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PHARMACOTHERAPEUTIC STRATEGIES FOR ENDOTHELIAL DYSFUNCTION CORRECTION WITH USE OF STATINES IN SYNDROME OF SYSTEMIC INFLAMMATORY RESPONSE

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Abstract

Objectives: The ways of pharmacological correction of cardiovascular complications in the syndrome of the systemic inflammatory response (SIRS) are not fully developed.

Goal: Determination of statins' new pharmacological effects and its combination with endothelioprotectors in the SIRS correction.

Materials and Methods: In experiments on mice, rats and rabbits, the anti-inflammatory, cardioprotective and endothelioprotective effects of statins and endothelioprotectors were explored. The modeling of endotoxin-induced endothelial dysfunction (EIED) was created by infecting rats with *Staphylococcus aureus* (strain 13407), subcutaneously (60 billion microbial bodies). To determine the activity of the inflammatory process, the indices of the C-reactive protein used. The involvement of the cytokine link of inflammation was assessed according to the plasma levels of TNF- α and IL-6. Modeling of L-NAME-induced pathology and further evaluation of endothelial and endothelium-independent vascular reactions were carried through according to a standard protocol.

Results: Simvastatin (9, 19, 35 mg/kg), atorvastatin (5, 9, 19 mg/kg), rosuvastatin (9, 19, 35 mg/kg) and nanoparticulated rosuvastatin (3, 6.3 and 11.6 mg/kg) proved a dose-dependent antiexudative effect on the model of formalin paw edema in mice. Similarly, the anti-inflammatory effect is evident in the exudative model on rabbits. The greatest effectiveness was demonstrated by rosuvastatin and its nanoparticulated form. Simvastatin 8.5 mg/kg, atorvastatin 4.3 mg/kg, rosuvastatin 8.5 mg/kg, and nanoparticulated rosuvastatin 11.6 mg/kg demonstrate a cardioprotective effect in the coronary-occlusive modeling of infarction in rats. In the process of cardioprotective effects' implementation the mechanisms of pharmacological preconditioning has significant importance. It was proved by the removal of effects with K⁺-ATP-ase channels blockade with glibenclamide (5 mg/kg) and iNOS blockade with aminoguanidine (40 mg/kg).

The use of inhibitors HMG-CoA reductase of simvastatin (2.2, 4.3 and 8.5 mg/kg), atorvastatin (1.1, 2.2 and 4.3 mg/kg), rosuvastatin (2.2, 4, 3 and 8.5 mg/kg) and nanoparticulated rosuvastatin (3, 6.3 and 11.6 mg/kg) on the background of endotoxin-induced pathology modeling leads to the development of a dose-dependent endothelioprotective effect, which is expressed in normalization of coefficient of endothelial dysfunction (CED), to prevention of adrenoreceptivity increase and to the exhaustion of the myocardial reserve, as well as to the normalization of biochemical markers of inflammation (C-reactive protein) and the level of pro-inflammatory cytokines. At the same time, positive dynamics of the final products of NO and eNOS expression was defined.

Applying of monotherapy with donator NO L-arginine (70 and 200 mg/kg), a nonselective inhibitor of arginase BEC (5 and 10 mg/kg), a selective inhibitor of arginase-2 arginasine (1 and 3 mg/kg) and recombinant darbepoetin (50 and 500 µg/kg) in the modeling of endothelial dysfunction, has revealed their high activity, expressed in preventing the increase in CED, adrenoreactivity, preservation of the myocardial reserve and normalization of the values of biochemical markers (Total NO, eNOS expression, C-reactive protein, IL-6, TNF). Herewith, the drugs had a dose-dependent effect and were approximately equally effective.

A vector analysis of the additive effects of the combined use of inhibitors of HMG-CoA reductase, simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin with L-arginine, inhibitors of arginase – BEC and arginasine, as well as darbepoetin demonstrated that, with endotoxin-induced pathology, the highest probabilistic percentage of addiction was found in combination of rosuvastatin with arginasine (3 mg/kg) and darbepoetin (500 µg/kg), relatively, 31.9 ± 2.8 and $30.2 \pm 2.9\%$.

Discussion: The inhibitors of HMG-CoA reductase have demonstrated cardioprotective (decrease of adrenoreactivity and depletion of myocardial reserve, normalization of blood pressure) and endothelioprotective (amplification of eNOS expression, increase of NO) the attributes, which manifested itself as well as in monotherapy and in combination with some endothelioprotectors (L-arginine, arginasine, BEC, darbepoetin) in varying degrees.

Keywords: endothelial dysfunction, nitric oxide, statins, endothelioprotectors, systemic inflammatory response syndrome.

Introduction

Inflammation is a distinctly coordinated systemic reaction of the body, focused on eliminating of the damaging agent. However, rather often an inflammatory reaction causes more damage to the body than the etiologic factor that had induced it [1]. More than 100 years ago, William Osler in his reflections about sepsis had correctly suggested that, except some rare cases, the cause of death in a septic process is not an infection, but the body's response to it. A contemporary pathophysiology explains this phenomenon through the concept of a systemic inflammatory reaction (SIRS), considered as a response of the organism to various flogogenic effects of infectious and non-infectious origin [2]. The scientific researches of the last decade led to the formation of an integral picture describing SIRS through a cascade of neurohumoral reactions, formed by complex mediator and cytokine nets. Describing the pathogenesis of systemic inflammation, several main stages can be identified: 1) presentation of lipopolysaccharide on the surface of the endothelium, massive stimulation of the pattern-recognition cells receptors of immune and reticuloendothelial systems; 2) the release

of inflammatory mediators (bradykinin, prostaglandins, histamine, proinflammatory cytokines) and the change of the expression molecules profile of intercellular interactions (integrins, selectins) followed by leukocyte adhesion and iNOS activation; 3) the growth of cytotoxic NO concentrations with the formation of active forms of nitrogen, the breakdown of intercellular contacts and the oxidation of LDL, the change of the antithrombotic properties of the endothelium to prothrombotic and the migration of leukocytes through the wall of micro vessels with further infiltration of tissues and the absorption of oxidized LDL; 4) hyperproduction of ROS, vasodilation, microthrombosis and systemic manifestations of SIRS; 5) death due to the gradual decompensation of life-support processes at the systemic level or recovery [3]. It is easy to see that one of the central participants of this continuum is the endothelium, which does not just produce the widest spectrum of humoral factors, but also realizes the processes of exudation, thrombosis and hemodynamics control. The more interesting fact is that the majority of patients who underwent abdominal catastrophes [4, 5, 6, 7], sepsis [8, 9, 10] and other infectious nosology associated with SIRS

[8, 11, 12] after regression of the pathological process on various terms are developing complications associated with endothelial

dysfunction (atherosclerotic vascular lesions, myocardial infarction, cerebral stroke) [13, 14] (Fig. 1).

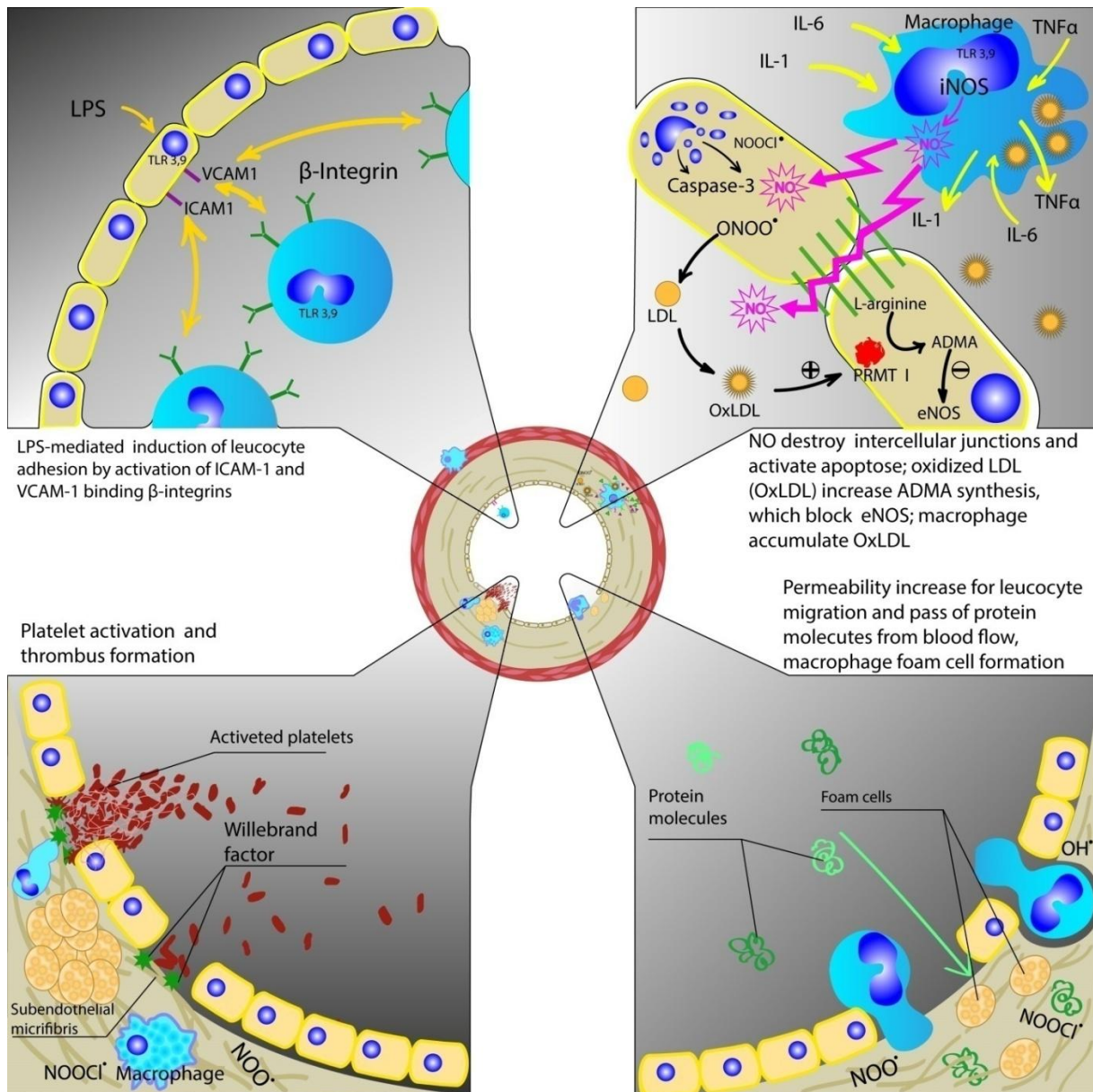


Fig. 1. Pathogenesis of vascular wall damage in SIRS.

Note: Pathogenesis of the formation of an inflammatory injury of the vascular wall with subsequent atherogenesis and thrombus formation. LPS binds to the Toll-receptors of the endothelial cell, enhancing the release of pro-inflammatory cytokines and the expression of adhesion receptors, to which leukocytes are attracted by β- integrins followed by iNOS activation and massive NO release, the latter forming the peroxynitrite (ONOO⁻) and nitrosyl chloride (NOOCl) radicals, damaging cellular structures and dissociating eNOS. As a result, intercellular contacts are destroying; oxidized by free radicals of LDL induce the cascade of reactions, leading to an even greater dissociation of eNOS. Disturbance of cells structures contributes to increased vascular permeability and migration of leukocytes, their absorption of oxidized LDL and formation of foamy cells – harbingers of atherosclerotic plaques. The destruction of the endothelium and exposure of the subendothelial layer creates conditions for the activation and aggregation of thrombocytes with thrombosis

At the same time, with the coherence of the pathogenetic regimens and the inclusion of multiple factors (VEGF, sFlt-1, autoantibody of receptor angiotensin II (type 1) (AT1-AA), cytokines (tumor necrosis factor (TNF) - α), endothelin, active forms of oxygen (ROS), thromboxane, 20-hydroxyicosatetraenoic acid (20-HETE), increased sensitivity to angiotensin II, etc.), the inadequacy of pharmacotherapeutic strategies pointed to correction of endothelial dysfunction in acute systemic inflammation is obvious. Besides, we can suppose the mechanisms underlying the ischemic preconditioning may play a role in pathogenetic approaches to the correction of multiple organ failure in endotoxin-induced pathology, including myocardium, brain, kidney, retina [15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25]. From this regard the inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) – reductase are very perspective. Being the gold standard of lipid-lowering therapy [26, 27, 28, 29, 30, 31, 32, 33, 34], statins continue to attract the attention of researchers because of their inherent pleiotropic effects [35, 36, 37], including cardioprotective [38, 39] anti-inflammatory [40, 41] and endothelioprotective [42]. These effects do not depend upon cholesterol decreasing, but caused by the main mechanism of action – inhibition of HMG-CoA reductase. It is concerned with the fact that a number of intermediate products in the chain of cholesterol synthesis, such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, have pro-inflammatory, prooxidant and several other negative attributes are realizing through the activation of secondary mediators Ras Rac1, Rho [43].

It is shown that activation of Rho is associated with inhibition of eNOS, and an increase of leukocytes migration through the vascular wall. The activation of Ras is connected with the synthesis initiation of pro-inflammatory cytokines, while the activation of Rac1 is connected with an increase in mitochondrial NADPH oxidase activity, what contributes to the oxidative stress development of [44].

Numerous researches with the use of different models had convincingly demonstrated that statins possess anti-apoptotic

activity and reduce the level of proinflammatory cytokines, chemokines and adhesion molecules. Antithrombotic effects of statins can improve the coagulopathy caused by sepsis. By influencing on the modification of proteins, they prevent the presentation of the antigen by the endothelial cell and recognition by the immune cells. The ability of statins to increase eNOS expression and reduce iNOS concentration has been also demonstrated. It has a great importance both for limiting the extent of damage in SIRS and for limiting the extent of endotoxin-induced endothelial dysfunction as well [45].

On the other hand, endothelium is a classic target of such pharmacological agents as L-arginine, BEC and darbepoetin. Theirs mono- and combination therapy has been proved as a successful in treating of a wide range of cardiovascular pathologies [46].

From this aspect, the pharmacological target "ADMA-eNOS", which was the research object of Kursk's and after of Belgorod's pharmacologists [47, 48, 49, 50, 51, 52], and also of their colleagues from Volgograd, has a definite interest [53, 54, 55, 56].

Methylated analogs of L-arginine – asymmetric dimethylarginine (ADMA) and monomethylarginine (L-NMMA) – are endogenous inhibitors of endothelial nitric oxide synthase (eNOS). In recent years, it has been found that ADMA concentrations in endotoxin shock are increasing and elevated concentrations of ADMA are one of the key risk factors of the cardiovascular nosological continuum [57].

Taking into consideration the fact that one of the main pathogenetic aspects of SIRS development is the imbalance between the inducible and endothelial isoforms of NOS [58], pharmacotherapeutic correction of endothelial dysfunction in systemic inflammation should be aimed at overcoming the internal inhibition of eNOS, including ADMA.

The researches on overcoming the inhibitory effect of ADMA by the introduction of L-arginine [59], tetrahydrobiopterin [60], a nonselective inhibitor of arginase L-norvaline [61], selective inhibitors of arginase-2

arginasine [62] and others has got a considerable development nowadays.

Based on this point of view, HMG-CoA reductase inhibitors and endothelioprotectors can potentially have complementary effects in prevention and treatment of endothelial dysfunction and other pathological conditions associated with systemic inflammation.

Accordingly, there are significant theoretical prerequisites for the use of drugs aimed at correcting endothelial function (in particular, statins and endothelioprotectors) in complex treatment and in prevention of SIRS.

Hereby one of the main elements of the proposed pharmacotherapeutic strategies is statins. This group of drugs is in demand all over the world and some of them are so-called blockbusters with sales volumes of more than 5-7 billion dollars per year in financial equivalent. From the chemical point of view, statins are represented by 8 international non-proprietary names, different in lipo- and hydrophilicity and also in a number of pharmacokinetic and toxicological indicators. Simvastatin and atorvastatin are the most prevalent. But rosuvastatin is up-to-date.

Objective: to study the effectiveness of pharmacological correction of endotoxin-induced pathology by statins using (simvastatin, atorvastatin, rosuvastatin, nanoparticulated rosuvastatin) and their combinations with L-arginine, BEC arginase inhibitors and selective inhibitor of arginase-2 arginasine, and also by recombinant darbepoetin.

Materials and Methods

The experiments were made on healthy matured rats of the Wistar line, weighing 180-250 g; laboratory mice weighing 18-22 g; and also rabbits weighing 2-2.5 kg. In the experiments, we used animals that passed through the quarantine regime of the Kursk State Medical University vivarium and did not have any external signs of any diseases. All animals were kept in the same conditions, on a normal diet. For the statistically reliable results, the groups were formed from 18-20 animals. The animals that were included into a control and into an experimental group were of the same age and came from the Stolbovaya cattery

of the Russian Academy of Medical Sciences. The spread in groups according to the initial mass did not exceed -10%. All experiments were carried out at the same time of the day, from 8 a.m to 12 a.m, in accordance with the principles set forth in the Convention for the Protection of Vertebrates used for experimental and other purposes (Strasbourg, France, 1986) and in accordance with the rules of laboratory practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 267 of June 19, 2003).

The investigation of the anti-inflammatory effect on the model of formalin edema of mice: The experiments were made on laboratory mice weighing 18-22 g. The anti-inflammatory effect was evaluated in conditions of acute aseptic inflammation of the formalin-induced mice, according to the degree of inhibition of the edema of the foot increasing by the drugs in comparison with the control group of untreated animals. Exudative edema was induced by subcutaneous injection into the right rear foot of mice with 0.02 ml of a 2% aqueous solution of formalin. The mass of the foot was measured in 4 hours (at the peak of the edema) after injection of phlogistics on electronic scales with an accuracy of 1 mg; as a control measure the left paw of the same animal was used, which was injected with an equal volume of isotonic NaCl solution in parallel with the injection of the flogogenic agent. The inhibitory effect was calculated by the formula:

$$Eing = \frac{(\Delta Mc - \Delta Me) \times 100\%}{\Delta Mc},$$

where *Eing* – the inhibitory effect, ΔMe and ΔMc – the average increase of the edematous foot mass in the control and experimental groups.

The anti-inflammatory effect investigation according to I.A. Oivin method. The experiments were made out on laboratory rabbits (3 rabbits in each group) weighing 2-2.5 kg. To investigate the anti-inflammatory activity of the drugs using the Oivin method, the rabbits were fixed; previously hair has been cut on the abdominal skin (13 x 5 cm piece). Tripanum coeruleum (permeability indicator) was injected into the

edge vein of the ear in the form of a 1% solution on a 0.9% solution of sodium chloride at a rate of 2 ml per 1 kg of animal weight. Then 6-12 drops of o-xylene were applied to the area of the abdominal skin. The indicator of the permeability of capillaries was the time of appearance of blue-stained spots on the skin and their diameter. By the difference in the time of appearance of the spots and their diameter before and after the injection of the drug, it was judged on its effect on the permeability of capillaries.

A simulating model of coronary-occlusive myocardial infarction in rats and evaluation of the magnitude of the necrosis zone.

The experiments were made on male Wistar rats of 200-250 g. The drug was injected intraperitoneally 1 time per day and 30 minutes before modeling myocardial infarction (MI). MI was reproduced on anesthetized animals by bandaging the descending branch of the left coronary artery on the lower edge of the left atrial appendage level, then the wound was layer-by-layer sutured.

On the 4th day, the animal was killed, the heart was removed, and crossed sections were made every 4-6 mm, and then incubated in a 1% solution of triphenyltetrazolium of blue in phosphate buffer pH 7.4 for 15 minutes at 37 ° C. This method makes it possible to visualize clearly the necrosis zone (not colored) for further examination.

Quantitative analysis and calculation of the area of necrotic tissue was carried out by means of the graphic editor Adobe Photoshop CS5 [63].

To analyze the participation of K⁺-ATPase channels and iNOS in the realization of the protective effects of pharmacological preparations, glibenclamide [64, 65] and aminoguanidine [66, 67] were used.

