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CONCOMITANT USE OF STATINS AND S-(2-BORON-ETHYL)-L-CYSTEINE ARGINASE INHIBITOR TO CORRECT ENDOTOXIN-INDUCED ENDOTHELIAL DYSFUNCTION

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Abstract: The concomitant use of non-selective *S-(2-boron-ethyl)-L-cysteine (BEC)* arginase inhibitor with simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of endotoxin-induced disorder modeling by Staphylococcus aureus (strain 13407) injection under the skin – 60 billions of microbial bodies – exhibits an endothelium- and cardio-protective action which is evident as endothelial dysfunction factor (EDF) increase prevention, adrenoreactivity, maintenance of myocardial reserve, and normalization of biochemical markers (total NO, expression of eNOS, C-reactive protein, IL-6, tumor necrosis factor). However, the concomitant therapy has shown no additive action of medications.
Key Words: endothelial dysfunction, statins, *S-(2-boron-ethyl)-L-cysteine*, simvastatin, atorvastatin, rosuvastatin, endotoxin.

Introduction: Great attention has been recently paid to endotoxin-induced cardiovascular injuries in abdominal surgery [1, 2, 3, 4]. In this respect, a clear sequence of events has been arranged: endotoxin shock with a multisystem disorder, -> release of proinflammatory cytokines, -> endothelial dysfunction ->, systemic vasculitis ->, vascular and endothelium permeability increase for lymphocytes ->, hyperlipoproteinemia ->, start of an atherosclerotic process [1, 2, 3, 4, 5, 6].

Under that logic, a similar algorithm could be used for any endotoxin-induced disorder regardless its cause considering critically high levels of proinflammatory cytokines [5, 6].

It is likely that concomitant use of HMG-CoA reductase inhibitors and arginase inhibitors is one of the pharmacological strategies to correct an endotoxin-induced endothelial dysfunction [7, 8, 9, 10].

Methods: tests have been run with white male Wistar rats weighing 200 to 250 grams. When creating a model of the endotoxin-induced endothelial dysfunction (EIED), the rats have been infected with Staphylococcus aureus (strain 13407) under the skin – 60 billions of microbial bodies – succeeded (in 24 hours) by sensibilization of the laboratory animal (0.1ml of staphylococcal anatoxin under the skin) followed by generalization of the infectious agent with a daily massage of the injection site. Then, a compression and pneumatic massage of the injection site was made daily within 10 minutes.

HMG-CoA reductase inhibitors in combination with an *S-(2-boron-ethyl)-L-cysteine (BEC)* arginase inhibitor (WIRUD GmgH, Hamburg) – 10mg/kg – have been daily injected intragastrically within 7 days. The animals have been divided into experimental groups (n = 10): 1 – intact ones; 2 – endotoxin-induced endothelial dysfunction (EIED); 3 – EIED + BEC 10mg/kg; 4 – EIED + simvastatin 8.5mg/kg; 5 – EIED + atorvastatin 4.3mg/kg; 6 – EIED + rosuvastatin 8.5mg/kg; 7 – EIED + nanaparticulated rosuvastatin 11.6mg/kg; 8 – EIED + simvastatin 8.5mg/kg + BEC 10mg/kg; 9 – EIED + atorvastatin 4.3mg/kg + BEC 10mg/kg; 10 – EIED + rosuvastatin 8.5mg/kg + BEC 10mg/kg; 11 – EIED + nanaparticulated rosuvastatin 11.6mg/kg + BEC 10mg/kg.

On the eighth day since the beginning of the test, the animals were catheterized into the left carotid

artery under anesthesia (300 mg/kg chloral hydrate) to register blood pressure (BP); the bolus dose of pharmacological agents has been injected into the femoral vein. Hemodynamic parameters: systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) have been measured constantly with the Biopac hardware and software system. Beside BP measuring, several functional tests have been run in the following sequence: 1. Test on endothelium-dependent vascular relaxation (intravenous injection of acetyl choline (ACh) solution dosing 40 mkg/kg at the rate of 0.1ml per 100g). 2. Test on endothelium-independent vascular relaxation (intravenous injection of sodium nitroprusside (SNP) solution dosing 30 mkg/kg at the rate of 0.1ml per 100g) [11, 12, 13, 14].

The experimental animals' endothelial dysfunction extent as well as level of dysfunction correction with the medications tested have been assessed according to the estimated factor of endothelial dysfunction (EDF) [11, 12, 13, 14].

To assess the cardioprotective activity, functional tests on adrenoreactivity [11, 12, 13, 14] and myocardial reserve exhaustion [11, 12, 13, 14] have been run.

The dynamics of biochemical markers (total NO, expression of eNOS, C-reactive protein, IL-6, tumor necrosis factor) of the animals with endotoxin-induced endothelial dysfunction have been assessed with standard reagent kits [11, 12, 13, 14].

