ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Endothelio- and Cardioprotective Effects Of HMG-CoA Reductase Inhibitors Under The Condition Of Endotoxin-Induced Endothelial Dysfunction.

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ABSTRACT

Application of the HMG-CoA reductase inhibitors, such as Simvastatin, Atorvastatin, Rosuvastatin and nanocapsular Rosuvastatin, leads to dose response endothelioprotective effect under the condition of endotoxin-induced endothelial dysfunction after administration of the Staphylococcus aureus strain 603. Endothelioprotective effect consists in recovery of the endothelial dysfunction coefficient, prevention of the adrenoreactivity increasing and of the miocardiac reserve reduction, and also normalization of the biochemical markers of inflammation (C-reactive protein) and proinflammatory cytokines. Also there is positive dynamic of the NO metabolism final products concentration and eNOS expression.

Keywords: endothelial dysfunction, HMG-CoA reductase inhibitors, Simvastatin, Atorvastatin, Rosuvastatin, endotoxin.

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ISSN: 0975-8585

INTRODUCTION

Despite a lot of studies cardiovascular disease are leading causes of death and disability. Thus, the focus of studies aimed at understanding the pathogenesis and development of preventive measures is shifted toward endothelial dysfunction (1, 2, 3) and the role of cytokines (4, 5, 6) in the atherosclerotic vascular lesions.

In addition to the above considerable attention has been given to endotoxin damage at different abdominal pathology (peritonitis, obstruction, acute pancreatitis, etc.) (7,8), sepsis (9), infectious diseases (10). At the same time a succession of events shows up as endotoxin shock with multiorgan dysfunction syndrome -> release of the pro-inflammatory cytokines -> endothelial dysfunction -> widespread vasculitis -> increase in vascular permeability and endothelial for lymphocytes -> hyperlipoproteinemia -> start of the atherosclerotic process (7,8,9,10).

Taking into account critical releasing of the pro-inflammatory cytokines under that logic analogous mechanism can be accepted for any endotoksin-induced pathology regardless of its cause.

However pharmacotherapeutic programs of correction of the endothelial dysfunction in acute systemic inflammation are not practice. For that matter ADMA-eNOS has a very interesting as a pharmacological target. It is fair to assume that HMG-CoA reductase inhibitors are one of the pharmacotherapeutic program to correction of the endotoxin-induced endothelial dysfunction.

PROCEDURE

The experiments were performed on the white male Wistar rats weighing 200-250 g. Simulation of the endotoxin-induced endothelial dysfunction (EIED) were performed by subcutaneous injection of 0,1 ml Staphylococcus aureus suspending (strain 603) in concentration 10 millions microbes for 1 ml.

HMG-CoA reductase inhibitors Simvastatin 2,2, 4,3, 8,5 mg/kg, Atorvastatin 1,1, 2,2, 4,3 mg/kg, Rosuvastatin 2,2, 4,3, 8,5 mg/kg and nanocapsular Rosuvastatin 3,0, 6,3, 11,6 mg/kg was given intragastrical. All substances were administered once a day within 7 days.

The animals were divided into groups (n = 10): 1 - control; 2 – endotoxin-induced endothelial dysfunction (EIED). 3 – EIED + Simvastatin 2,2, 4,3, 8,5 mg/kg; 4 - EIED + Atorvastatin 1,1, 2,2, 4,3 mg/kg; 4 - EIED + Rosuvastatin 2,2, 4,3, 8,5 mg/kg; 5 - EIED + nanocapsular Rosuvastatin 3,0, 6,3, 11,6 mg/kg.