A simulating model of endotoxin-induced endothelial dysfunction.

In the modeling of endotoxin-induced endothelial dysfunction, the rats were infected with *Staphylococcus aureus* (strain 13407), subcutaneously with 60 billion microbial bodies, with followed by (after 24 hours) sensitization of the laboratory animal (0.1 ml of staphylococcal anatoxin subcutaneously),

followed by generalization of the infectious agent by daily injection place massage. Further, a compression, pneumatic massage of the injection place was performed daily during 10 minutes [68].

To determine the activity of the inflammatory process, the indices of the C-reactive protein were used. The involvement of the cytokine link of inflammation was estimated according to the plasma content of TNF- α and IL-6, determined by immunoassay methods using the Alfa-TNF- α -IFA-Best reagents kits; "IL-6-IFA-Best" [69, 70, 71].

Modeling of L-NAME-induced gestosis and estimation of endothelium-dependent and endothelium-independent vascular reactions (M.V. Pokrovsky and co-auth., 2006).

N-nitro-L-arginine methyl ether (L-NAME) was injected to males daily, once a day, intraperitoneally at a dose of 25 mg/kg. On the 8th day from the beginning of the experiment, an L-NAME-induced and hyperhomocysteine-induced endothelial dysfunction a catheter was put in (under anesthesia by chloral hydrate 300 mg/kg) into the left carotid artery to record hemodynamic parameters. Hemodynamic parameters: systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured continuously, using the Biopac hardware complex (USA) and the computer program "Acqknowledge 3.8.1". Bolus injection of pharmacological agents was made into the right femoral vein. The following functional tests were performed: intravenous acetylcholine (40 μ g/kg) [72] and sodium nitroprusside (30 μ g/kg) [73].

A study of myocardial contractility after pathology modeling was made in narcotized rats on controlled respiration. The left ventricle cavity was probed with a needle through the apex of the heart and by means of the hardware complex "Biopac" (USA), the computer program "Acqknowledge 3.8.1", the parameters of cardiohemodynamics (left ventricular pressure, maximum reduction rate (+ dp / dt max), maximum relaxation rate (-dp / dt max), heart rate (heart rate) were registered.

To estimate the functional capabilities of the myocardium in animals, burthen tests were performed in the following sequence:

1. Adrenoreactivity test – intravenous one-stage injection of an adrenaline hydrochloride solution $1 \cdot 10^{-5}$ mol/l, calculated on the basis of 0.1 ml per 100 g of body weight [74].

2. Resistance test – clamping of the ascending aorta by 30 sec. [75].

Biochemical markers of endothelial dysfunction.

A modification of the method for the determination of stable metabolites of NO was used; it allows one-step quantitative determination of total nitrates and nitrites after deproteinization of blood serum [76]. The principle of the method is in simultaneous reduction of nitrates to nitrites in the presence of chloride bath tubing and the reaction of

diazotization followed by the development of color.

The level of expression of endothelial nitric oxide synthase (e-NOS) was determined in a cell lysate by using the Hendrickson method [77]. Detection of the eNOS band was carried out by the method of enhanced chemiluminescence (ECL).

Justification of doses and the experiment design:

In this research, the study of endothelial and cardioprotective activity of statins in doses was made; anti-inflammatory effects were detected before [78, 79]. In chronic experiments in the modeling of EIED and L-NAME-induced endothelial dysfunction, drugs were injected at appropriate doses once a day intraperitoneally or intragastrically for 7 days 30 minutes after L-NAME injection.

Table 1
The correlation of doses of inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin for humans and experimental animals (rabbits, rats and mice) [80]

Drug	Human doze, mg	Rabbit mg/kg	Rat mg/kg	Mouse mg/kg
Simvastatin	5	2.2	4.3	9
	10	4.3	8.6	19
	20	8.5	17.2	35
Atorvastatin	2.5	1.1	2.2	5
	5	2.2	4.3	9
	10	4.3	8.6	19
Rosuvastatin	5	2.2	4.3	9
	10	4.3	8.6	19
	20	8.5	17.2	35
Nano-rosuvastatin	-	0.73	3	6.2
	-	1.43	6.3	13.9
	-	2.83	11.6	23.6

Simvastatin "Zokor"[®] tablets, film-coated 20 mg, 28 pcs. packing contour mesh 14, cardboard pack 2 code EAN: 4601969006711

No. P N013094 / 01, 2011-10-24 Merck Sharp & Dohme B.V. (Netherlands).

Atorvastatin "Lipitor" Tablets of round form 10, 20, 40 mg in a shell in a blister in cardboard bundle No. 30. Manufacturer: Gedeke / Parke-Davis, Pfizer, Inc. (Germany).

Rosuvastatin "Crestor" Crestor[®] tablets, film-coated 10 mg, 7 pcs. blister 7, a pack of cardboard 1 code EAN: 7321838721251 No. П N015644 / 01, 2009-03-24 AstraZeneca UK Ltd. (United Kingdom).

Nanoparticulated-rosuvastatin, substance. Manufactured in V.N. Orekhovich Research Institute of Biomedical Chemistry (Russian Federation).

Glibenclamide Maninil® 5 tablets 5 mg, 120 pcs. a bottle (bottle) 120, a pack of cardboard 1 code EAN: 4013054000922 No. П N011519 / 01, 2012-03-07 Berlin-Chemie AG / Menarini Group (Germany).

Aminoguanidine Aminoguanidine hydrochloride (Sigma-Aldrich) CAS Number 1937-19-5 Linear Formula NH₂ NHC (= NH) NH₂ • HCl9.

A study of *L-arginine* produced by EUROBIOPHARM GmgH (Hamburg) was made by intraperitoneal injection at doses of 70 and 200 mg/kg/day. This dose is selected in accordance with the literature data, where the dosage range for the L-arginine activity is in the range of 30 mg/kg to 200 mg/kg [81].

A nonselective inhibitor of arginase – *S*-(2-boro-ethyl) -*L*-cysteine (BEC), (WIRUD GmgH, Hamburg) was injected at a dose of 10 mg/kg/day. According to modern researchers, BEC is able to inhibit arginase [81] exactly from this dosage.

Recombinant darbepoetin “Aranesp” injection solution 0.3 mg, 1 unit syringes 0.6 ml Amgen Europe B.V. (Netherlands). It was injected intraperitoneally at doses of 50 and 500 µg/kg for 7 days. (K.M. Reznikov, co-authors, 2017) [83]

Selective inhibitor of arginase-2 arginase (laboratory code ZB49-0010) synthesized in TVC “ChemRar” was administered intraperitoneally at a dose of 1-3 mg/kg/day.

Design of the experiment on the study of cardio and endothelioprotective activity of inhibitors of HMG-Co-A-reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in combination with L-arginine, BEC. Arginase and darbepoetin consists of the following blocks:

1. Control group.
2. Simulation of EIED or L-NAME-induced deficiency of nitric oxide.
3. Estimation of endothelial and cardioprotective effects of inhibitors of HMG-Co-A-reductase of simvastatin and L-arginine, BEC, arginase and darbepoetin.
4. Appraisal of the effectiveness of endothelial and cardioprotective effects of the combined use of HMG-CoA reductase inhibitors and L-arginine, BEC, arginase and darbepoetin.

The protocol consists of the following sections:

1. Simulation of EIED and or L-NAME-induced deficiency of nitric oxide and its correction with the help of inhibitors of HMG-CoA reductase and L-arginine, BEC, arginase and darbepoetin.

2. On the 8th day in the conditions of etaminal-sodium anesthesia, the appraisal of endothelium-dependent and endothelium-independent reactions of arterial pressure on intravenous one-stage administration of acetylcholine and sodium nitroprusside.

3. Connection of the animal to the artificial ventilation of the lungs left ventricular catheterization, registration of the LDL, + dp / dt, -dp / dt, HR, IFS. Stress tests for assess the functional reserves of myocardial contractility.

4. Removal of the animal from the experiment and taking blood from the thoracic aorta for biochemical studies.

5. Taking samples of myocardial tissue for morphological examination and determination of the diameter of cardiomyocytes.

Statistical processing of the research results

For graded data, the normality of the distribution was determined by using the Shapiro-Wilk criteria. In the case of normal distribution, the significance of the differences was assessed using a one-dimensional analysis of variance or dispersion analysis for repeated measurements, followed by the Dunnet method of multiple comparisons. In a case if there wasn't normal distribution, a nonparametric analogue of the Friedman dispersion analysis was used with subsequent processing by the method of multiple comparisons according to Newman-Keils or Dunnet. The non-parametric Wilcoxon test for coupled samples and Mana-Whitney were also used [84, 85].

The alternative data was worked on with the help of Fisher's exact probability method and the χ^2 criterion. To estimate the correlation between anti-inflammatory activity and endothelioprotection, the Pearson correlation coefficient was used.

The veracity of the observed changes in the parameters, caused by experimental drugs, both as absolute and in percentage of the initial level as well, was find out with the help of the

residual method of the variable statistics by finding the mean weight of the shifts (M); the arithmetic mean (+ m) and the probable error of the mean (P) according to Student tables. Differences were estimated as significant, starting from $p < 0.05$. For calculations, we used the program for statistical analysis Microsoft Excel 7.0.

Discussion and Results of the Research

Investigation of the anti-inflammatory effect of inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the model of formalin edema of mice foot and exudative model of inflammation in rabbits according to Oivin.

The use of inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin

and nanoparticulated rosuvastatin causes a dose-dependent antiexudative effect, appeared in decreasing of the gain of weight degree of the foot comparing to intact animals (Figure 2).

The calculated coefficient of inhibitory effect (CIE) with an increase in the dose of simvastatin from 9 to 35 mg/kg had been increasing from $12.7 + 4.6$ to $46.3 + 4.9$ cu. ($p < 0.05$). For atorvastatin, the initial effective anti-inflammatory dose was the less and amounted to 5 mg/kg for CIE $23.3 + 4.5$ cu. With dose increasing up to 19 mg/kg, CIE became $39.7 + 4.5$ cu. Rosuvastatin on this exudative inflammation model also demonstrated a dose-dependent effect which can be compared with The most evident antiexudative effect was seen in using of nanoparticulated rosuvastatin in the dose range of 4 times lower than rosuvastatin (Figure 2).

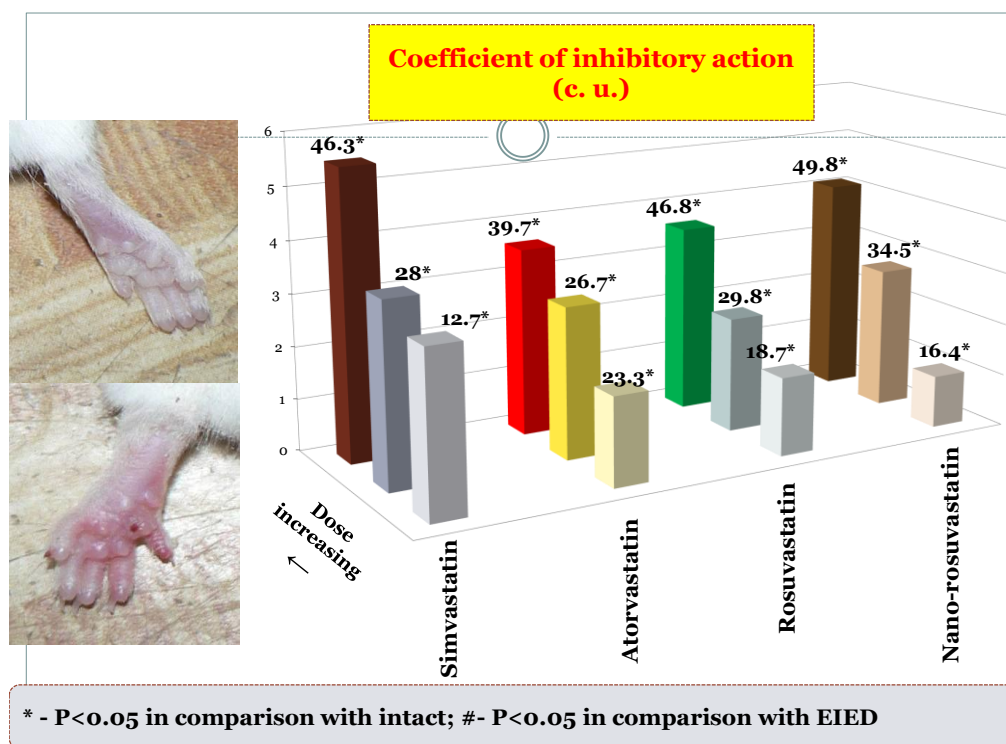


Fig. 2. The anti-inflammatory effect of inhibitors of HMG-CoA-reductase of simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the model of formalin edema of the foot of mice

The study results of the anti-inflammatory drugs activity according to the E.A. Oivin method are shown in Figure 3.

The study results of the anti-inflammatory drugs activity according to the E.A. Oivin method are shown in Figure 3.

Simvastatin in doses of 4.3 and 8.5 mg/kg had a dose-dependent anti-inflammatory effect, so the result of this was a decreasing of spots area to 3.5 ± 0.3 and 3.1 ± 0.3 cm²; and same time an increasing the time of its coming out to 397.1 ± 12.3 and 397.1 ± 12.3 seconds. In comparison with the control values, 5.4 ± 0.3

cm² and 236.1 ± 6.4 seconds. Atorvastatin, as well as in experiments on mice, started to show an anti-inflammatory effect at a lower dose of 1.1 mg/kg and exerted more evident effect, reaching its maximum at 4.3 mg/kg. Rosuvastatin turned out to be the most effective

at doses of 2.2, 4.3 and 8.5 mg/kg, retarding the time of spots coming out up to 501.9 ± 21.7, 531.5 ± 13.8 and 601.7 ± 13, 6 seconds compared with the control numbers of 236.1 ± 6.4 seconds.

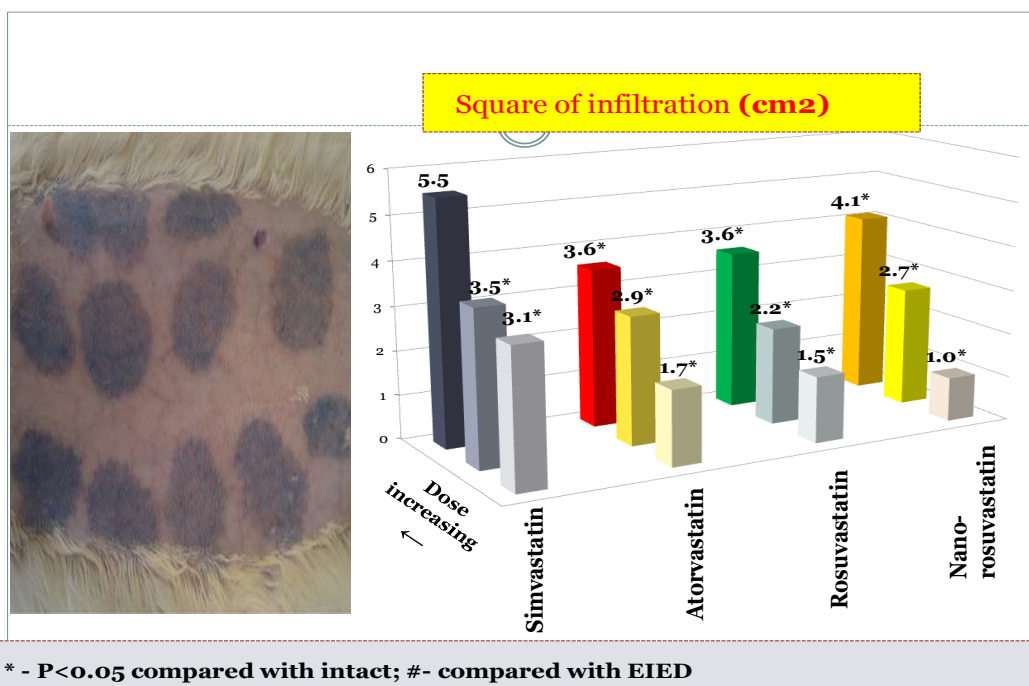


Fig. 3. Anti-inflammatory effect of inhibitors of HMG-CoA-reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the model of anti-exudative action in rabbits

The size of the spots was also minimal and amounted to a larger dose of 1.5 ± 0.2 cm². Nanoparticulated rosuvastatin (0.73, 1.43 and 2.83 mg/kg) showed comparable efficacy with a dosage of rosuvastatin base substance 4 times exceeding that of nanoparticulated form.

Thus, statins demonstrate a dose-dependent anti-inflammatory (on the model of formalin edema of the foot in mice) and anti-exudative effect (on the model of exudative inflammation in rabbits according to Oivin's method). The highest activity was evident in nanoparticulated rosuvastatin in the dose range (0.73, 1.43 and 2.83 mg/kg), which is 4 times lower than in the experiments with the base substance (Figure 3).

The investigation of the cardioprotective effect of inhibitors of HMG-CoA-reductase of simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in the simulation model of coronary-occlusive myocardial infarction in rats.

The application of the ligature to the descending branch of the left coronary artery in

rats in the control group of animals led to myocardial necrosis, the size of which was 12.71 ± 0.59% of the total myocardium size (Figure 4).

The use of inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin had a dose-dependent cardioprotective effect. In this instance, the statins we were studying appeared to be approximately equally effective (Figure 4).

The blockade of K⁺-ATPase channels by glibenclamide (5 mg/kg) completely stopped the cardioprotective effect of inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in the simulation model of coronary-occlusive infarction in rats (Figure 4). The use of aminoguanidine (40 mg/kg) for iNOS blockade had also completely stopped the cardioprotective effect of the statins which were studied (Figure 4). This indicates the participation of the effects of mechanisms for

the realization of pharmacological preconditioning as the "first window" (blockade of K⁺-ATPase channels with

glibenclamide (5 mg/kg) and the "second window" (iNOS blockade with aminoguanidine 40 mg/kg).

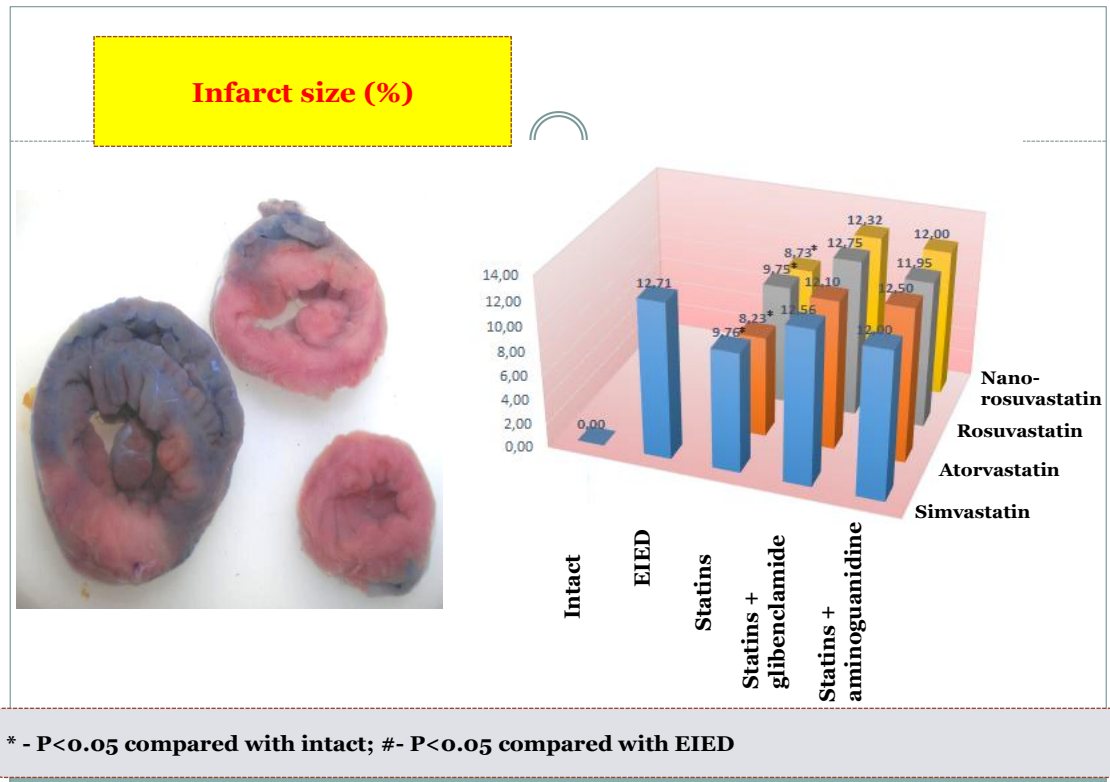


Fig. 4. Cardioprotective effect of inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the model of coronary-occlusive infarction in rats

Thereby, the written above indicates that statins have a dose-dependent cardioprotective effect in the simulation model of coronary-occlusive infarction in rats. In cardioprotective effects actualization, the mechanisms of pharmacological preconditioning are of considerable importance, as it became evident with remove of effects during blockade of K⁺-ATPase-channels with glibenclamide and blockade of iNOS with aminoguanidine.