Accuracy of changes in absolute values has been defined by the difference method of the variation statistics with finding the mean shifts (M), the arithmetical mean (±m), and error probability (p) using Student's t-distribution tables. Differences have been assessed as accurate at $p<0.05$. Statistical data have been calculated with Microsoft Excel 7.0.

Results: Daily intragastric monotherapy of non-selective L-norvaline arginase inhibitor (10mg/kg) on the background of modeling a sepsis-induced endothelial dysfunction has moderately normalized the EDF and had no remarkable influence on BP values (Table 1). The EDF was 2.5±0.4 units. HMG-CoA reductase inhibitors – simvastatin (8.5mg/kg), atorvastatin (4.3mg/kg), rosuvastatin (8.5mg/kg), and nanoparticulated rosuvastatin (11.6mg/kg) – in the most efficient doses, have significantly improved the EDF and had no effect on the BP (Table 1). The EDF values were in the range of 2.3 to 1.5 units.

The concomitant use of BEC with statins has shown no additive effect on the EDF and BP. The values have been even a bit higher than in case of the statin monotherapy though they have remarkably differed from the intact animals in statistics (Table 1).

At the same time, a positive dynamics of the indices of contractility has been revealed when running EKG stress tests in animals with sepsis-induced endothelial dysfunction (Table 2). Activity of BEC was second to statins both regarding prevention of adrenoactivity increase and maintenance of dilatation reserve. The concomitant use of BEC with simvastatin, atorvastatin, rosuvastatin, and nanoparticulated rosuvastatin has resulted in no additive enhancement while chamber pressure parameters, in case of EKG stress tests, have approached additive enhancement in the BEC series and have been behind HMG-CoA reductase inhibitors (Table 2).

A similar trend has been found out regarding parameters of biochemical markers of the animals with sepsis-induced endothelial dysfunction (Table 3).

The most significant protective action of the concomitant use of BEC with HMG-CoA reductase inhibitors - simvastatin, atorvastatin, rosuvastatin, and nanoparticulated rosuvastatin – has been evident regarding the level of C-reactive protein and values of proinflammatory cytokines IL-6 and tumor necrosis factor with their levels being comparable in any experiment series with pharmacotherapy. We have not seen additive action of the concomitant use for this model of disorders (Table 3).

Conclusion: The concomitant use of non-selective BEC arginase inhibitor with simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on the background of sepsis-induced disorder modeling by Staphylococcus aureus (strain 13407) injection under the skin – 60 billions of microbial bodies – exhibits an endothelium- and cardio-protective action which is evident as EDF increase prevention, adrenoreactivity, maintenance of myocardial reserve, and normalization of biochemical markers (total NO, expression of eNOS, C-reactive protein, IL-6, tumor necrosis factor). However, the concomitant therapy has shown no additive action of medications.

Table 1

Influence of the concomitant use of non-selective BEC arginase inhibitor with simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on dynamics of hemodynamic parameters of animals with endotoxin-induced endothelial dysfunction (M±м, n=10)

Group of Animals	SBP	DBP	EDF
Intact	129.4±2.2	89.2± 1.1	1.1 ± 0.1
Endotoxin-Induced Endothelial Dysfunction (EIED) (n=10)	117.6±2.3*	85.0±2.1	3.7±0.5*
EIED + BEC 10mg/kg (n=10)	115.3±2.4	79.1±2.2	2.5±0.4*
EIED + Simvastatin 8.5mg/kg (n=10)	127.3±2.8	87.1±1.9	2.3±0.5*#
EIED + Atorvastatin 4.3mg/kg(n=10)	130.0±3.3	85.8±2.2	2.1±0.3*#
EIED + Rosuvastatin 8.5mg/kg (n=1)	135.0±3.8	83.1±2.1	1.7±0.5*#
EIED + Nanoparticulated Rosuvastatin 11.6mg/kg (n=10)	129.6±4.3	84.9±2.0	1.5±0.2*#
EIED + BEC 10mg/kg + Simvastatin 8.5mg/kg (n=10)	122.1±3.0	84.1±2.1	2.6±0.4*
EIED + BEC 10mg/kg + Atorvastatin 4.3 mg/kg(n=10)	125.3±3.2	82.3±2.0	2.5±0.3*
EIED + BEC 10mg/kg + Rosuvastatin 8.5mg/kg (n=1)	126.3±3.1	81.9±2.1	2.7±0.4*
EIED + BEC 10mg/kg + Nanoparticulated Rosuvastatin 11.6mg/kg (n=10)	127.3±3.2	84.2±2.4	2.5±0.3*

Note: SBP – systolic blood pressure (mmHg), DBP – diastolic blood pressure (mmHg), EDF – endothelial dysfunction factor (units), * – accurate difference with the group of intact animals ($p<0.05$); # – accurate difference with the group of endotoxin-induced endothelial dysfunction (EIED) ($p<0.05$).