On the day 8 from an initiation of experiments under anaesthetic (chloral hydrate 300 mg/kg) a catheter in the left carotid artery for recording of indexes of blood pressure (BP) was entered. Bolus introduction of pharmacological agents was made into a femoral vein. Hemodynamic indexes: the systolic arterial pressure (SAP), a diastolic arterial pressure (DAP) and cardiac contractions rate metered continuously with a hardware-software complex «Biopac». Besides BP measuring a series of the functional trials was led in introduced succession: 1. endothelium dependent vasorelaxation test (intravenous entering of a solution of acetylcholinum (AH) in a dose of 40 mkg/kg); 2. endothelium independent vasorelaxation test (intravenous entering of a solution of sodium nitroprussidum (NP) in a dose of 30 mkg/kg) [1, 11, 12, 13, 14, 15].

Functional tests for adrenoreactivity (11, 12) and miocardiac reserve (9, 10) reduction were performed.

Level of endothelial dysfunction at the experimental animals, and also a level of its correction by researched drugs valued on coefficient of endothelial dysfunction (CED) [1, 11, 12, 13, 14, 15].

Dynamic of the biochemical markers (NO total, eNOS expression, CRP, IL-6) in animals with endotoxin-induced endothelial dysfunction were determined by standard chemical kits.

The results were expressed as the mean (M) ± the standard error of mean (m). Differences were considered significant at $p < 0.05$.

ISSN: 0975-8585

FINDINGS OF THE STUDY

Administration of the HMG-CoA reductase inhibitors such as Simvastatin, Atorvastatin, Rosuvastatin and nanocapsular Rosuvastatin under the condition of endotoxin-induced endothelial dysfunction showed a great dose response endothelioprotective effect, which expressed in reducing of coefficient of endothelial dysfunction and normalization of systolic and diastolic blood pressure (tbl. 1). So, in group with EIED CED was 3,7±0,5, when in groups of animals which was treated by the stiff doses of Simvastatin (8,5 mg/kg), Atorvastatin (4,3 mg/kg), Rosuvastatin (8,5 mg/kg) and nanocapsular Rosuvastatin (11,6 mg/kg) it accordingly was 2,3±0,5, 2,1±0,3, 1,7±0,5 и 1,5±0,2 c.u. and approximated to the level of the control group (1,1 ± 0,1). Rosuvastatin and nanocapsular Rosuvastatin were more efficient.

Table 1: Influence of the HMG-CoA reductase inhibitors such as Simvastatin, Atorvastatin, Rosuvastatin and nanocapsular Rosuvastatin on the dynamic of haemodynamic forces in animals under the condition of endotoxin-induced endothelial dysfunction (M±m, n=10).

Groups of animals	SBP	DBP	CED
Control	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 + Simvastatin 2,2 mg/kg (n=10)	117,1±3,3	82,1±2,3	3,6±0,4*
4,3 mg/kg (n=10)	121,6±2,0	83,0±2,2	2,9±0,3*#
8,5 mg/kg (n=10)	127,3±2,8	87,1±1,9	2,3±0,5*#
EIED + Atorvavstatin 1,1 mg/kg (n=10)	115,1±3,0*	86,7±2,0	3,5±0,4*
2,2 mg/kg (n=10)	121,6±2,9	82,9±2,3	2,7±0,4*#
4,3 mg/kg (n=10)	130,0±3,3	85,8±2,2	2,1±0,3*#
EIED +Rosuvastatin 2,2 mg/kg (n=10)	118,9±3,3*	87,3±2,8	3,3±0,3*
4,3 mg/kg (n=10)	127,0±3,9	86,0±2,0	2,4±0,4*#
8,5 mg/kg (n=10)	135,0±3,8	83,1±2,1	1,7±0,5*#
EIED + nanocapsular Rosuvastatin 3 mg/kg (n=10)	120,1±4,0	87,0±2,0	3,2±0,3*
nanocapsular Rosuvastatin 6,3 mg/kg (n=10)	127,9±3,3	84,1±2,1	2,5±0,3*#
nanocapsular Rosuvastatin 11,6 mg/kg (n=10)	129,6±4,3	84,9±2,0	1,5±0,2*#

Annotation: SBP – systolic blood pressure (mm Hg), DBP – diastolic blood pressure (mm Hg), CED - coefficient of endothelial dysfunction (c.u.), * - significant difference from control group (p<0,05), # - significant difference from EIED group (p<0,05).