The investigation of endothelioprotective effects of inhibitors of HMG-CoA reductase of simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in the simulation model of endotoxin-induced endothelial dysfunction.

The simulation model of endotoxin-induced endothelial dysfunction in experimental animals

When a model of endotoxin-induced endothelial dysfunction was created, rats were

infected with *Staphylococcus aureus* (strain 13407), subcutaneously with 60 billion microbial bodies, followed by (after 24 hours) sensitization of the laboratory animal (0.1 ml of staphylococcal anatoxin subcutaneously), after that generalization of the infectious agent by means of a daily massage at the injection place was made. A way of a chronic septic process in laboratory animals RU 2143749 C1 6 G09B23 / 28 [86] learning. This model allows having a long-term dynamic process of endotoxin intoxication with 100% survival of experimental animals learning.

The temperature of the rats body during the learning period was higher than the intact group for 2-3 ° C, reaching the maximum increase by the sixth day of the experiment (40.1 ± 0.2 ° C) and decreasing to the initial values by the 15th days of the experiment (38.2 ± 0.1 ° C). This fact demonstrates the dynamics

of the generalized sluggish inflammatory process, subsiding by itself to this date.

During the EIED technique working out, we paid special attention to the dynamics of the coefficient of endothelial dysfunction (CED) as an integral indicator characterizing the development of pathology. For this purpose, experiments were performed on endothelium-dependent (acetylcholine 40 µg/kg) and endothelium-independent (nitroprusside 30 µg/kg); vascular relaxation in animals with EIED on the 3rd, 6th, 9th, 12th and 15th days with the subsequent calculation of CED.

It was discovered that EIED affects the functioning of the endothelium, in particular, the endothelium-dependent vasodilatation caused by acetylcholine. On the third day CED was 1.1 ± 0.1 , which did not differ from the parameter in the control group – 1.1 ± 0.2 , and can be explained by activation of microorganisms and macrophages with inducible NOS with a sufficiently intensive formation of nitric oxide in the initial period septic process. Hereinafter, by the sixth day of

the experiment, the endothelial dysfunction rate significantly has increased in comparison with intact and was 2.1 ± 0.3 . In some animals, the values reached 5 and even 6. Further, a gradual decrease in the coefficient of endothelial dysfunction was observed: on the 9th day, 1.8 ± 0.4 , 12 - 1.8 ± 0.5 , and finally on the 15th day the value of CED was coming to the level of animals without any pathology – $1, 3 \pm 0. 1$.

According to the given results, for further experiments, we performed functional tests on the 7th day after the EIED simulation. Comparing to intact animals, on the 7th day after the simulation EIED had a negative effect on the rats on the indicators of central hemodynamics and showed a statistically significant decrease in SBP and DBP. Simultaneously, when the endothelium-dependent (acetylcholine) and endoterium-independent (nitroprusside) relaxation tests had been done, the CED was increased to 3.7 ± 0.5 , which is 3 times higher than in intact animals (Table 2).

Table 2

Changes in hemodynamic parameters in animals with endotoxin-induced endothelial dysfunction (M ± m, n = 10)

Group	Systolic blood pressure	Diastolic blood pressure	Coefficient of endothelial dysfunction (CED)
Intact	129.4±2.2	89.2± 1.1	1.1 ± 0.1
Endotoxin-induced endothelial dysfunction	117.6±2.3*	85.0±2.1*	3.7±0.5*

Note: SBP – systolic blood pressure (mmHg), DBP – diastolic arterial pressure (mmHg), CED – coefficient of endothelial dysfunction (unit units), * – significant difference with a group of intact animals (p <0.05).

Adrenoreactivity in animals with EIED was 20% higher, and the myocardial reserve was 30% lower than in intact animals.

Analyzing the values of biochemical markers, reflecting the metabolism of nitric oxide, C-reactive protein and pro-inflammatory cytokines the most significant changes has been found. Thus, the total content of final metabolites of NO (Total NO) in animals with EIDD from 116.8 ± 10.3 to 182.3 ± 12.4 µmol/l were highly increased. In contrast, eNOS expression has been decreasing significantly.

This fact may indicate a more significant contribution of the inducible isoform of the enzyme activation (iNOS) to an enlargement of NO content. Integral marker of inflammatory processes in the vascular wall C-reactive protein (CRP) has been increasing its level more than 7 times, which indicates the development of systemic vasculitis. A similar dynamics can be observed in the case of the inflammatory cytokines IL-6 and TNF, the values of which increased 16 and 2 times, respectively (Table 3).

Table 3

Changes in the values of biochemical markers (Total NO, Expression of eNOS, C-reactive protein, IL-6, TNF- α) in animals with endotoxin-induced endothelial dysfunction (EIED) (M \pm m, n = 10)

Groups of animals	NOx	eNOS expression	C-reactive protein	IL-6	TNF- α
Intact	116.8 \pm 10.3	5.4 \pm 0.21	0.05 \pm 0.01	0.43 \pm 0.17	8.4 \pm 2.5
Endotoxin-induced endothelial dysfunction	182.3 \pm 12.4*	0.04 \pm 0.01*	0.38 \pm 0.01*	6.87 \pm 1.93*	17.8 \pm 3.7*

Note: NOx is the final metabolite of NO (μ mol/L); eNOS expression (%); level of CRP-C-reactive protein (mg/l); IL-6 – interleukin 6 (pg/ml) TNF- α -tumor necrosis factor α (pg/ml), * – significant difference with a group of intact animals (p <0.05).

Thereby, the modeling of endotoxin-induced pathology by the injection of strain 13407 of *Staphylococcus aureus* leads to the development of endothelial dysfunction with an increase of CED 3.7-fold, a decrease in myocardial reserve and an augmentation of adrenergic reactivity, as well as an enlargement of stable NO metabolites against an increase in inflammatory markers of C-reactive protein and pro-inflammatory cytokines IL-6 and TNF- α . Decreased expression of eNOS indicates a preferential involvement of the enzyme form iNOS in this process.

The endothelioprotective effects of the inhibitors of HMG-CoA-reductase of simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin investigation in modeling endotoxin-induced endothelial

dysfunction.

The use of inhibitors of HMG-CoA reductase of simvastatin, atorvastatin, rosuvastatin, and nanoparticulated rosuvastatin in EIED modeling revealed an evident dose-dependent endothelioprotective effect, manifested in a significant decrease in CED, against normalization of systolic and diastolic blood pressure values. Thus, in the simulation of EIED, CED was 3.7 \pm 0.5, whereas in large doses of simvastatin (8.5 mg/kg), atorvastatin (4.3 mg/kg), rosuvastatin (8.5 mg/kg) and nanoparticulated rosuvastatin (11.6 mg/kg), respectively, 2.3 \pm 0.5, 2.1 \pm 0.3, 1.7 \pm 0.5 and 1.5 \pm 0.2 c.u., which was close to the values in intact animals (1.1 \pm 0.1). Wherein, the most effective drugs rosuvastatin and its nanoparticulated form were (Figure 5).

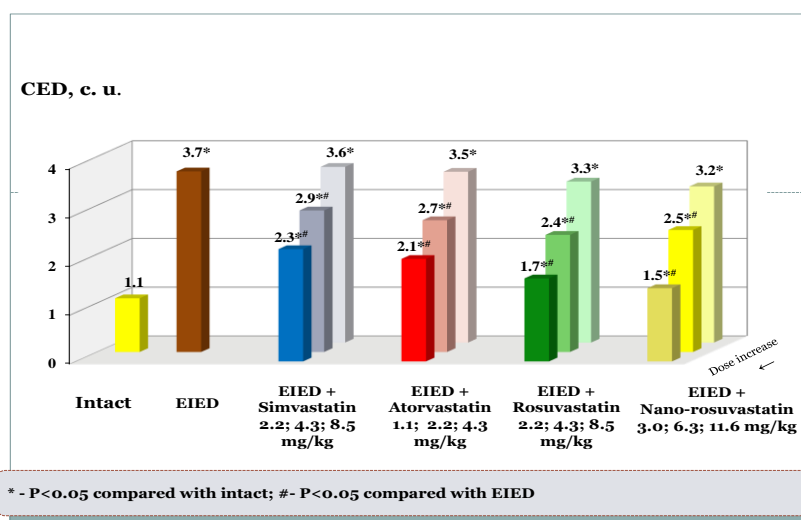


Fig. 5. Indices of endothelial dysfunction coefficient (CED) with the use of inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of endotoxin-induced endothelial dysfunction modeling

Parallel to this, conducting stress tests in animals with EIED indices a positive dynamics of contractility was observed. As a result, the prevention of an increase in adrenoreactivity and a decrease in the myocardial reserve was revealed. At the same time, as in the case of CED, rosuvastatin (8.5 mg/kg) and its nanoparticulated form (11.6 mg/kg) proved to

be the most effective. The most evident endothelial and cardioprotective effect of inhibitors of HMG-CoA reductase of simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin was found out in the values of biochemical markers in animals with EIED (Figure 6).

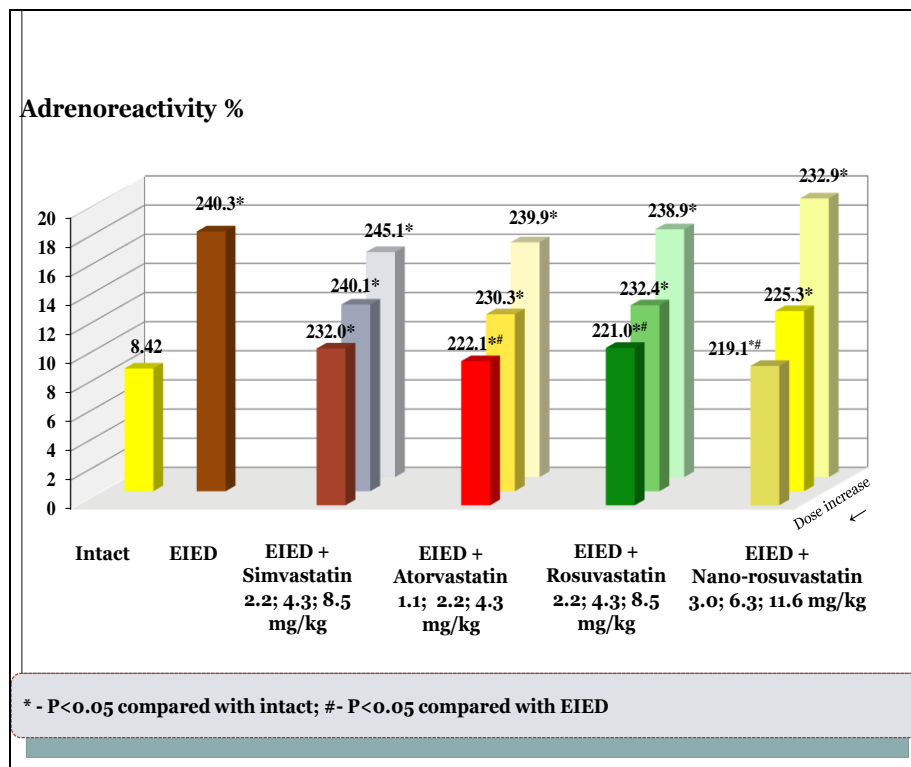


Fig. 6. Adrenoreactivity indicators with the use of inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of modeling endotoxin-induced endothelial dysfunction

The level of final metabolites of NOx and eNOS expression, subject to a high increase (NOx) (Figure 7) and decrease (Expression of eNOS) (Figure 8) in the simulating of endotoxin-induced pathology at the effect of statins were normalized on the 8th day and reached values not differing from intact animals at the maximum doses of HMG-CoA reductase. It should be noticed that the greatest effect the nanoparticulated form of rosuvastatin had. This fact supports the hypothesis of a

change in the volume of distribution of rosuvastatin by the blood stream restriction (Figure 9).

Systemic inflammatory reaction marker the C-reactive protein has showed a 7.5-fold increase in EIED, but being under the influence of medium and large doses of inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin, it was reduced to values statistically not different from intact animals (Figure 9).

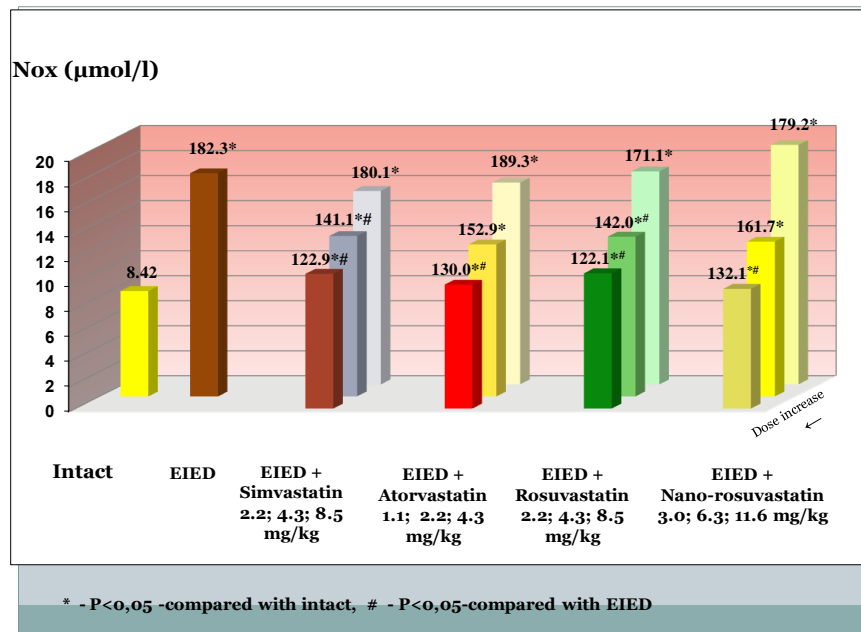


Fig. 7. Concentration of nitrogen oxide metabolites (NO_x) using the inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of endotoxin-induced endothelial dysfunction modeling.

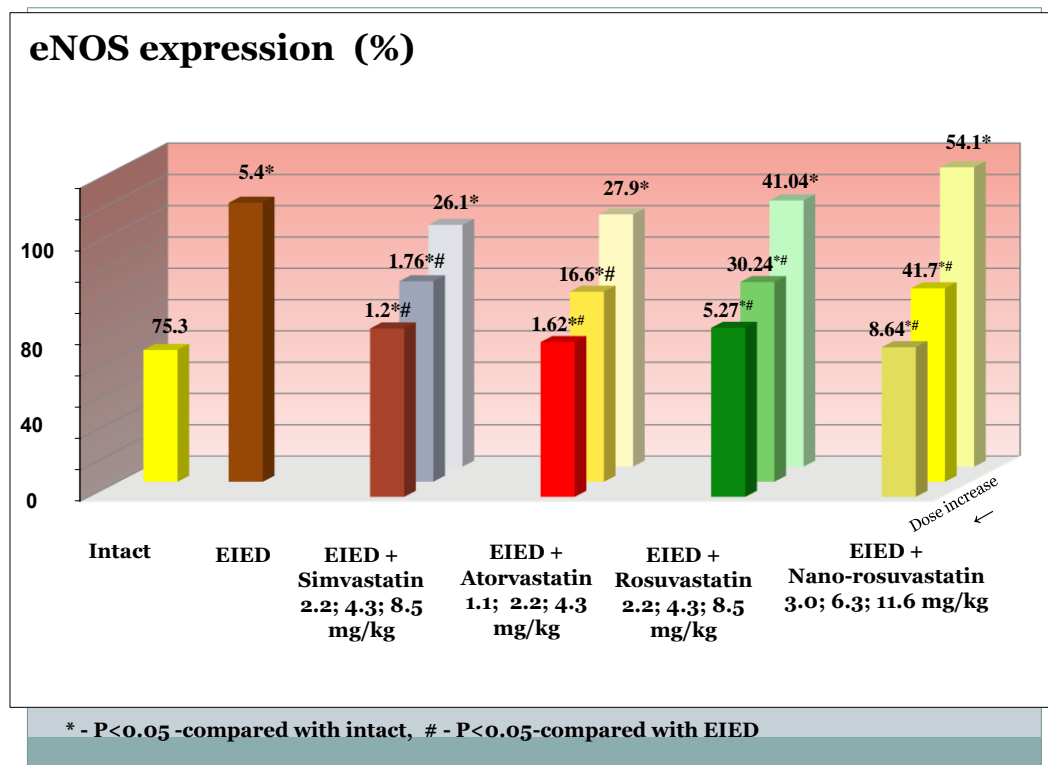


Fig. 8. Expression of NO-synthase (eNOS) using inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin against the background of endotoxin-induced endothelial dysfunction modeling

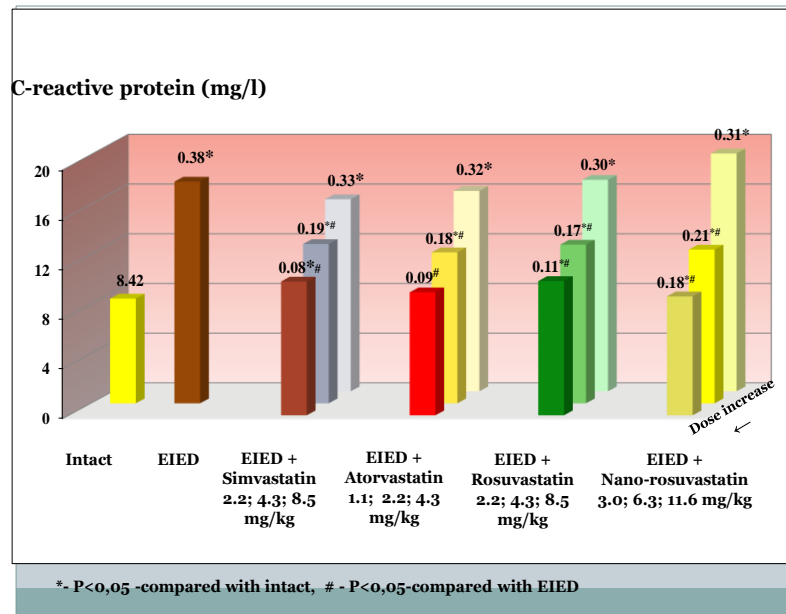


Fig. 9. C-reactive protein (CRP) indices with the use of inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of endotoxin-induced endothelial dysfunction modeling

The proinflammatory cytokines IL-6 and TNF- α , during the EIED simulating, had increased 15 and 2.1 times. The use of medium and large doses of inhibitors of HMG-CoA reductase, simvastatin, atorvastatin,

rosuvastatin and nanoparticulated rosuvastatin had been exerting an evident normalizing effect and their values had been approximating to the same values in intact animals (Figure 10).