Table 2

Influence of the concomitant use of non-selective BEC arginase inhibitor with simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on dynamics of indices of contractility in case of EKG stress tests in animals with endotoxin-induced endothelial dysfunction (M±м, n=10).

Group of Animals	Adrenoreactivity (mmHg)	Myocardial Reserve Exhaustion (%)
Intact	201.5±9.4	112.7±10.9
Endotoxin-Induced Endothelial Dysfunction (EIED) (n=10)	240.3±8.7*	79.4±3.9*
EIED + BEC 10mg/kg (n=10)	220.7±8.3*	92.4±5.7*
EIED + Simvastatin 8.5mg/kg (n=10)	232.0±8.9*	87.4±3.7*
EIED + Atorvastatin 4.3mg/kg(n=10)	222.1±8.5*#	97.0±4.9*
EIED + Rosuvastatin 8.5mg/kg (n=1)	221.0±8.4*#	109.4±5.7*#
EIED + Nanoparticulated Rosuvastatin 11.6mg/kg (n=10)	219.1±8.7*#	99.9±6.3*#
EIED + BEC 10mg/kg + Simvastatin 8.5mg/kg (n=10)	232.6±7.5*#	89.1±3.6*#
EIED + BEC 10mg/kg + Atorvastatin 4.3 mg/kg(n=10)	231.9±8.4*#	88.9±3.9*#
EIED + BEC 10mg/kg + Rosuvastatin 8.5mg/kg (n=1)	223.9±9.6*#	88.5±4.9*#
EIED + BEC 10mg/kg + Nanoparticulated Rosuvastatin 11.6mg/kg (n=10)	226.5±8.4*#	90.1±5.0*#

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

Table 3

Influence of the concomitant use of non-selective BEC arginase inhibitor with simvastatin, atorvastatin, rosuvastatin and nanoparticulated rosuvastatin on value dynamics of biochemical markers (total NO, expression of eNOS, C-reactive protein, IL-6, tumor necrosis factor) of animals with endotoxin-induced endothelial dysfunction (M±м, n=10).

Group of Animals	NOx	Expression of eNOS	CRP Level	IL-6	TNF
Intact	116.8±10.3	5.4±0.21	0.05±0.01	0.43±0.17	8.42±2.51
Endotoxin-Induced Endothelial Dysfunction (EIED) (n=10)	182.3±12.4*	0.04±0.01*	0.38±0.01*	6.87±1.93*	17.83±3.79*
EIED + BEC 10mg/kg (n=10)	141.4±12.7*#	1.97±0.10*#	0.21±0.02*#	2.78±1.79*#	10.23±2.08*#
EIED + Simvastatin 8.5mg/kg (n=10)	122.9±8.4*#	1.93±0.12*#	0.08±0.01*#	1.03±0.62*#	10.76±1.70*#
EIED + Atorvastatin 4.3mg/kg(n=10)	130.0±10.9*#	2.07±0.21*#	0.09±0.01*#	1.27±0.33*#	9.89±1.79*#
EIED + Rosuvastatin 8.5mg/kg (n=1)	122.1±9.9#*	3.04±0.35*#	0.11±0.01*#	1.17±0.33*#	10.80±1.99*#
EIED + Nanoparticulated Rosuvastatin 11.6mg/kg (n=10)	132.1±10.3*#	4.01±0.56*#	0.18±0.01*#	1.48±0.24*#	9.56±1.87*#
EIED + BEC 10mg/kg + Simvastatin 8.5mg/kg (n=10)	145.7±10.7*#	2.19±0.37*#	0.38±0.06*#	2.54±0.24*#	9.12±1.12*#
EIED + BEC 10mg/kg + Atorvastatin 4.3 mg/kg(n=10)	143.5±9.9*#	2.41±0.34*#	0.42±0.08*#	1.94±0.19*#	8.79±0.91*#
EIED + BEC 10mg/kg + Rosuvastatin 8.5mg/kg (n=1)	139.1±9.5*#	2.52±0.41*#	0.57±0.09*#	1.89±0.21*#	8.42±0.87*#
EIED + BEC 10mg/kg + Nanoparticulated Rosuvastatin 11.6mg/kg (n=10)	117.8±10.0*#	2.97±0.41*#	0.83±0.09*#	1.87±0.20*#	7.56±0.79*#

Note: NOx – terminal metabolites NO (μmol/l); Expression of eNOS (%);CRP Level – level of C-reactive protein (mg/l); IL-6 – interleukin-6 (pg/mL); TNF – tumor necrosis factor (pg/mL), * – accurate difference with the group of intact animals ($p<0.05$); # – accurate difference with the group of endotoxin-induced endothelial dysfunction (EIED) ($p<0.05$).

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