Table 2: Influence of the HMG-CoA reductase inhibitors such as Simvastatin, Atorvastatin, Rosuvastatin and nanocapsular Rosuvastatin on the dynamic of contractility after the tolerance tests in animals with endotoxin-induced endothelial dysfunction (M±m, n=10)

Group of animals	Adrenoreactivity (mm Hg)	miocardiac reserve reduction (%)
Control	201,5±9,4	112,7±10,9
Endotoxin-induced endothelial dysfunction (EIED) (n=10)	240,3±8,7*	79,4±3,9*
EIED + Simvastatin 2,2 mg/kg (n=10)	245,1±10,5*	79,9±4,2*
4,3 mg/kg (n=10)	240,1±9,7*	82,1±3,9*
8,5 mg/kg (n=10)	232,0±8,9*	87,4±3,7*
EIED + Atorvavstatin 1,1 mg/kg (n=10)	239,9±9,0*	81,7±4,0*
2,2 mg/kg (n=10)	230,3±9,7*	85,0±3,6*
4,3 mg/kg (n=10)	222,1±8,5*#	97,0±4,9*
EIED +Rosuvastatin 2,2 mg/kg (n=10)	238,9±9,8*	89,0±4,9*
4,3 mg/kg (n=10)	232,4±9,7*	95,7±5,8*
8,5 mg/kg (n=10)	221,0±8,4*#	109,4±5,7*#
EIED + nanocapsular Rosuvastatin 3 mg/kg (n=10)	232,9±9,3*	90,3±5,0*
nanocapsular Rosuvastatin 6,3 mg/kg (n=10)	225,3±7,6*	95,3±5,7*
nanocapsular Rosuvastatin 11,6 mg/kg (n=10)	219,1±8,7*#	99,9±6,3*#

Annotation: * - significant difference from control group (p<0,05), # - significant difference from EIED group (p<0,05).

Also positive dynamic of the contractility was determined after the tolerance tests in animals with EIED (tbl. 2). So, the prevention of adrenoreactivity increasing and miocardiac reserve reduction was determined. Rosuvastatin (8,5 mg/kg) and nanocapsular Rosuvastatin (11,6 mg/kg) were more efficient in this case, too (tbl. 2).

More significant effects of the HMG-CoA reductase inhibitors such as Simvastatin, Atorvastatin, Rosuvastatin and nanocapsular Rosuvastatin were observed on biochemical markers in animals with EIED (tbl. 3). Simulation of the sepsis leads to significant increasing of the NO final metabolites and decreasing of the eNOS expression. But the level of NO final metabolites and eNOS expression were normalized and approximated to the level of the control group after administration of the stiff doses at the 8 day of the HMG-CoA reductase inhibitors treatment of the endotoxin-induced endothelial dysfunction (tbl. 3). It is important that nanocapsular Rosuvastatin was more efficient. It attests to the fact that volume of distribution of the Rosuvastatin is limited by circulatory system (tbl. 3).

Table 3: Influence of the HMG-CoA reductase inhibitors such as Simvastatin, Atorvastatin, Rosuvastatin and nanocapsular Rosuvastatin on the dynamic of biochemical markers (NO total, eNOS expression, CRP, IL-6) in animals with endotoxin-induced endothelial dysfunction (M±m, n=10)