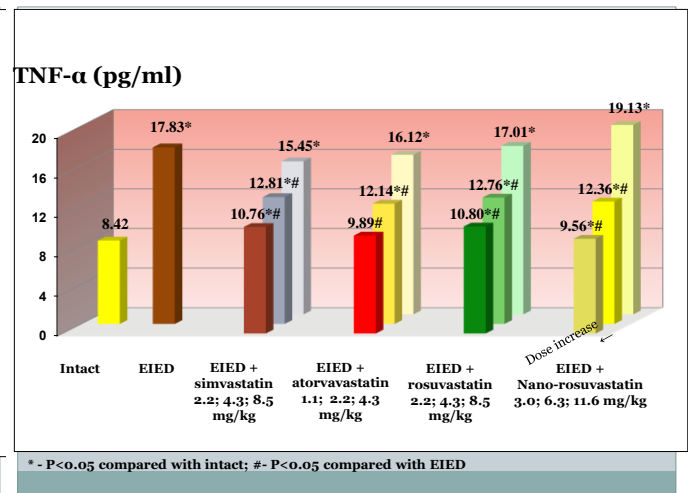
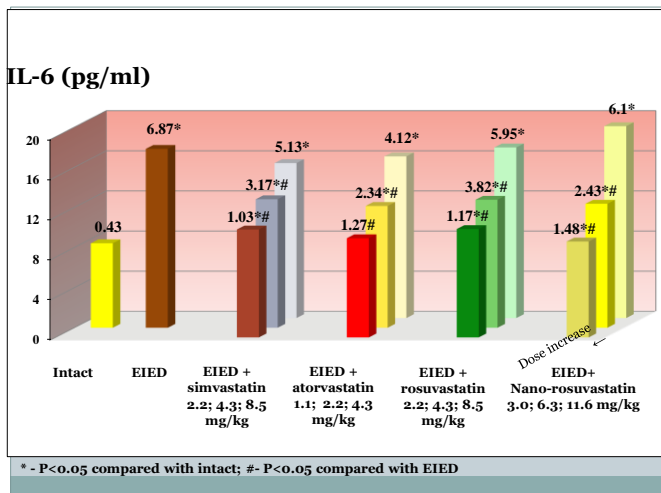


Fig. 10. The concentration of proinflammatory cytokines IL-6 and TNF- α with the use of inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of endotoxin-induced endothelial dysfunction modeling

The most pronounced protective effect was provided by the nanoparticulated form of rosuvastatin 11.6 mg/kg.

The modeling of L-NAME-induced endothelial dysfunction was accompanied by an

increase in SBP and DBP, an increase in adrenergic reactivity, a decrease in the myocardial reserve, biochemical analysis showed a disturbance in the metabolism of nitric oxide, histological findings revealed that modeling L-

NAME-induced NO deficiency on the 8th day resulted in bright pronounced hypertrophy of myocardiocytes, and spasm of arterioles. Inhibitors of HMG-CoA reductase of

simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin effect on the model of L-NAME-induced deficiency of nitric oxide is shown in Figure 11.

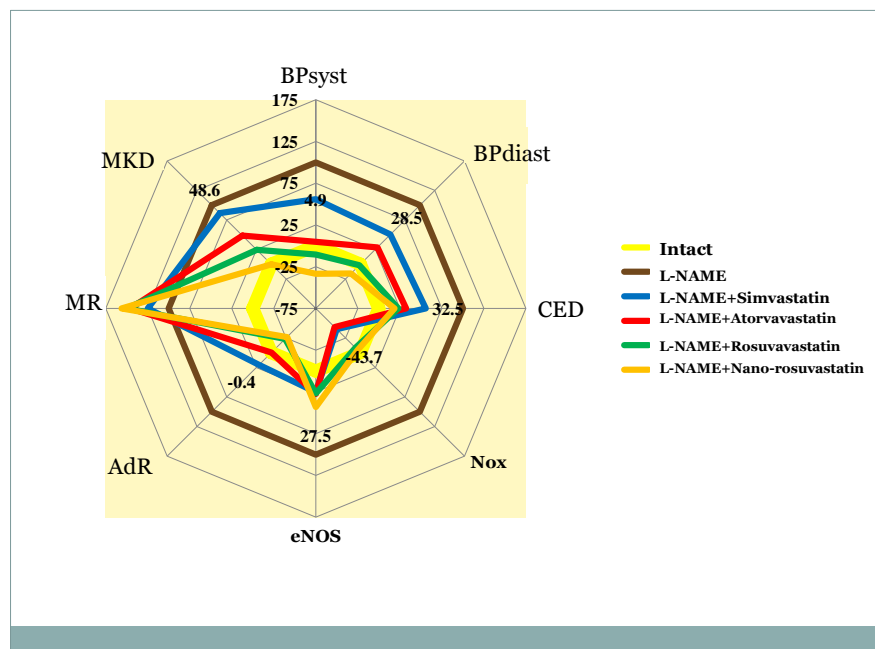


Fig. 11. Endothelioprotective effects of HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in L-NAME-induced endothelial dysfunction.

Note: BPSyst – systolic blood pressure; BPdiast – diastolic blood pressure; CED – coefficient of endothelial dysfunction; NOx is the concentration of the nitrite ion in the plasma; eNOS – expression of eNOS; AdR – adrenoreactivity; MR – myocardial reserve; MKD is the myocardiocytes diameter (% of the group of intact animals)

HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin, and nanoparticulated rosuvastatin had been causing a dose-dependent endothelial and cardioprotective effect. The result of it came out in decreasing of systolic blood pressure values had been reaching to statistically significant values and were 167.2 ± 10.1 , 140.3 ± 9.6 , 132.2 ± 7.7 and 120.1 ± 6.4 mm Hg., but as for the controlled group, it was 190.3 ± 6.7 mm Hg. A similar dynamics was found in the evaluation of diastolic blood pressure. At the same time, CED dose-dependent had been decreasing reliably and under nanoparticulated rosuvastatin influence had been reaching 1.9 ± 0.6 cu., what was almost corresponded to intact animals and was practically 3 times less than in control 5.4 ± 0.6 cu.

In experiments with stress tests, inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated

rosuvastatin had been also demonstrating cardioprotective effects and were preventing increasing in adrenoreactivity. Thus, in the control with simulating a model of L-NAME-induced nitric oxide deficiency, the maximum intraventricular pressure during the test for adrenoreactivity was 247.3 ± 4.8 mm Hg, and with rosuvastatin and nanoparticulated rosuvastatin correction 187.9 ± 10.2 and 186.4 ± 10.7 mm Hg. respectively, which is significantly less than in the control and close to the values in intact animals.

The cardioprotective effect of inhibitors of HMG-CoA reductase of simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin had been also seen in improving the indicators contractility during the resistance stress test. Thus, the values of intraventricular pressure in the control group with L-NAME-induced pathology were $66.0 \pm 4.6\%$ on 25th second of the test, whereas in the experimental

groups with simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin, respectively, they were 88.1 ± 5.8 ; 91.7 ± 6.3 ; 92.4 ± 6.7 and $93.5 \pm 7.4\%$, which is significantly higher than in the control and does not differ from the values in intact animals of $83.6 \pm 4.3\%$.

Inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin, and nanoparticulated rosuvastatin had been causing a dose-dependent endothelioprotective effect, which was consisted in preventing the increase in the values of the final metabolites of NOx and reducing eNOS expression. Wherein rosuvastatin and its nanoparticulated form had the most significant effect.

So, the use of inhibitors of HMG-CoA reductase in simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin during simulating a model of EIED (introduction of *Staphylococcus aureus* strain 13407), and in simulating a model of ADMA-like L-NAME-induced deficiency of NO leads to a dose-dependent endothelioprotective effect development, which consists in CED normalization, adrenergic reactivity increasing prevention and the myocardial reserve depletion as well, and at the same time it leads to biochemical markers of inflammation

(C-reactive protein) and the level proinflammatory cytokines normalization. During the experiment positive dynamics of the final products of NO and eNOS expression was found. At the same time, the values of AD significantly were decreasing, and in the experiments with nanoparticulated rosuvastatin they had reached the set values.

Investigation of the effect of monotherapy with NO donor L-arginine, a nonselective inhibitor of arginase-S-(2-boroethyl) -L-cysteine (BEC), a selective inhibitor of arginase-2 arginase and darbepoetin in endotoxin-induced endothelial dysfunction in experimental animals.

Monotherapy with donator NO L-arginine in the dose range of 70, 200 mg/kg daily intragastric on the background of the EIDE modeling normalized CED and did not significantly affect the blood pressure indicators. Inhibitors of arginase BEC (5 and 10 mg/kg) and arginase (1 and 3 mg/kg) also normalized CED and did not affect BP. The greatest effect was provided by recombinant erythropoietin at a dose of 500 µg/kg. So CED was 1.9 ± 0.2 USD, while in the group with EIED 3.7 ± 0.5 c. u. (Table 4).

Table 4

Effect of monotherapy with L-arginine, BEC, arginase and darbepoetin on the dynamics of hemodynamic parameters in animals with endotoxin-induced endothelial dysfunction (EIED)

(M ± m, n = 10)

Group	SBP	DBP	CED
Intact	129.4±2.2	89.2± 1.1	1.1 ± 0.1
EIED	117.6±2.3*	85.0±2.1	3.7±0.5*
EIED + L-arginine 70 mg/kg	121.9±3.2	80.7±2.0	3.1±0.4*
EIED + 200 mg/kg	118.5±2.1	76.3±2.1	2.1±0.3*#
EIED + BEC 5 mg/kg	114.1±3.0*	83.5±2.0	2.9±0.4*
EIED + BEC 10 mg/kg	120.5±2.7	85.7±2.3	2.2±0.3*#
EIED + Arginase 1 mg/kg	117.8±3.2*	88.2±2.7	3.0±0.3*
EIED + Arginase 3 mg/kg	126.2±3.4	85.1±2.1	2.1±0.3*#
EIED + Darbepoetin 50 µg/kg	121.2±3.1	86.9±2.1	2.5±0.3*
EIED + Darbepoetin 500 µg/kg	126.3±3.2	83.4±2.3	1.9±0.2*#

Note: SBP – systolic blood pressure (mmHg), DBP – diastolic arterial pressure (mmHg), CED – coefficient of endothelial dysfunction (unit units), * – significant difference with a group of intact animals (p <0.05); # – significant difference with the endotoxin-induced endothelial dysfunction (EIED) group (p <0.05).

The positive dynamics of the indices of contractility in carrying out stress tests in animals with EIDD was found in parallel. Thus, prevention of an increase in adrenoreactivity and a decrease in the myocardial reserve was revealed. At the same time, as in CED, arginine (3 mg/kg) and darbepoetin (500 µg/kg) were the most effective.

The most pronounced endothelial and cardioprotective effect of monotherapy with L-arginine, BEC, arginine and darbepoetin was manifested in the values of biochemical markers in animals with EIDD. Thus, under the influence of monotherapy with L-arginine,

BEC, arginasine and darbepoetin, the level of final metabolites of NOx in the modeling of sepsis increased significantly less than in control experiments, and in large doses did not differ from intact animals. Similarly, monotherapy with these drugs prevented the decrease of eNOS expression. The most significantly protective effect of monotherapy with L-arginine, BEC, arginasine and darbepoetin was manifested in relation to the level of C-reactive protein and the values of proinflammatory cytokines IL-6 and TNF-α (Table 5).

Table 5

Effect of monotherapy with L-arginine, BEC, arginasine and darbepoetin on the dynamics of the values of biochemical markers (total NO, eNOS expression, C-reactive protein, IL-6, TNF-α) in animals with endotoxin-induced endothelial dysfunction (EIED) (M ± m, n = 10)

Group	NOx	eNOS	CRP	IL-6	TNF-α
Intact	116.8±10.3	5.4±0.21	0.05±0.01	0.43±0.17	8.42±2.51
EIED	182.3±12.4*	0.04±0.01*	0.38±0.01*	6.87±1.93*	17.83±3.79*
EIED + L-arginine	165.4±9.7*	1.17±0.27*	0.13±0.01*	3.12±1.12*	11.13±2.17*
70 mg/kg	132.7±11.3*#	2.14±0.22*#	0.17±0.02*#	2.23±1.67*#	10.23±2.08*#
200 mg/kg					
EIED + BEC					
5 mg/kg	167.1±12.4*	2.15±0.32*	0.12±0.02*	3.98±1.23*	10.16±2.37*
10 mg/kg	133.7±10.1*#	3.21±0.75*#	0.14±0.01*#	2.37±1.17*#	9.97±1.36*#
EIED + arginasine					
1 mg/kg	146.0±13.1*	2.39±0.74*	0.16±0.03*	2.24±1.27*	12.89±2.06*
3 mg/kg	125.1±97.3*#	4.16±1.15*#	0.10±0.02*#	2.03±0.98*#	10.54±1.72*#
EIED + darbepoetin					
50 µg/kg	145.1±11.7*	2.75±0.64*	0.21±0.02*	2.11±1.12*	9.58±2.29*
500 µg/kg	122.5±10.5*#	4.19±0.72*#	0.17±0.01*#	1.72±0.97*#	8.20±2.26*#

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

The obtained results allowed to determine the choice of doses. L-arginine 200 mg/kg, BEC-10 mg/kg, arginine-3 mg/kg and darbepoetin 500 µg/kg for studies on the effectiveness of combined use with inhibitors of HMG-CoA reductase by simvastatin, atorvastatin, rosuvastatin and nano-pararticular rosuvastatin.

Thus, the use of monotherapy with a donor NO L-arginine, inhibitors of arginase and

darbepoetin EIED on background modeling by introducing strain 13407 *Staphylococcus aureus* exhibits endotelio- and cardioprotective action, expressed in preventing increase CED adrenoreactivity, conservation and normalization of myocardial reserve biochemical marker values (Totals NO, expression of eNOS, CRP, IL-6, TNF-α). In this case, the drugs had a dose-dependent effect and were approximately equally effective.

Investigation of the effect of combined therapy with the L-arginine and inhibitors of HMG-CoA reductase by simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in endotoxin-induced endothelial dysfunction.

Monotherapy with donator NO L-arginine (200 mg/kg) daily intragastric on the

background of the EIED modeling normalized CED and did not significantly affect blood pressure (table 4). Inhibitors of HMG-CoA reductase, 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 significantly improved CED and did not affect blood pressure (Figure 5).

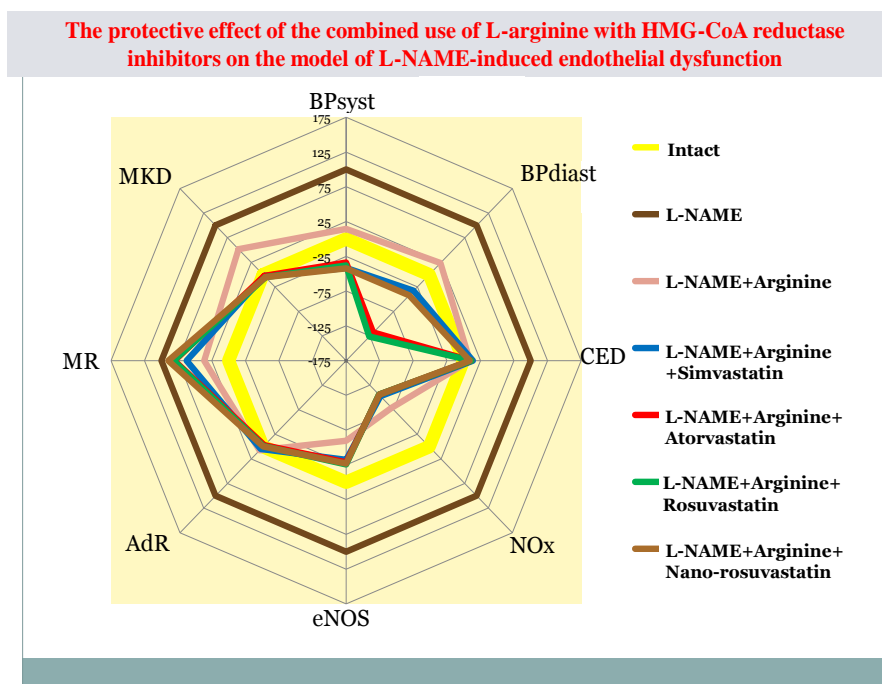


Fig. 12. Endothelioprotective effects of combined use of L-arginine with inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in L-NAME-induced endothelial dysfunction

Note: BPsyst – systolic blood pressure; BPdiast – diastolic blood pressure; CED – coefficient of endothelial dysfunction; NOx – concentration of nitrite ions in plasma; eNOS – expression of eNOS; ADR – adrenergic reactivity; MR – myocardial reserve; DMK is the diameter of myocardiocytes (% of the group of intact animals)

The combined use of L-arginine with HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin showed the most pronounced endothelioprotective effect, which was

reflected in the indices of hemodynamic parameters of systolic and diastolic blood pressure, as well as CED, which in the EIED group did not differ statistically from intact animals (Figure 13).

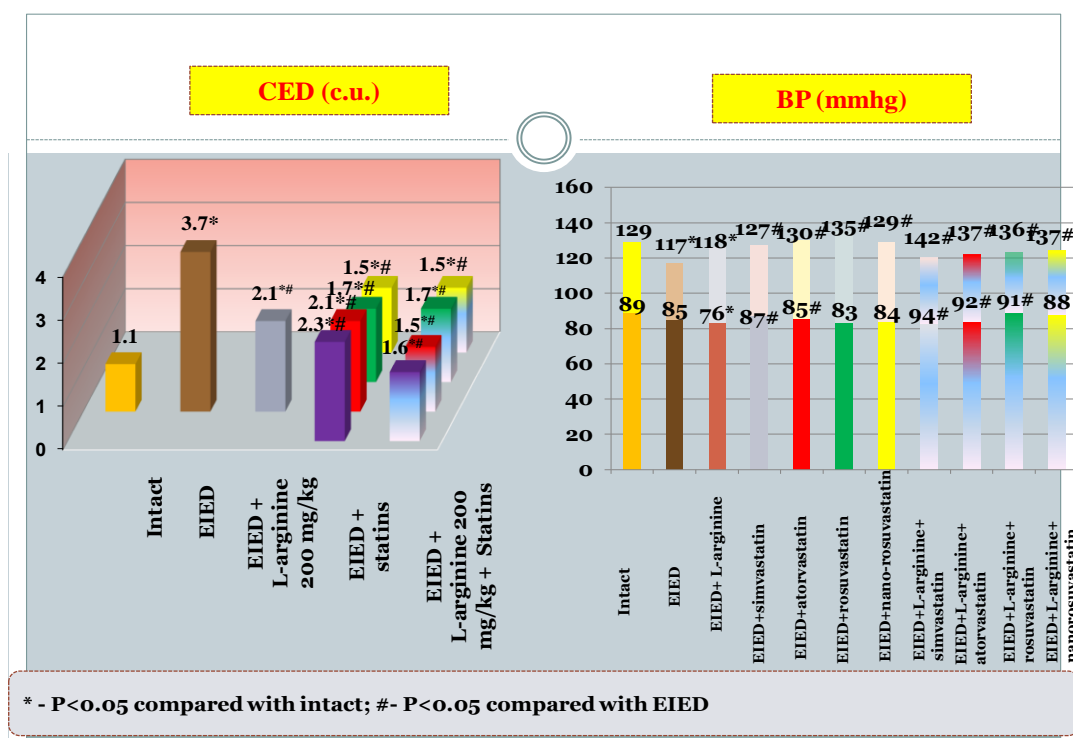


Fig. 13. The indices of endothelial dysfunction coefficient (CED) and blood pressure (BP) when combined use of L-arginine and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of endotoxin-induced endothelial dysfunction (EIED) modeling

At the same time, a positive dynamics of contractility indices was found in carrying out stress tests in animals with EIED. Thus, the prevention of an increase in adrenoreactivity and a decrease in the myocardial reserve both with the use of L-arginine and with its combined use of inhibitors of HMG-CoA-reductase by simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin was detected. At the same time, as with CED, the combined use of drugs led to the fact that the values did not differ from those of intact animals (Figure 14).

The most significantly protective effect of the combined use of the NO L-arginine donor with the HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin was manifested with respect to the level of C-reactive protein

and pro-inflammatory cytokines IL-6 and TNF- α , the values of which did not differ from such in intact animals (Figures 15-17).

