. [PubMed] [Full text]
10. Yang K.Y., Liu K.T., Chen Y.C. [et al.] Plasma soluble vascular endothelial growth factor receptor-1 levels predict outcomes of pneumonia-related septic shock patients: a prospective observational study. Crit. Care. Vol. 15. № 1. (2011): P. R11. [PubMed] [Full text]
11. Chemomortseva E.S., Smimova E.I., Pokrovskaya T.G. [et al.] Cardio- and the endothelioprotective effects of macrolide antibiotics in the experiment. Kuban Research Medical Bulletin. № 1-2 (94-95). (2007): 184-7. [eLIBRARY] [Full text]
12. Pokrovsky M.V., Ivlitskaya I.L., Provotorov V.Ya. Efficiency of statins and L-norvalin in correction of violations of contractility function of a myocardium and haemodynamic indicators at animals with endotoxin model of sepsis. Questions of science and education: theoretical and practical aspects. Samara. (2015): 363-5. [eLIBRARY]

13. Ivlitskaya I.L., Pokrovsky M.V., Provotorov V.Ya., Pokrovskaya T.G. Immunomodulation, endotelyo- and cardioprotective effects of statins and L-norvaline in conditions endotoxin-induced endothelial dysfunction. Medical Immunology. Vol. 17. № 4. (2015): 377–8. [eLIBRARY] [Full text]

14. Korokin M.V., Pokrovskii M.V., Kochkarov V.I. Research of endothelio-and cardioprotective effects of enalapril, lozartan and amlodipin at modelling hyperhomocystein induced endothelial dysfunction. I.P. Pavlov Russian Medical Biological Herald. №1. (2014): 60-65. [eLIBRARY] [Full text]

15. Svistunov A.A., Osadchuk M.A., Vasilyeva I. N. Rozuvastatin in therapy of patients with coronary heart disease in out-patient practice. Atmosphere. Cardiology news. №3 (2015): 48-56. [eLIBRARY] [Fulltext]

16. Ivlitskaya I.L., Pokrovsky M.V., Provotorov V.Ya., Endotelioprotektive effects of statin and L-norvalin at animals with endotoxin-induced endothelial dysfunction. Questions of science and education: theoretical and practical aspects: materials international scientific conference Research Center Povolzh. Sci. corporation, (25 Dec. 2015. Samara): 361-3 [eLIBRARY]

17. Chernomortseva E.S. Influence of macrolides on the functional possibilities of endothelium and myocardium with the experimental model of l-name induced endothelial dysfunction. Kursk scientific and practical bulletin «Man and his health». №1 (2010): 30-4. [eLIBRARY] [Full text]

18. Merzhvinsky I.A. Role of lymphatic system in pathogenesis and treatment of sepsis: doctorate dissertation. (2002): 35. [eLIBRARY]

19. Korokin M.V., Pokrovskii M.V., Kochkarov V.I. Research of endothelio-and cardioprotective effects of enalapril, lozartan and amlodipin at modelling hyperhomocystein induced endothelial dysfunction. I.P. Pavlov Russian Medical Biological Herald. №1 (2014): 60-5. [eLIBRARY] [Full text]

20. Markov H.M. Vascular effects of lipoproteins and nitrogen oxide: cellular and molecular mechanisms. Pathological physiology and experimental therapy. №3 (2006): 2-7. [eLIBRARY]

21. Torreilles J., Guérin M.C. Does nitric oxide stress exist? C R Seances Soc Biol Fil. Vol.189. №3. (1995): 389-400. [PubMed]

22. Rahman S.H., Ibrahim K., Larvin M. et al. Association of antioxidant enzyme gene polymorphisms and glutathione status with severe acute pancreatitis/ Gastroenterology. – 2004. – Vol. 126, N 5. – P. 1312-1322. [PubMed] [Full text]

23. Bochenina Yu.A., Kuznetsov G.E., Tenchurina L.R. Dynamics of change of vasomotor function an endothelium and indicators of remodeling of heart at patients with chronic heart failure against treatment of rozuvastatin. Consilium Medicum. Vol.16. №10. (2014): 98-102. [eLIBRARY] [Full text]

24. Dobrovolsky A.V. Rozuvastatin – an effective and safe modern gipolipid preparation. Russian medical journal. Vol.20, №14. (2012): 728-33. [eLIBRARY] [Full text]

25. Skachilova S.Y., Kesarev O.G., Danilenko L.M., Bystrova N.A., Dolzhikov A.A., Nikolaev S.B. Pharmacological correction of L-NAME-induced oxide deficiency with derivatives of 3-(2,2,2-trimethylhydrazinium) propionate. Research result: pharmacology and clinical pharmacology. 2016. Vol. 2, №1 (2): 36-41. [Full text]

26. Yakushev V.I., Filippenko N.G., Kizilova I.V., Korokin M.V., Beskhmelnitsyna E.A., Litvinova A.S. Studying dosedependent endothelio- and cardioprotective activity of selective arginase II inhibitor in hyperhomocysteineinduced endothelial dysfunction. Research result: pharmacology and clinical pharmacology. 2016. Vol. 2, №1 (2): 42-45. [Full text]

27. Denisyuk T.A., Lazareva G.A., Provotorov V.Yu., Shaposhnikov A.A. Endothelium and cardioprotective effects of HMG-Co-Areductase in combination with L-arginine in endothelial dysfunction modeling. Research result: pharmacology and clinical pharmacology. 2016. Vol. 2, №1 (2): 4-8. [Full text]