Group of animals	NOx	eNOS expression	CRP level	IL-6
Control	116,8±10,3	5,4±0,21	0,05±0,01	0,43±0,17
Endotoxin-induced endothelial dysfunction (EIED) (n=10)	182,3±12,4*	0,04±0,01*	0,38±0,01*	6,87±1,93*
EIED + Simvastatin 2,2 mg/kg (n=10)	180,1±9,9*	0,09±0,01*	0,33±0,01*	5,13±1,07*
4,3 mg/kg (n=10)	141,1±10,0*#	0,13±0,02*#	0,19±0,02*#	3,17±0,95*#
8,5 mg/kg (n=10)	122,9±8,4*#	1,93±0,12*#	0,08±0,01*#	1,03±0,62*#
EIED + Atorvavstatin 1,1 mg/kg (n=10)	189,3±13,7*	0,12±0,01*	0,32±0,02*	4,12±0,91*
2,2 mg/kg (n=10)	152,9±11,2*	1,23±0,15*#	0,18±0,01*#	2,34±0,43*#
4,3 mg/kg (n=10)	130,0±10,9*#	2,07±0,21*#	0,09±0,01*#	1,27±0,33*#
EIED +Rosuvastatin 2,2 mg/kg (n=10)	171,1±14,2*	0,39±0,02*	0,30±0,03*	5,95±1,29*
4,3 mg/kg (n=10)	142,0±10,1*#	2,24±0,15*#	0,17±0,02*#	3,82±0,90*#
8,5 mg/kg (n=10)	122,1±9,9#*	3,04±0,35*#	0,11±0,01*#	1,17±0,33*#
EIED + nanocapsular Rosuvastatin 3 mg/kg (n=10)	179,2±12,0*	0,64±0,03*	0,31±0,02*	6,1±1,43*
nanocapsular Rosuvastatin 6,3 mg/kg (n=10)	161,7±11,7*	3,09±0,23*#	0,21±0,01*#	2,43±0,95*#
nanocapsular Rosuvastatin 11,6 mg/kg (n=10)	32,1±10,3*#	4,01±0,56*#	0,18±0,01*#	1,48±0,24*#

Annotation: NOx - NO final metabolites (mcmole/l), eNOS expression (%),CRP level – the level of the C- reactive protein (mg/ml); IL-6 - interleukin 6 (pg/ml); * - significant difference from control group ($p<0,05$), # - significant difference from EIED group ($p<0,05$).

CRP as a marker of the systemic inflammatory reaction was increased 7,5 times in group of animals with EIED, but after administration of the average and stiff doses of the HMG-CoA reductase inhibitors such as Simvastatin, Atorvastatin, Rosuvastatin and nanocapsular Rosuvastatin it didn't has a significant difference from control group.

Pro-inflammatory cytokine IL-6 was increased 15 times in group of animals with EIED. It didn't has a significant difference from control group after administration of the average and stiff doses of the HMG-CoA reductase inhibitors such as Simvastatin, Atorvastatin, Rosuvastatin and nanocapsular Rosuvastatin. Nanocapsular Rosuvastatin in dose 11,6 mg/kg was more efficient (tbl. 3).

CONCLUSION

Thus application of the HMG-CoA reductase inhibitors, such as Simvastatin, Atorvastatin, Rosuvastatin and nanocapsular Rosuvastatin, leads to dose response endothelioprotective effect under the condition of endotoxin-induced endothelial dysfunction after administration of the Staphylococcus aureus strain 603.

Endothelioprotective effect consists in recovery of the endothelial dysfunction coefficient, prevention of the adrenoreactivity increasing and of the miocardiac reserve reduction, and also normalization of the biochemical markers of inflammation (C-reactive protein) and proinflammatory cytokines. Also there is positive dynamic of the NO metabolism final products concentration and eNOS expression.

In opinion of many researchers pleiotropic effects of the HMG-CoA reductase inhibitors are not involving with its hypolipidemic activity. Mevalonate is a major metabolite not only for the synthesis of cholesterol but the synthesis isoprenoid intermediates such as farnesylphosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). These molecules are involved in the activation and intracellular transport proteins of Ras and Rho, which are crucial for the proliferation, cell differentiation, including smooth muscle cells and cells of the immune system.

ACKNOWLEDGEMENTS

The research was partially supported by the Ministry of Education and Science of the Russian Federation (grant agreement No. 14.578.21.0012, unique identifier Agreement RFMEFI57814X0012.), grant of the President of the Russian Federation №MD-4711.2015.7 and № МК-3136.2014.4.

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