Thus, the use of combined use of L-arginine with HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin against the background of modeling of EIED and L-NAME-induced deficiency of NO exhibits endothelial and cardioprotective effect, manifested in preventing CED, adrenoreactivity, preservation of the myocardial reserve and normalization of the values of biochemical markers (Total NO, expression of eNOS, CRP, IL-6, TNF- α). Combined therapy was so effective that the values obtained with combined therapy did not differ from those obtained in intact animals (Figure 12).

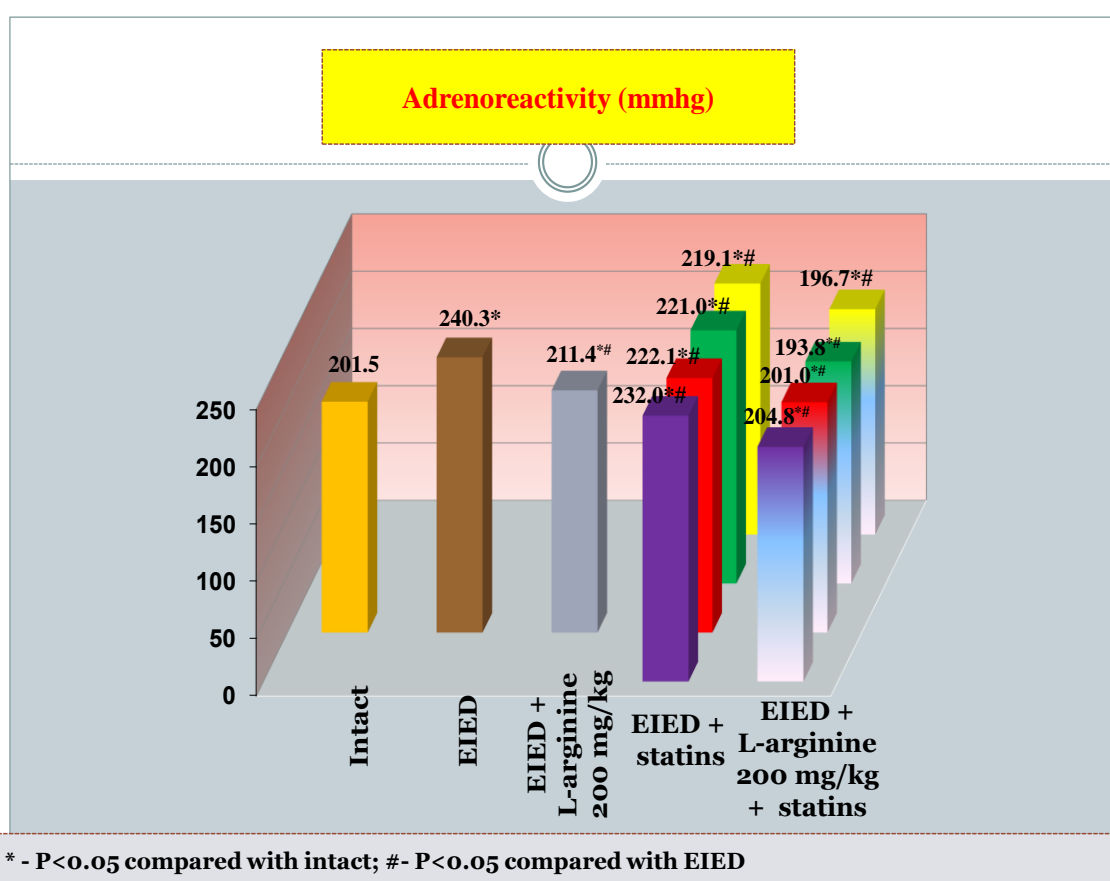


Fig. 14. Adrenoreactivity indicators for combined use of L-arginine and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin against the background of endotoxin-induced endothelial dysfunction modeling

In the experiments with loading tests, the combined use of L-arginine with HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin also shows an additive cardioprotective effect, prevention of adrenoreactivity growth and depletion of the myocardial reserve.

A similar additive effect of combined use of L-arginine and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin forms, interaction with metabolites of nitrogen NOx and eNOS expression.

In parallel with the dynamics of the additive effect of combined application of L-arginine and inhibitors of HMG-CoA reductase, simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin,

a positive additive effect on the reduction of hypertrophy of myocardiocytes was found.

Thus, the use of combined use of L-arginine with HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin against the background of the EIED and L-NAME-induced deficiency shows NO endothelial and cardioprotective effect, which is manifested in preventing CED increase, adrenoreactivity, preservation of the myocardial reserve and normalization of biochemical markers (Total NO, expression of eNOS, CRP, IL-6, TNF- α). Combined therapy was so effective that the values obtained with combined therapy did not differ from those in intact animals.

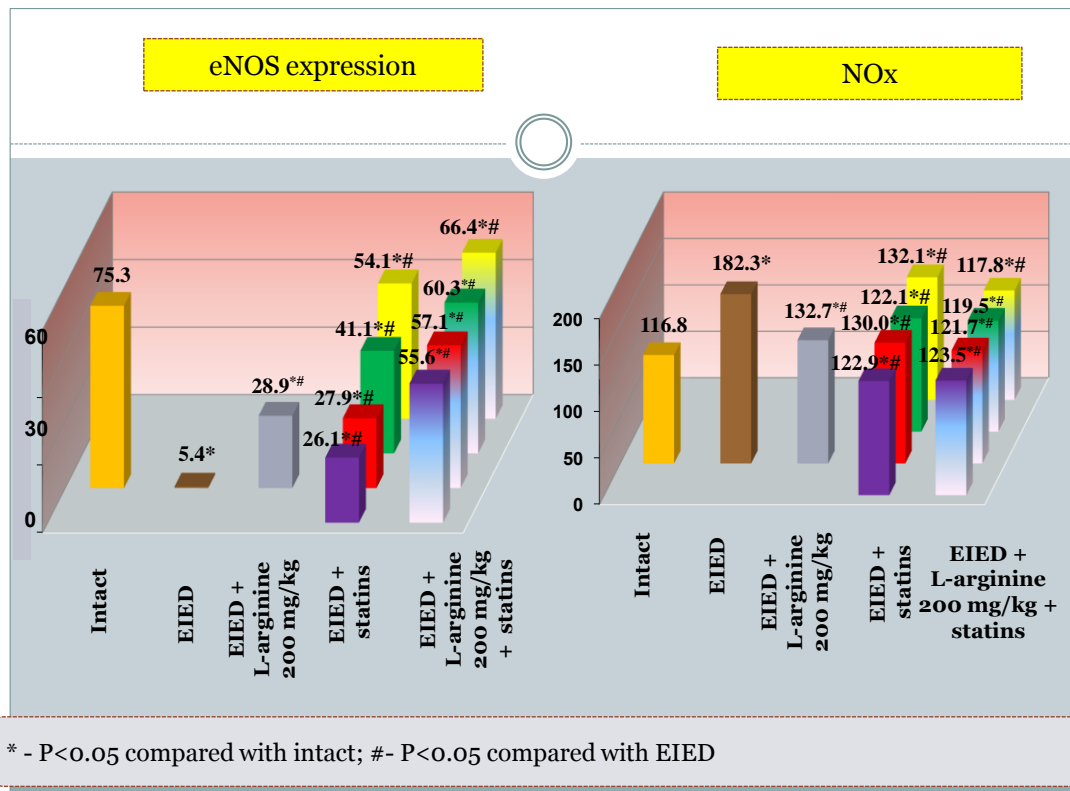


Fig. 15. Nitric oxide concentration (NOx) and NO synthase expression (eNOS) in combined use of L-arginine and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in the context of endotoxin-induced endothelial dysfunction modeling

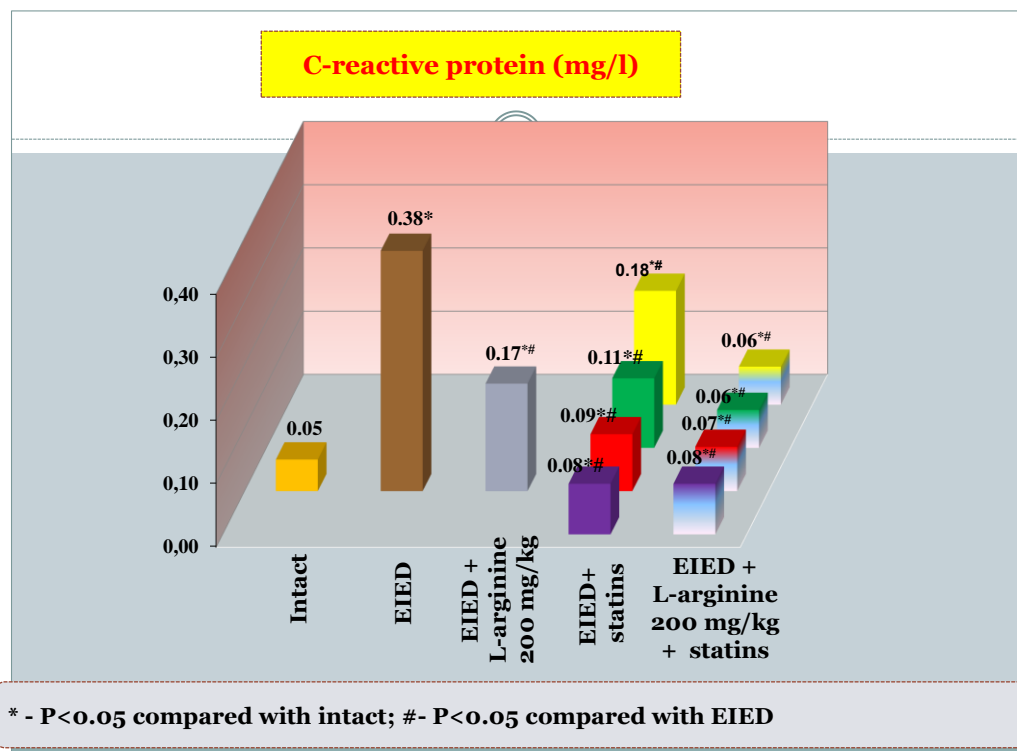


Fig. 16. C-reactive protein (CRP) indices with combined use of L-arginine and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in the context of endotoxin-induced endothelial dysfunction modeling

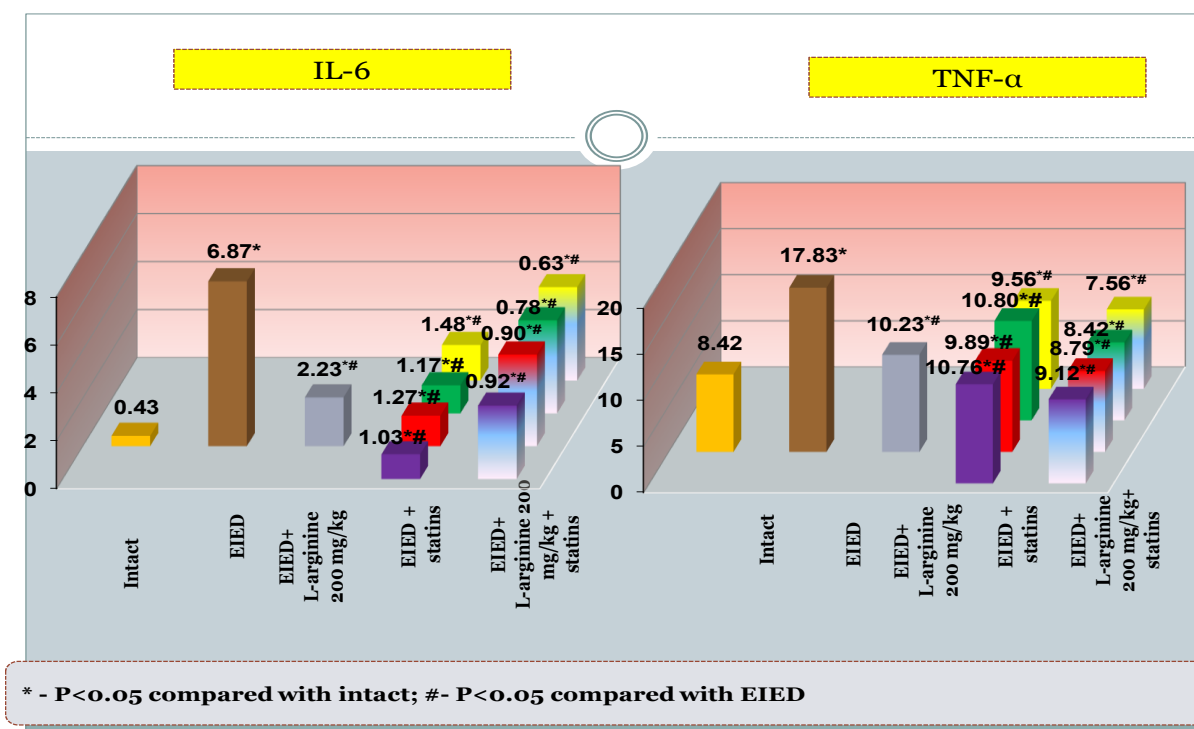


Fig. 17. Concentration of proinflammatory cytokines IL-6 and TNF- α in combined use of L-arginine and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of modeling endotoxin-induced endothelial dysfunction (EIED)

Study of the effect of combined therapy with a nonselective inhibitor of arginase S-(2-boro-ethyl)-L-cysteine (BEC), with simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in endotoxin-induced endothelial dysfunction model.

Monotherapy with a nonselective inhibitor of arginase S-(2-boro-ethyl)-L-cysteine (BEC) daily intragastric moderately normalized CED and did not significantly affect blood pressure (Table 4). The CED value was 2.5 ± 0.4 c. u. Inhibitors of HMG-CoA reductase, 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 significantly improved CED and did not affect blood pressure. The CED values were in the range 2.3 – 1.5 c. u.

The combined use of BEC with statins did not show an additive effect with respect to CED and BP. Values were even slightly higher than with statin monotherapy, however, were statistically significantly different from intact animals (Figure 18).

Parallel to this, a positive dynamics of contractility indices was observed when carrying out stress tests in animals with EIED (Figure 19). BEC was inferior in activity to statins both in terms of preventing an increase in adrenoreactivity, and in terms of conserving an expansion reserve. The combined use of BEC with simvastatin, atorvastatin, rosuvastatin, and nano-pararticular rosuvastatin did not lead to an additive enhancement of the effect, and the values of intraventricular pressure when loading tests were close to those in the BEC series and inferior to the HMG-CoA reductase inhibitors. A similar trend has been observed with regard to the values of biochemical markers in animals with EIED (Figures 20-22).

The most significantly protective effect of the combined use of BEC with inhibitors of HMG-CoA reductase by simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin was manifested with respect to the level of C-reactive protein whose level was comparable in all series of experiments. (Figure 21).

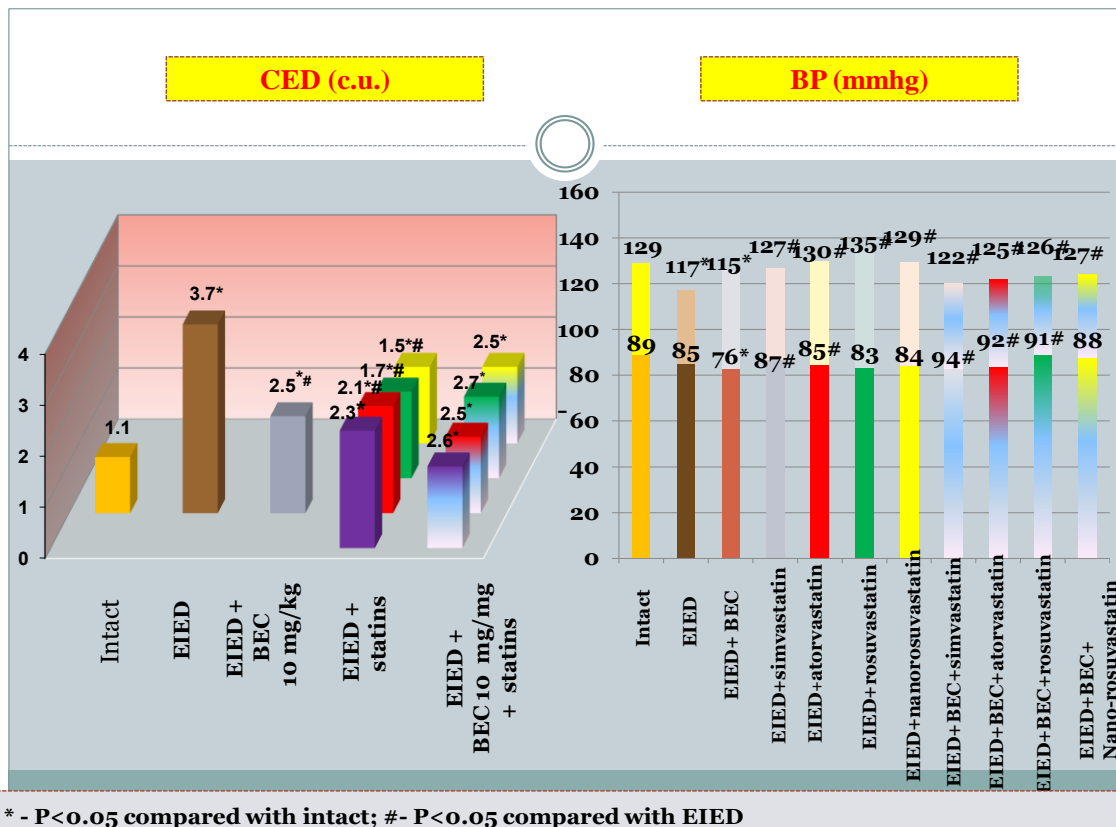


Fig. 18. The indices of the endothelial dysfunction coefficient (CED) and arterial pressure (BP) when combined with the use of S- (2-boro-ethyl) -L-cysteine (BEC) and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in endotoxin-induced endothelial dysfunction model

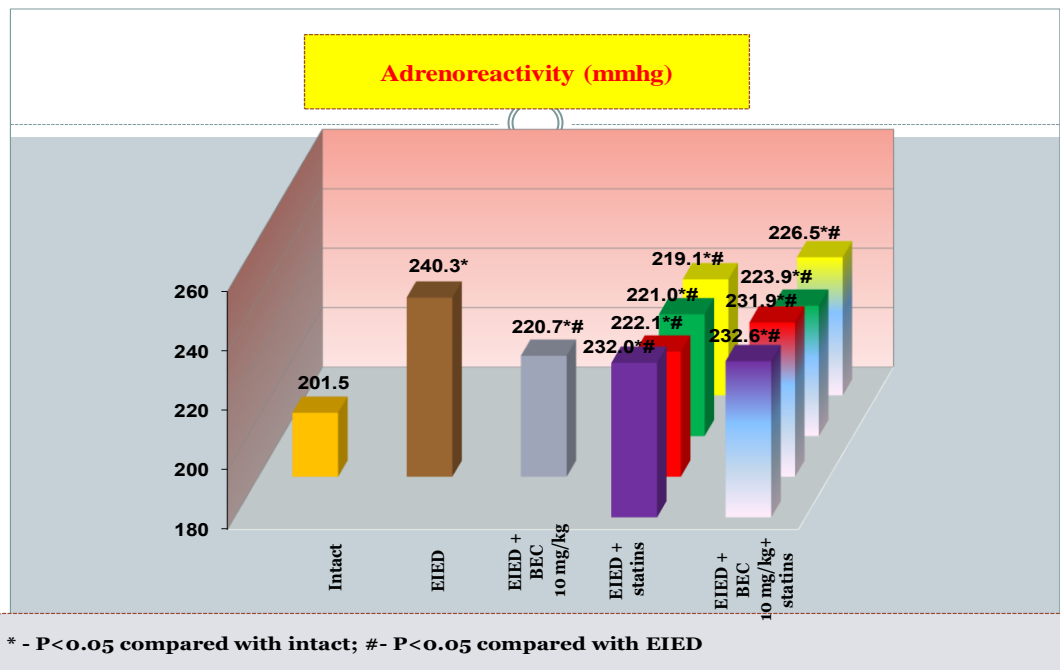


Fig. 19. Adrenoreactivity in the combined use of S- (2-boro-ethyl) -L-cysteine (BEC) and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin against endotoxin-induced endothelial dysfunction modeling

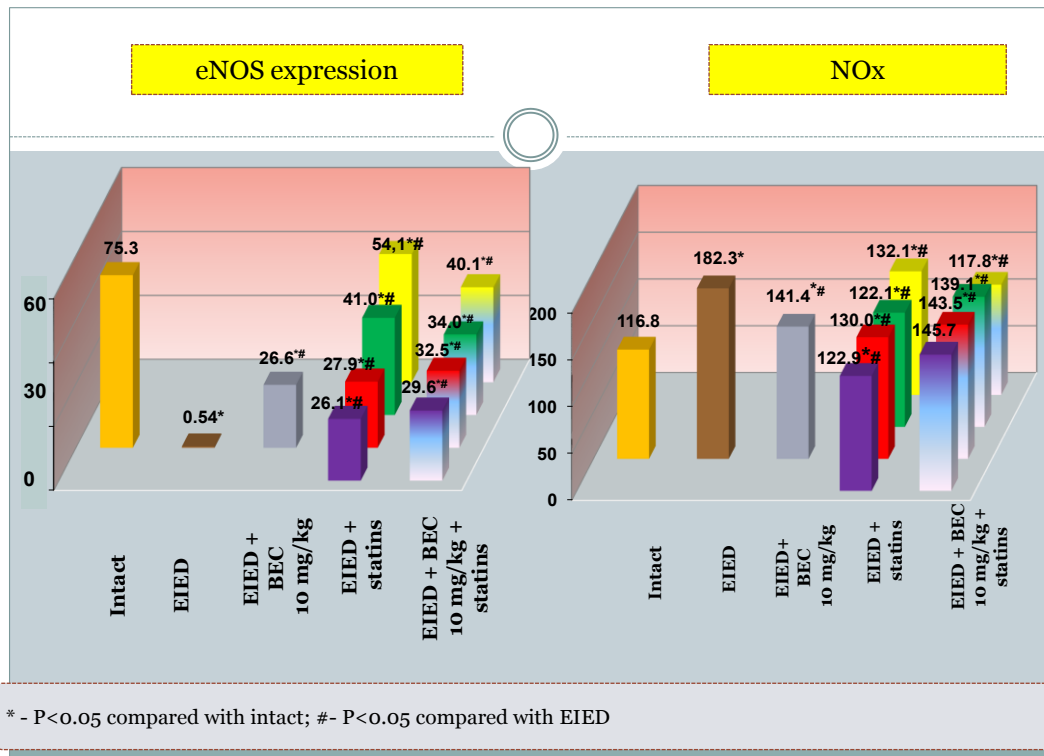


Fig. 20. Nitric oxide concentration (NOx) and NO synthase expression (eNOS) when combined with the use of S-(2-boro-ethyl)-L-cysteine (BEC) and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of modeling endotoxin-induced endothelial dysfunction

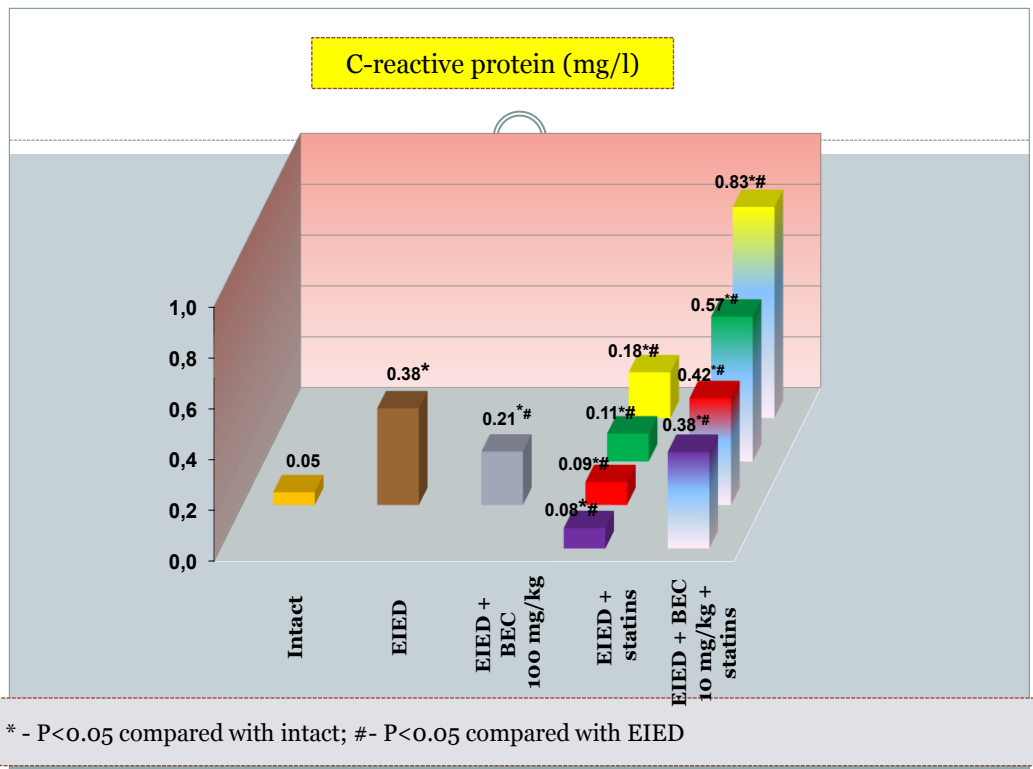


Fig. 21. C-reactive protein (CRP) indices in the combined use of S-(2-boro-ethyl)-L-cysteine (BEC) and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin against endotoxin-induced endothelial dysfunction

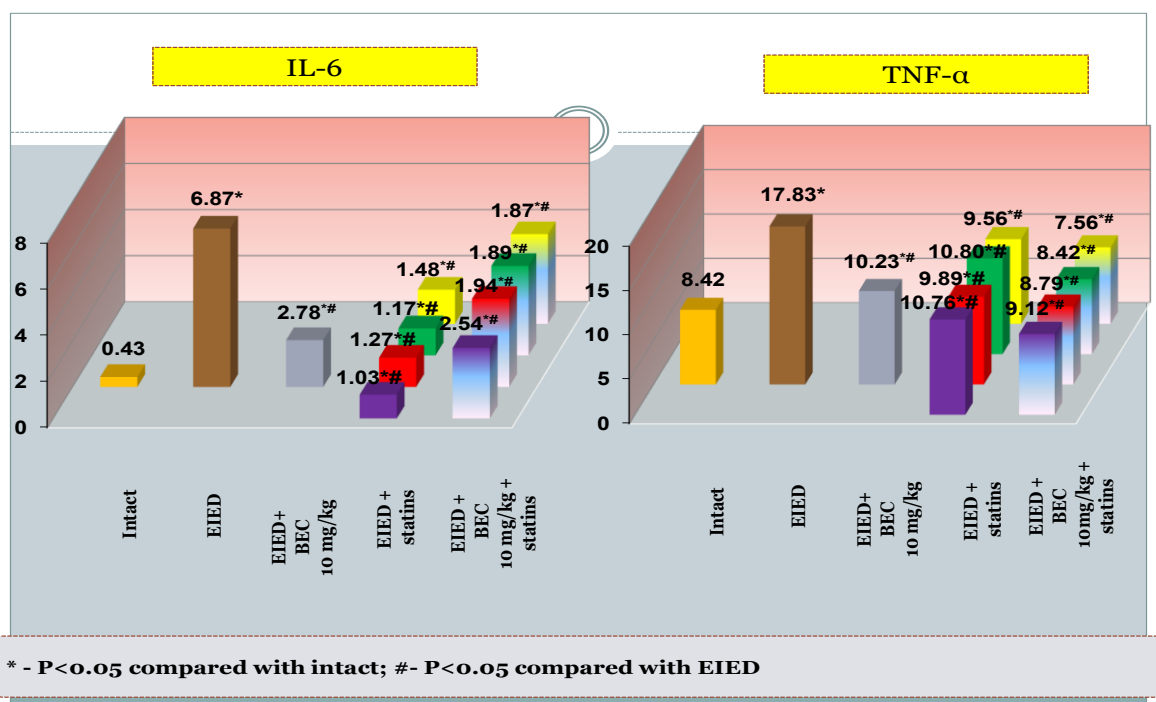


Fig. 22. Concentration of proinflammatory cytokines IL-6 and TNF-α when combined use of S- (2-boro-ethyl) -L-cysteine (BEC) and inhibitors of HMG-CoA reductase of simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of modeling endotoxin-induced endothelial dysfunction (EIED)

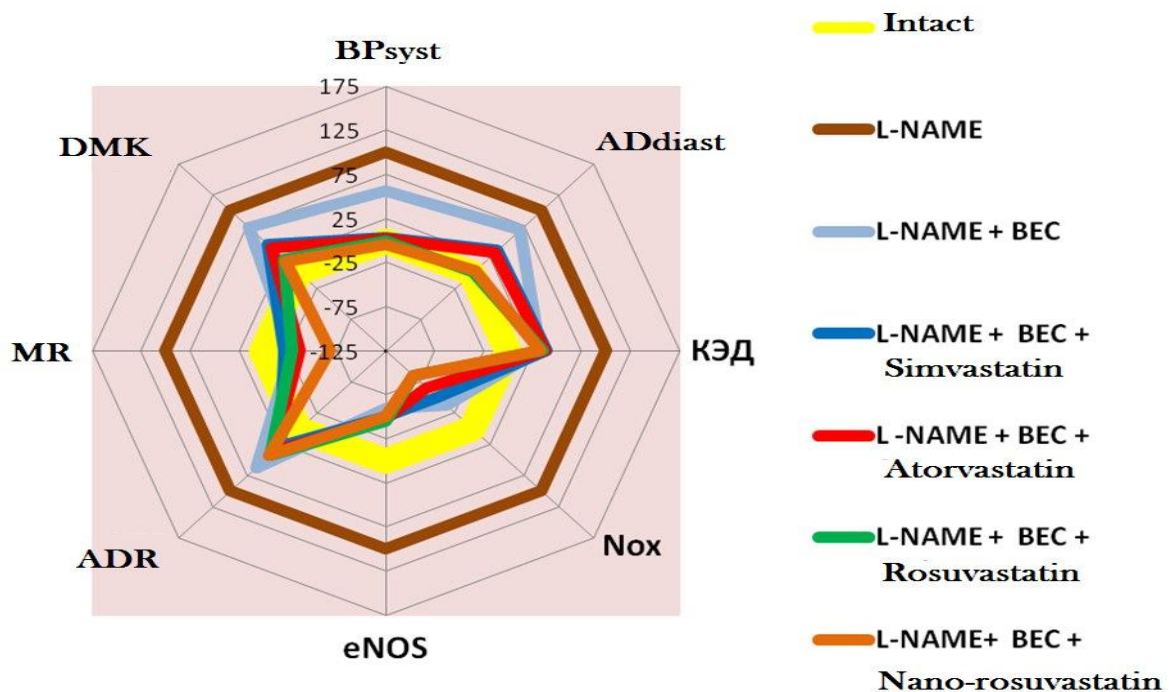


Fig. 23. Protective effect of the combined use of S- (2-boro-ethyl) -L-cysteine (BEC) with HMG-CoA reductase inhibitors on the model of L-NAME-induced endothelial dysfunction

Note: BPSyst – systolic blood pressure; BPdiast – diastolic blood pressure; CED – coefficient of endothelial dysfunction; NOx – concentration of nitrite ions in plasma; eNOS – expression of eNOS; ADR – adrenoreactivity; MR – myocardial reserve; DMK is the diameter of myocardiocytes (% of the group of intact animals)

The effect of combined therapy with a non-selective inhibitor of arginase BEC (10 mg/kg) with inhibitors of HMG-CoA reductase by simvastatin (8.5 mg/kg), atorvastatin (4.3 mg/kg), rosuvastatin (8.5 mg/kg) and nanoparticulated rosuvastatin (11.6 mg/kg) on the model of L-NAME-induced deficiency of nitric oxide is shown in Figure 23.

BEC moderately reduced systolic and diastolic blood pressure and 2-fold decreased CED, the value of which was 2.5 ± 0.5 c.u., whereas in control 5.4 ± 0.6 c.u. (table 4). HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin caused more pronounced endothelial and cardioprotective effects in preventing the increase in blood pressure and CED (Figure 5).

The combined use of a nonselective inhibitor of BEC arginase with simvastatin, atorvastatin, rosuvastatin, and nanoparticulated rosuvastatin also had a protective effect, but did not show an additive effect. In the experiments with loading tests, the combined use of BEC with HMG-CoA reductase inhibitors also revealed a cardioprotective effect, preventing an increase in adrenoreactivity and depletion of the myocardial reserve. At the same time, the combined use of drugs did not increase the cardioprotective effect, and the adrenoreactivity values even increased slightly compared to statin monotherapy. A similar additive effect of the combined use of BEC and inhibitors of HMG-CoA-reductase of simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin was also observed with respect to the values of the final NO_x metabolites and eNOS expression. In parallel with the dynamics of the cardioprotective effect of the combined use of BEC with HMG-CoA reductase inhibitors, a positive effect has been found with respect to the reduction of hypertrophy of myocardiocytes.

Thus, the use of the combined use of a nonselective inhibitor of arginase BEC with simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin against the

background of endotoxin-induced pathology modeling, and L-NAME-induced NO deficiency, exhibits endothelial and cardioprotective effects in preventing CED, adrenoreactivity, preservation of the myocardial reserve and normalization of the values of biochemical markers (Total NO, eNOS expression, C-reactive protein, IL-6, TNF- α). At the same time, combined therapy showed no additive effect of drugs (Figure 23).

Study of the effect of combined therapy with a selective inhibitor of arginase-2 arginasine with simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in endotoxin-induced endothelial dysfunction.

Monotherapy with a selective inhibitor of arginase-2 arginasine (3 mg/kg) daily intragastric on the background of modeling EIED moderately normalized CED and did not significantly affect blood pressure (Figure 19). The average value of CED was 2.1 ± 0.3 cu. Inhibitors of HMG-CoA reductase, 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 significantly improved CED and did not affect blood pressure. The CED values were in the range 2.3 – 1.5 cu.

The combined use of a selective inhibitor of arginase-2 arginasine (3 mg/kg) with statins showed the additivity of the effect against CED and BP. Values were even slightly higher than with monotherapy with statins, however, were statistically significantly different from intact animals (Figure 24). In parallel, a positive dynamics of contractility indices was found in carrying out stress tests in animals with endotoxin-induced endothelial dysfunction. The selective inhibitor of arginase-2 arginasine (3 mg/kg) was superior to statins both in terms of preventing an increase in adrenoreactivity and in terms of conserving an expansion reserve. The combined use of arginasine with simvastatin, atorvastatin, rosuvastatin, and nanoparticulated rosuvastatin resulted in an additive enhancement (Figure 25).

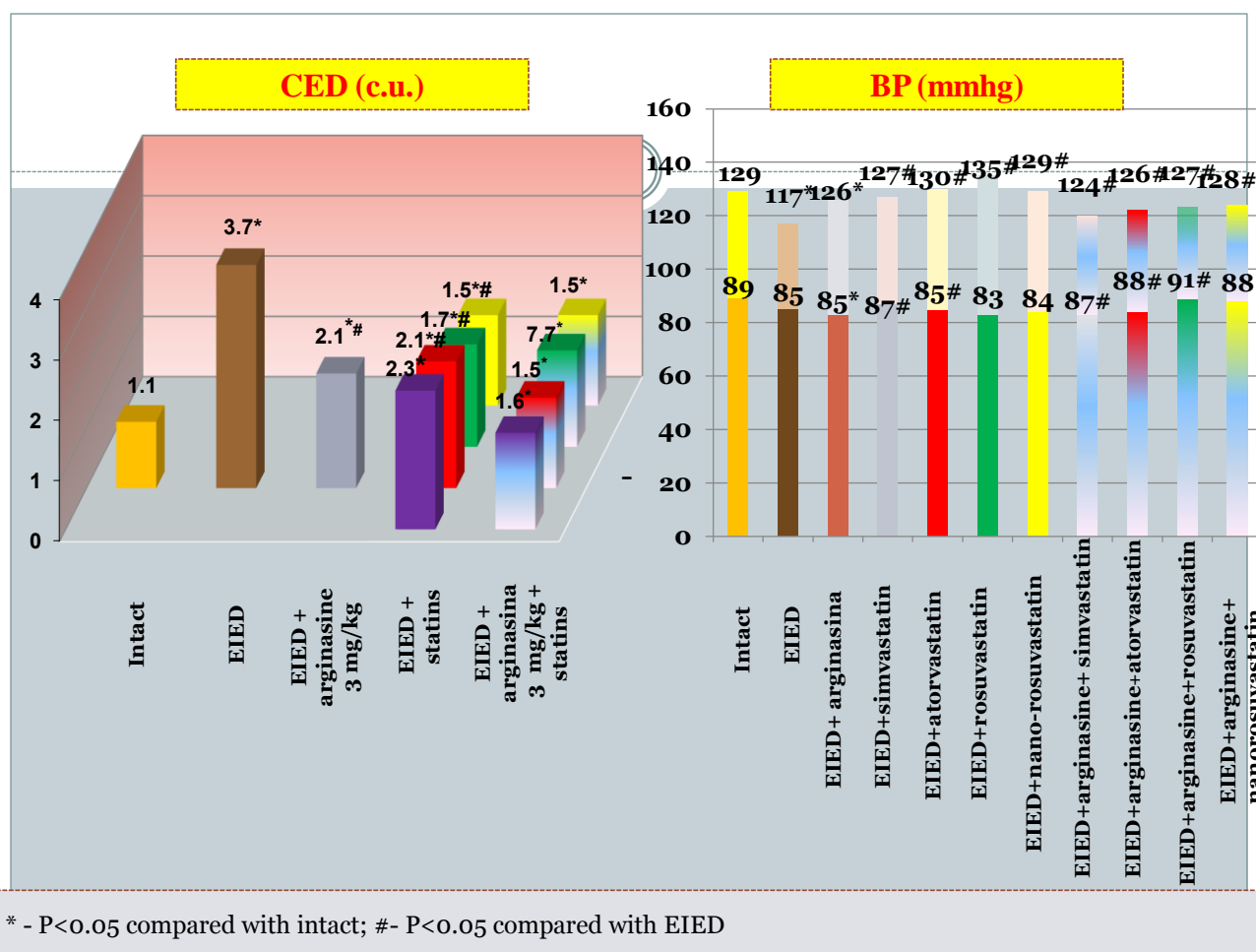


Fig. 24. Indices of endothelial dysfunction coefficient (CED) and arterial pressure (BP) when combined use of arginase and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of endotoxin-induced endothelial dysfunction (EIED) modeling

A similar trend has been observed with respect to the values of biochemical markers in animals with endotoxin-induced endothelial dysfunction (Fig. 26).

The most significantly protective effect of the combined use of a selective inhibitor of arginase-2 arginase (3 mg/kg) with inhibitors of HMG-CoA reductase by simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin was manifested in relation to the level of C-reactive protein and the values of proinflammatory cytokines IL-6 and TNF- α , the levels of which were comparable in all series of experiments that received pharmacotherapy (Figures 27, 28). We did not find an additive effect when combined in this model of pathology.

In the model of L-NAME-induced nitric oxide deficiency, arginine (3 mg/kg) moderately reduced systolic and diastolic blood

pressure and reduced CED by a factor of 1.7 ± 0.6 , while in control $5.4 \pm 0,6$ c.u. Inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin caused more pronounced endothelial and cardioprotective effects in preventing the increase in blood pressure and CED.

The combined use of a selective inhibitor of arginase-2 arginase (3 mg/kg) with simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin also had a protective effect and showed an additive effect, manifested in a decrease in the endothelial dysfunction coefficient, prevention of NOx concentration decrease, and improvement of indices myocardial contractility in conducting functional tests for adrenergic reactivity and resistance loading, reduction of hypertrophy of myocytes (Figure 29).

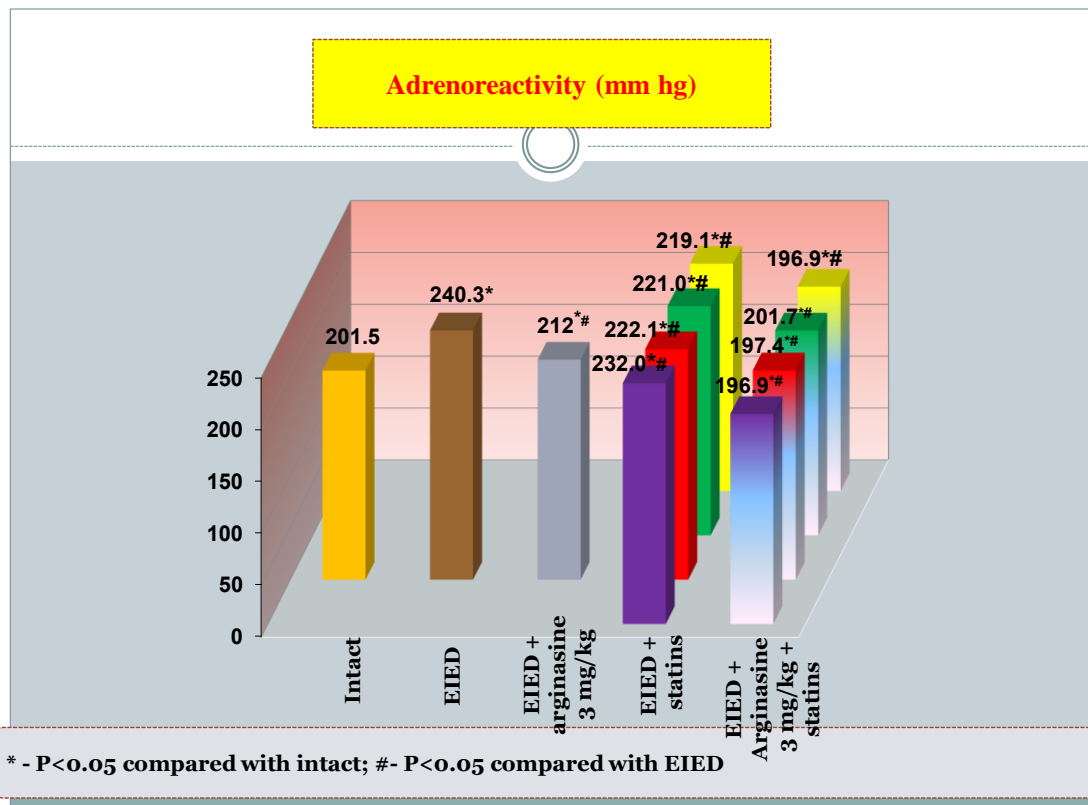


Fig. 25. Adrenoreactivity in the combined use of arginase and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in the context of modeling endotoxin-induced endothelial dysfunction (EIED)

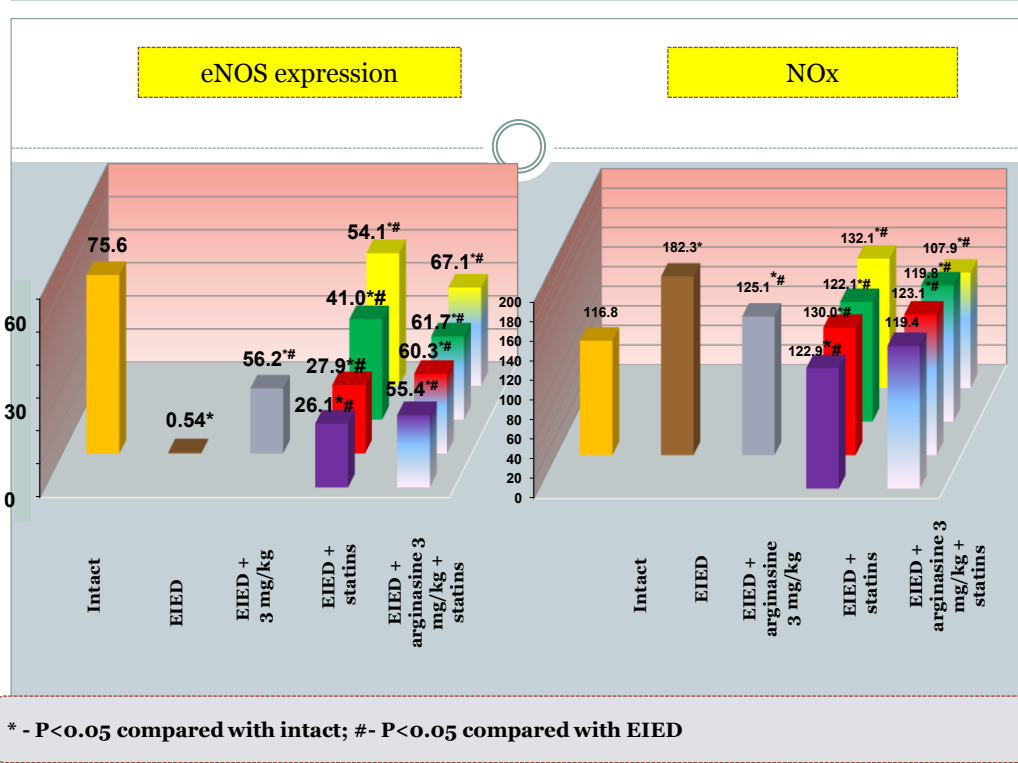


Fig. 26. Nitric oxide concentration (NOx) and NO synthase expression (eNOS) combined with the use of arginase and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of modeling endotoxin-induced endothelial dysfunction (EIED)

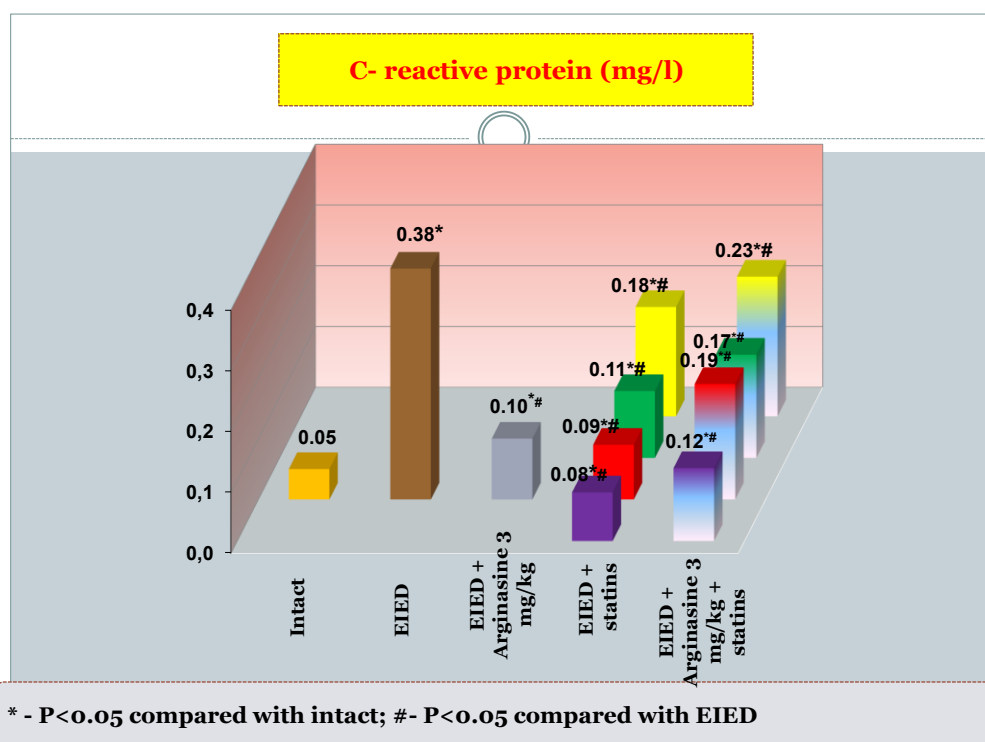


Fig. 27. Concentration of C-reactive protein (CRP) with the combined use of arginase and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in the context of endotoxin-induced endothelial dysfunction (EIED) modeling

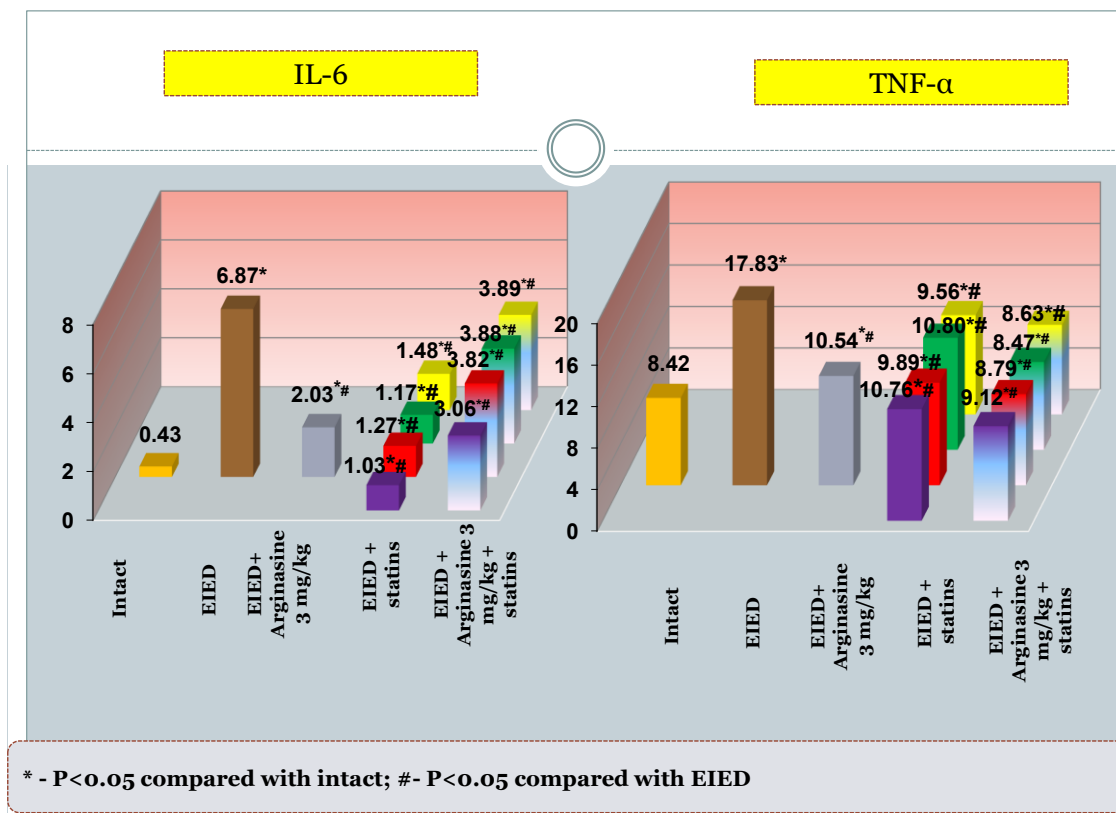


Fig. 28. Concentration of IL-6 TNF-α with the combined use of arginase and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in the context of modeling endotoxin-induced endothelial dysfunction (EIED)

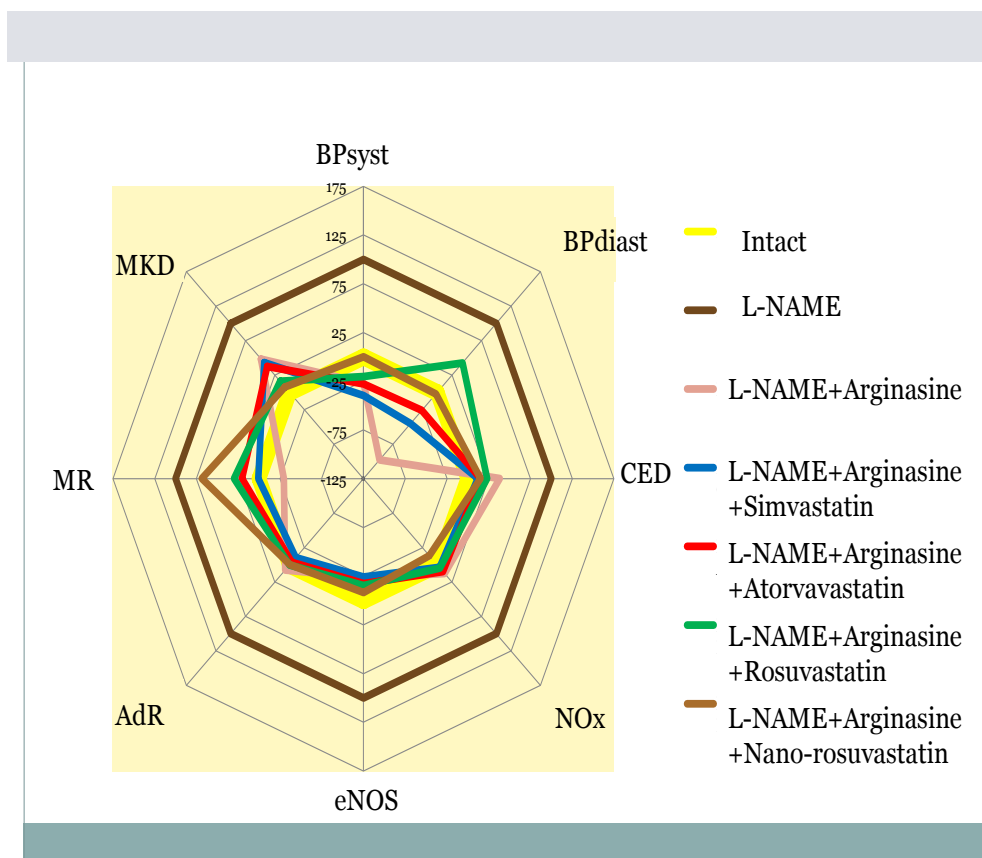


Fig. 29. Protective action of combined arginine with HMG-CoA reductase inhibitors on the model of L-NAME-induced endothelial dysfunction

Note: BPsyst – systolic blood pressure; BPdiast – diastolic blood pressure; CED – coefficient of endothelial dysfunction; NOx – concentration of nitrite ions in plasma; eNOS – expression of eNOS; AdR – adrenoactivity; MR – myocardial reserve; DMK is the diameter of myocardiocytes (% of the group of intact animals)

Thus, the use of the combined use of a selective inhibitor of arginase-2 arginasine (3 mg/kg) with simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin against endotoxin-induced pathology modeling, and L-NAME-induced NO deficiency, exhibits ectothelial and cardioprotective effects, expressed in preventing the increase in CED, adrenoactivity, preservation of the myocardial reserve and normalization of the values of biochemical markers (Total NO, eNOS Expression, C-reactive protein, IL-6,

TNF- α). At the same time, combined therapy revealed the additive effect of drugs.

Study of the effect of combined therapy with darbepoetin, simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in endotoxin-induced endothelial dysfunction.

The combined use of darbepoetin (500 μ g/kg) with statins showed additivity of the effect against CED and BP. Values were even slightly higher than with statin monotherapy, however, were statistically significantly different from intact animals (Figure 30).

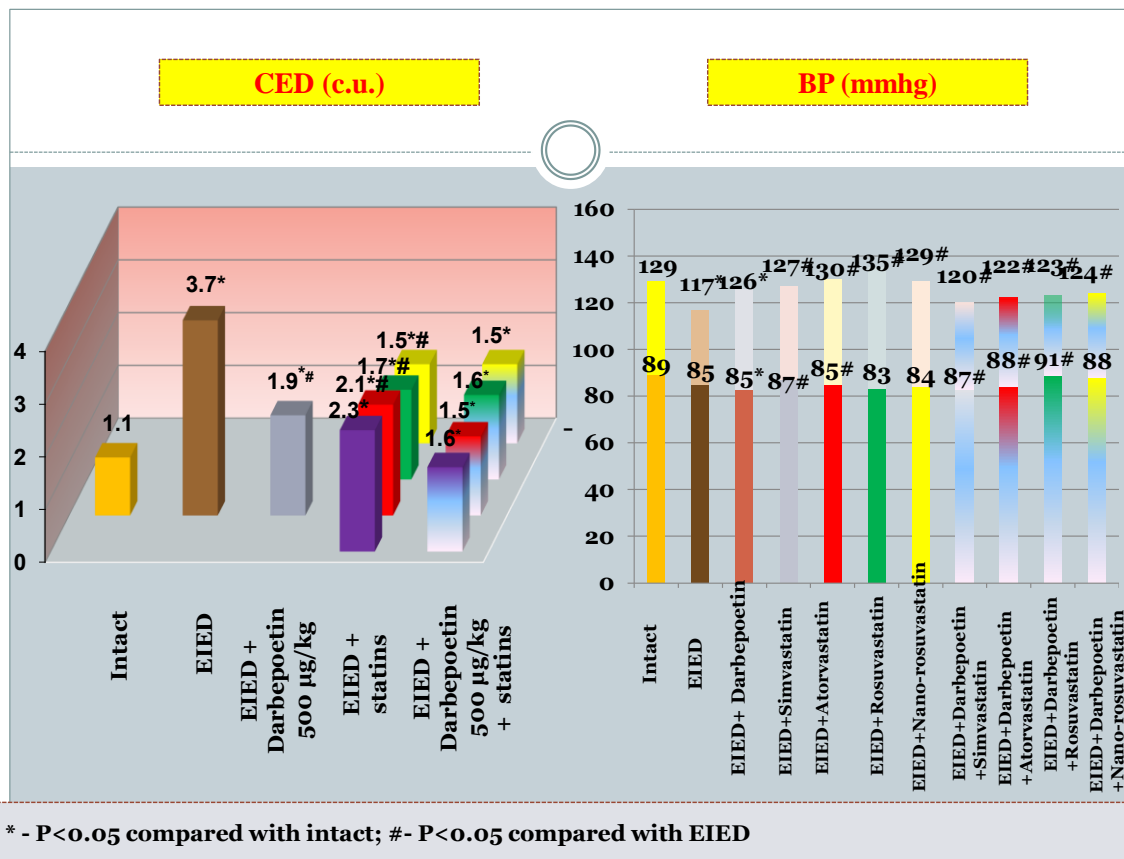


Fig. 30. The indices of endothelial dysfunction coefficient (CED) with the combined use of darbepoetin and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in the context of modeling endotoxin-induced endothelial dysfunction (EIED)

The most significantly protective effect of the combined use of darbepoetin (500 µg/kg) with inhibitors of HMG-CoA reductase by simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin was manifested in relation to the level of C-reactive protein and the values of proinflammatory cytokines IL-6 and TNF- were comparable in all series of experiments that received pharmacotherapy. We did not find the additive effect of combined use on this model of pathology (Figures 31-33).

Thus, the use of the combined use of darbepoetin (500 µg/kg) with simvastatin,

atorvastatin, rosuvastatin and nanoparticulated rosuvastatin against the background of endotoxin-induced pathology modeling, the introduction of strain 13407 of *Staphylococcus aureus* shows endothelial and cardioprotective effects, which is manifested in preventing CED and adrenoreactivity, preservation of the myocardial reserve and normalization of the values of biochemical markers (Total NO, eNOS expression, C-reactive protein, IL-6, TNF-α). At the same time, combined therapy revealed the additive effect of drugs (Figures 31-33).

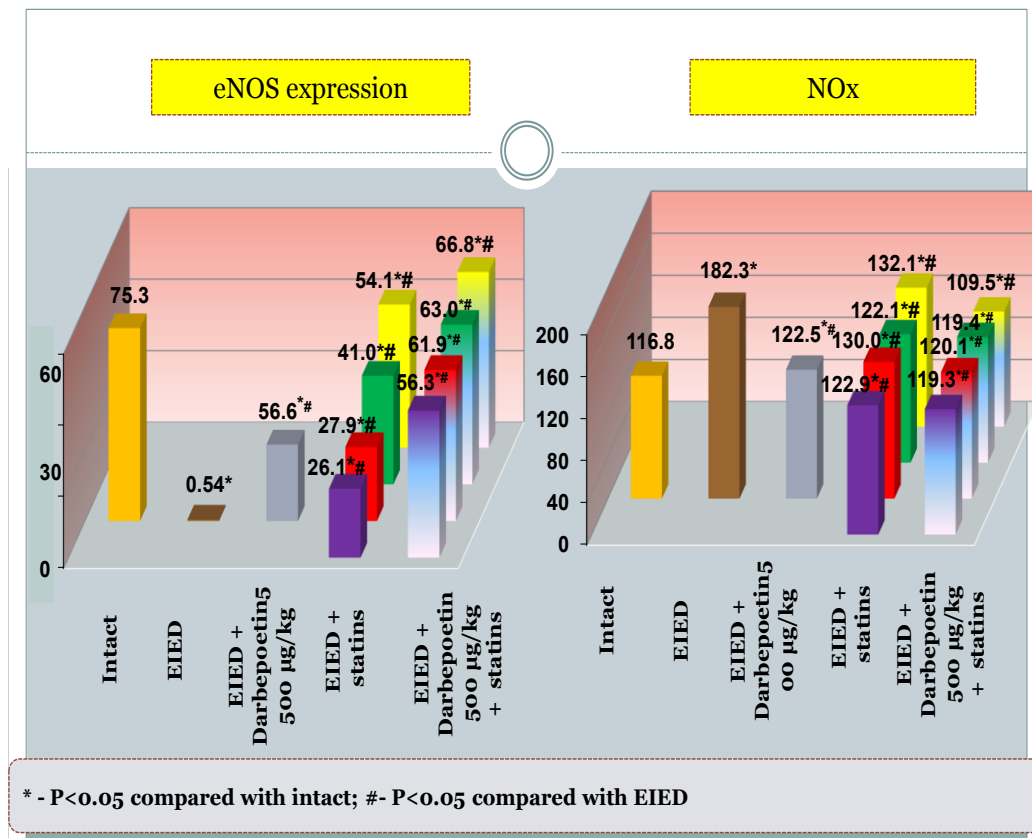


Fig. 31. Nitric oxide (NOx) concentration and NO-synthase (eNOS) expression in combined use of darbepoetin and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin against endotoxin-induced endothelial dysfunction (EIED) modeling

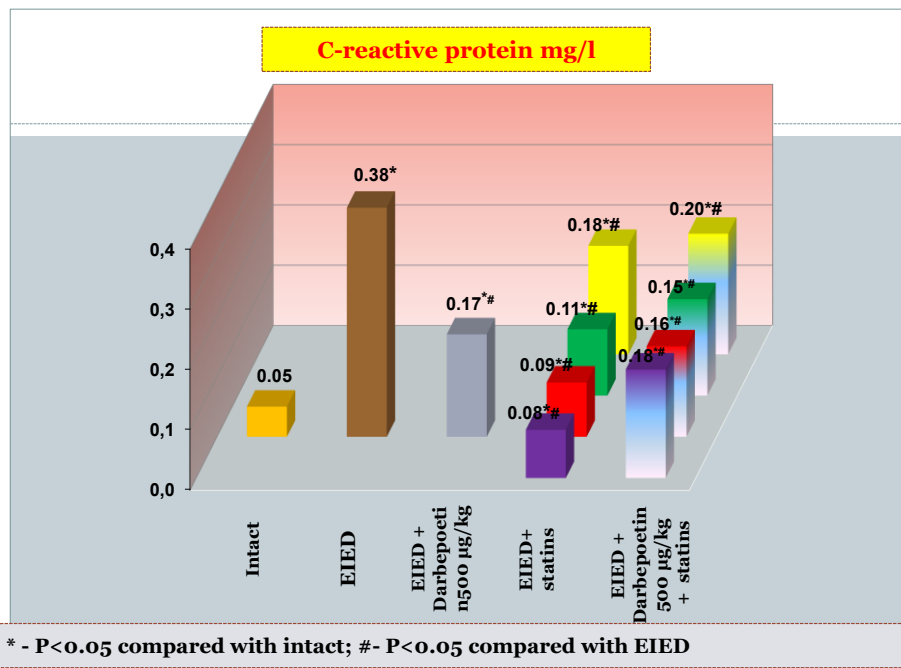


Fig. 32. Concentration of C-reactive protein (CRP) in the combined use of darbepoetin and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in the context of modeling endotoxin-induced endothelial dysfunction (EIED)

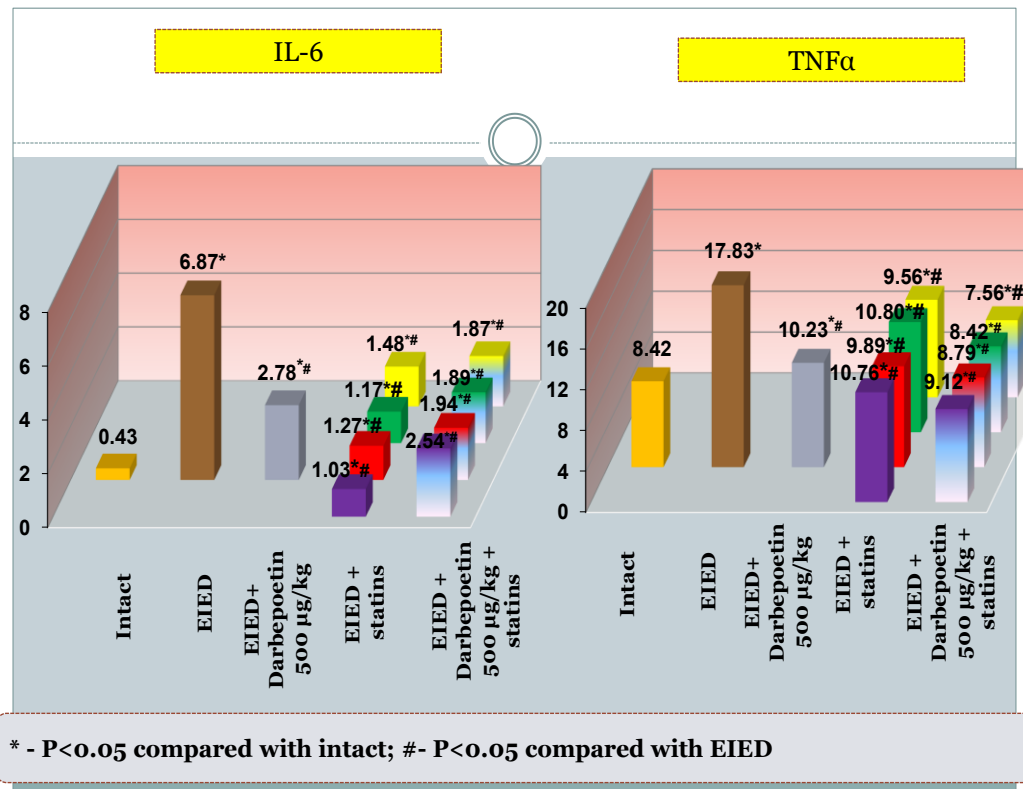


Fig. 33. Concentration of IL-6 and TNF-α with the combined use of darbepoetin and HMG-CoA reductase inhibitors simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin in the context of modeling endotoxin-induced endothelial dysfunction (EIED)

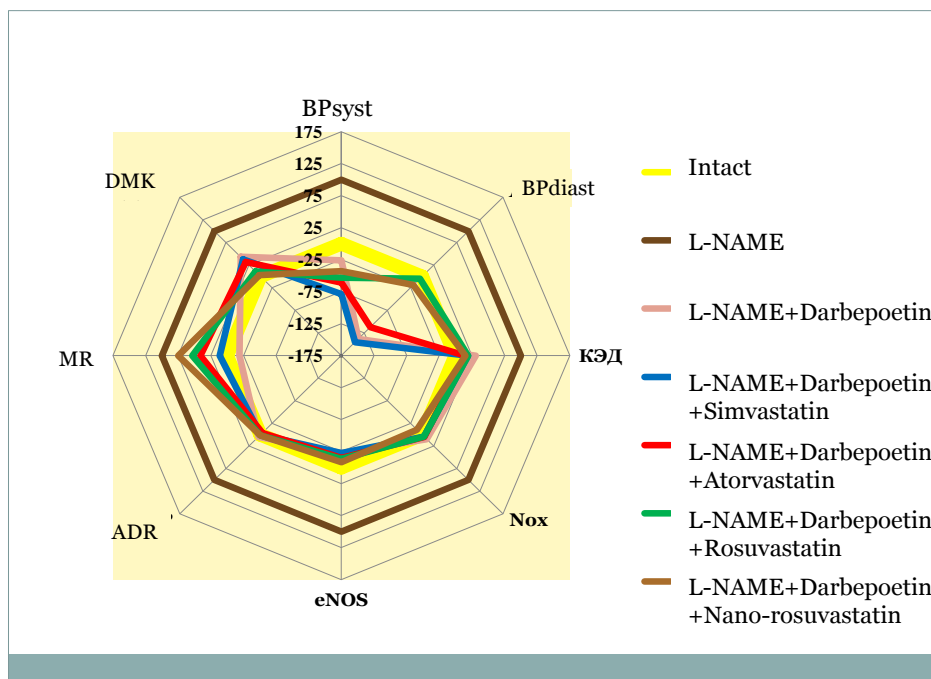


Fig. 34. Protective effect of the combined use of Darbeopetin with HMG-CoA reductase inhibitors on the model of L-NAME-induced endothelial dysfunction

Note: BPyst – systolic blood pressure; BPdiast – diastolic blood pressure; CED – coefficient of endothelial dysfunction; NOx – concentration of nitrite ions in plasma; eNOS – expression of eNOS; ADR – adrenoreactivity; MR – myocardial reserve; DMK is the diameter of myocardiocytes (% of the group of intact animals)

In the model of L-NAME-induced deficiency of nitric oxide, darbepoetin (500 µg/kg) moderately reduced systolic and diastolic blood pressure and reduced CED by a factor of 1.8, with a value of 1.8 ± 0.3 , whereas in control 5.4 ± 0.6 c.u. Inhibitors of HMG-CoA reductase simvastatin, atorvastatin, rosuvastatin, and nanoparticulated rosuvastatin caused a more pronounced endothelial and cardioprotective effect in preventing the increase in blood pressure and CED (Figure 34).

The combined use of darbepoetin (500 µg/kg) with simvastatin, atorvastatin, rosuvastatin, and nanoparticulated rosuvastatin also had a protective effect and showed an additive effect (Figure 28), expressed in a decrease in the endothelial dysfunction coefficient, prevention of NOx concentration increase, and improvement in myocardial contractility when conducting functional tests for adrenergic activity and resistance load; reduction of hypertrophy of cardiomyocytes.

Further analysis of combinations of inhibitors of HMG-CoA reductase and endothelioprotectors was carried out on the basis of the resulting areas obtained as a result

of integral vector analysis, the values of which are presented in Table 6. Comparing the areas of vector diagrams, we obtained distinct differences between the group of intact animals, animals with endotoxin-induced endothelial dysfunction modeling and groups of animals with pharmacological correction of the above preparations.

Further, a probabilistic percentage of the additions (Padd) of the tested combinations (Table 6) was determined using an integral vector analysis of combinations of HMG-CoA reductase inhibitors and endothelioprotectors.

The highest probability percentage of the additive for simvastatin was in combination with the selective inhibitor of arginase-2 with arginine – $21.0 \pm 1.9\%$. Similarly, atorvastatin worked at $22.6 \pm 2.1\%$. It is noteworthy that the maximum values of the additive effect were found for combinations of rosuvastatin with arginine (3 mg/kg) and darbepoetin (500 µg/kg), respectively, 31.9 ± 2.8 and $30.2 \pm 2.9\%$. Nanoparticulated rosuvastatin also showed the greatest addiction with arginase and darbepoetin 30.2 ± 2.9 and $26.0 \pm 2.7\%$, respectively (Table 6).

Table 6

Probabilistic percent of additions (Padd) of the studied combinations in the modeling of ED

Experimental group	Padd (%)
ED + L-arginine 200 mg/kg + Simvastatin 8,5 mg/kg	3.4±0.3
ED + BEC 10 mg/kg + Simvastatin 8,5 mg/kg	-0.01±0.1
ED + Arginase 3 mg/kg + Simvastatin 8,5 mg/kg	21±1.9#
ED + Darbepoetin 500 µg/kg + Simvastatin 8,5 mg/kg	9.2±1.1#
ED + L-arginine 200 mg/kg + Atorvastatin 4,3 mg/kg	14.2±1.7#
ED + BEC 10 mg/kg + Atorvastatin 4,3 mg/kg	0.0±0.1
ED + Arginase 3 mg/kg + Atorvastatin 4,3 mg/kg	22.6±2.1#
ED + Darbepoetin 500 µg/kg + Atorvastatin 4,3 mg/kg	13.4±1.8#
ED + L-arginine 200 mg/kg + Rosuvastatin 8,5 mg/kg	23.5±2.7#
ED + BEC 10 mg/kg + Rosuvastatin 8,5 mg/kg	0.01±0.1
ED + Arginase 3 mg/kg + Rosuvastatin 8,5 mg/kg	31.9±2.8#
ED + Darbepoetin 500 µg/kg + Rosuvastatin 8,5 mg/kg	30.2±2.9#
ED + L-arginine 200 mg/kg Nanorosuvastatin 11,6 mg/kg	21±2.4#
ED + BEC 10 mg/kg + Nanorosuvastatin 11,6 mg/kg	0.0±0.1
ED + Arginase 3 mg/kg + Nanorosuvastatin 11,6 mg/kg	30.2±2.9#
ED + Darbepoetin 500 µg/kg + Nanorosuvastatin 11,6 mg/kg	26.0±2.7#

Note: ED – endothelial dysfunction; Padd – probability percentage of additions (%); * – $p < 0.005$ in comparison with ED; # – $p < 0.005$ compared with the endothelioprotective agent.

Discussion

The results of this research allow us to affirm that simulating a model of endotoxin-induced pathology created by injecting strain 13407 *Staphylococcus aureus*, leads to the development of endothelial dysfunction with changes in the response to endothelium-dependent and endothelium-independent vascular tests; to increasing of stable NO metabolites with inflammatory markers values raise: C-reactive protein, IL -6 and TNF- α . So, these phenomena correspond to the clinical picture of endotoxin-induced multi-organ pathology typical for after abdominal disasters with the development of systemic vasculitis and a further cascade of atherosclerotic changes in the vascular bed. The use of inhibitors of HMG-CoA reductase of simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin during simulating a model of endotoxin-induced pathology leads to a dose-dependent endothelioprotective effect development, which is expressed in CED normalization, adrenergic reactivity prevention and myocardial reserve depletion, as well as to normalization of biochemical markers of

inflammation and level of proinflammatory cytokines. The experiment had demonstrated positive dynamics of the final products of NO and expression of eNOS. It was proved that rosuvastatin and its nanoparticulated form had the most significant effect. Statins has both anti-inflammatory and cardioprotective effects. In the implementation of anti-inflammatory and cardioprotective effects, the mechanisms of pharmacological preconditioning are of great importance. This fact was proved with effects removal in the blockade of K⁺-ATP-ase channels and blockade of iNOS.

The combined use of L-arginine, a nonselective inhibitor of arginase, a selective inhibitor of arginase-2, arginasine, and recombinant darbepoetin with HMG-CoA reductase inhibitors had showed that the drugs enhance the endothelioprotective effects of each other. The combination of rosuvastatin with arginasine and darbepoetin gives the most additive effect. A possible explanation of the research results can be expressed as the analysis of application points of the drugs and the suggestion of their pharmacodynamic interaction (Figure 35).

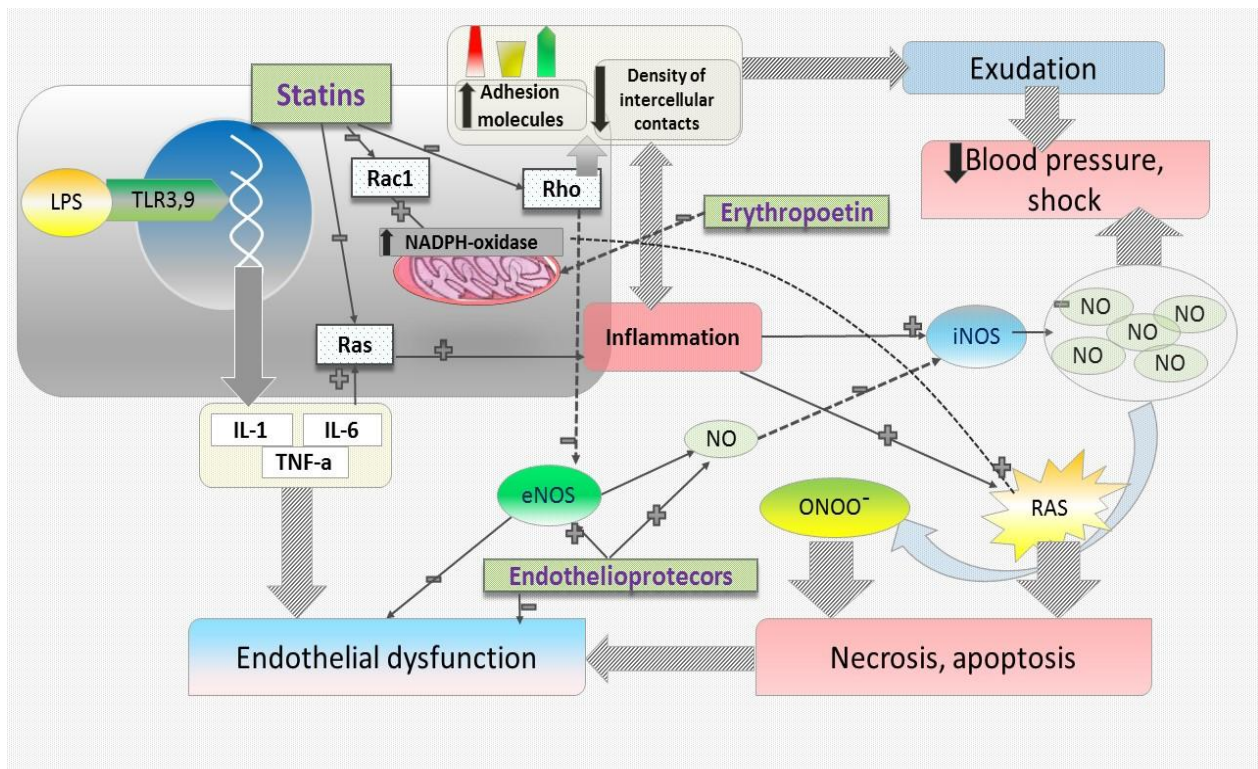


Fig. 35. Hypothetical ways of pharmacodynamic interaction of HMG-CoA reductase inhibitors, erythropoietin preparations and endothelioprotectors

By inhibiting the intracellular factors Rac1, Ras and Rho, statins reduce the production of reactive oxygen species and the production of pro-inflammatory cytokines, and same time they increase the activity of eNOS. Erythropoietin affects mitochondria through the secondary mediators by suppressing their activity. This also reduces the production of free radicals. Endothelioprotectors by activating eNOS, increase the correlation of eNOS / iNOS, which leads to decreasing of nitrosative stress and to improvement of endothelial function. As for a perspective of further research of this topic the authors assume to investigate therapeutic and prophylactic measures in patients with endotoxin-induced pathology, including peritonitis, abscesses and others purulent-septic complications of the abdominal cavity and small pelvis, as well as different acute and chronic intoxications accompanied by systemic vasculitis and endothelial dysfunction with the use of HMG-CoA reductase inhibitors and their combination with endothelioprotectors of various mechanisms of action.

Conflicts of Interest

The authors have no conflict of interest to declare.

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