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Research Article

Sarpogrelate and atorvastatin synergistically ameliorate hyperlipidemia-induced aortic damage in LDL receptor deficient (LDL-R$^{-/-}$) mice

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Abstract: Hyperlipidemia is a major risk factor for blood vessel damage, which can lead to cardiovascular diseases. Statins are the first-line treatment for hyperlipidemia and atherosclerosis. However, most patients receiving statins do not reach the low-density lipoprotein-cholesterol (LDL-c) goal. Sarpogrelate (SP) has been shown to reduce blood lipids and oxidative stress. Therefore, this study investigated the effects of SP plus atorvastatin compared to atorvastatin alone on aortic damage in LDL-R$^{-/-}$ mice with hyperlipidemia. Male LDL-R$^{-/-}$ mice were randomly divided into four groups: normal diet-fed mice (control group), high-cholesterol diet-fed mice (H group), high-cholesterol diet-fed mice treated with atorvastatin (HA group), and high-cholesterol diet-fed mice treated with atorvastatin + SP (HAS group). Total cholesterol (TC) and LDL-c levels were lower in the HA and HAS groups. SP plus atorvastatin was more effective in reducing TC and LDL-c levels, compared to atorvastatin alone. Morphological and immunohistochemical analyses showed that lipid deposition and macrophage infiltration were significantly suppressed in the HAS group, compared to those in the HA group. CD36 expression was lower in aotra tissues of mice in the HA and HAS groups than in the H group. Furthermore, macrophages and pro-inflammatory cytokine levels were lower in the HA and HAS groups than in the H group. There were significant differences in macrophages, pro-inflammatory cytokine and CD36 levels between HA and HAS groups. Therefore, SP plus atorvastatin might be more effective in ameliorating hyperlipidemia-induced aortic damage in LDL-R$^{-/-}$ mice, compared to atorvastatin alone.

Keywords: hyperlipidemia, aortic damage, LDL-R$^{-/-}$ mice, sarpogrelate, atorvastatin

Introduction

LDL-R$^{-/-}$ mice are considered a well-accepted model of hyperlipidemia [1].

Hyperlipidemia leads to the development of cardiovascular disease (CVD), which involves disorders of the heart and blood vessels, and causes various fatal events [2,3]. Hyperlipidemia accelerates lipid deposition, atherosclerosis, and chronic inflammation [4, 5]. However, the underlying pathophysiological mechanisms of the relationship between Hyperlipidemia and aortic damage are not yet fully understood.

Statins, including atorvastatin, are a class of drugs which exhibit a powerful hypocholesterolemic effect, as revolutionized cholesterol-lowing agents. Treatment with statins has been reported to markedly reduce morbidities and mortalities of major cardiovascular events in patients [6, 7]. However, most patients treated with statins do not reach the low-density lipoprotein-cholesterol (LDL-c) goal [8, 9] due to poor compliance, variability in drug response, inadequate titration of applied doses, and safety issues associated with high doses [8, 9]. Therefore, the present study investigated whether a combination of two different pharmacological drugs, statins and a non-lipid modifying agent, can achieve the LDL-c goal. Sarpogrelate (SP), a serotonin (5-HT) receptor antagonist, has been shown to reduce platelet aggregation and thrombus formation [8-12]. Recently, the ability of SP to delay atherosclerosis progression has gained much attention [13]. Several studies have shown that SP can upregulate endothelial nitric oxide synthase (eNOS) expression [14] and lower blood lipid levels and blood viscosity [15] in rabbits. SP treatment has been effective in reducing restenosis in patients with acute coronary syndrome [16]. In a study comparing the effects of sarpogrelate and placebos in patients with stable angina, restenosis rates, after coronary stenting in the SP group, were significantly reduced from 28.6 to 4.3% [17]. Another study investigating the effects of SP and aspirin treatment in patients with acute coronary syndrome showed that restenosis rates, after percutaneous balloon angioplasty, decreased in the SP group from 57 to 37% [16, 18]. In addition, a previous study showed that SP exhibited anti-inflammatory and insulin-sensitizing effects [17]. Therefore, the present study investigated the synergistic effects of SP plus atorvastatin, compared to atorvastatin alone, in aortic damage in LDL-R$^{-/-}$ mice with hyperlipidemia.

Materials and methods

Animal experiments

LDL-R$^{-/-}$ mice were purchased from Beijing Vital River Lab Animal Technology CO., LTD. (Beijing, China). All mice were housed in a room with 12/12-hour light-dark cycles at a controlled temperature (24°C). Male LDL-R$^{-/-}$ mice (8 weeks old) were randomly divided into four groups, as follows: mice fed a normal diet (control group, n = 8), mice fed a high-cholesterol diet (H group, n = 8), mice fed a high-cholesterol diet + atorvastatin (10 mg/kg/day; Pfizer, New York, USA) (HA group, n = 8), and mice fed a high-cholesterol diet + atorvastatin (10 mg/kg/day) + SP (50 mg/kg/day; Mitsubishi Tanabe Pharma, Osaka, Japan) (HAS group, n = 7). High-cholesterol diet contained 1.5% cholesterol and 15% fat. The experimental diet was purchased from Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, China). Mice in all groups were fed with the appropriate diet for 8 weeks. Blood samples were obtained from the inferior vena cava, collected in serum tubes, and stored at -80°C until use. Longitudinal sections of the aotras were fixed in 10% formalin and embedded in paraffin for histological evaluation. The remaining aotras were snap-frozen in liquid nitrogen for mRNA isolation and immunoblotting analyses. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals. This study was approved by the Ethical Committee of the affiliated Zhongshan Hospital of Dalian University of China.

Serum lipoprotein profile

The blood samples were collected and serum was prepared by centrifugation at 3000 rpm for 15 min. Following the manufactural instruction, Total Cholesterol Assay Kit (COD-PAP method) (Nanjing Jiancheng Biological Technology Institute, China) was used for detecting serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and Triglyceride Assay Kit (GPO-PAP method) (Nanjing Jiancheng Biological Technology Institute, China) for triglyceride (TG).

Histological analysis

Paraffin-embedded aorta tissues were cut into 5 μm-thick cross-sections and deparaffinized prior to staining using a standard protocol. Hematoxylin and eosin staining was used to for detecting the lesion area at the aortic . Immunohistochemical staining was performed according to the manufacture's description (Zsbio, Beijing, China) with the antibody against CD68 (rabbit anti-CD68 antibody, 1:200; Proteintech, Wuhan，China), NIH Image J software was used for quantification.

RNA isolation and real-time RT-PCR

Total RNA was isolated from aorta using the ISOGEN (Nippon Gene, Tokyo, Japan) according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from total RNA using a first-strand cDNA synthesis kit (SuperScript VILO cDNA Synthesis Kit; Life Technologies Carlsbad, CA, USA), according to the manufacturer's protocol. Gene expression was analysed quantitatively by real-time RT-PCR using fluorescent SYBR Green technology (Light Cycler; Roche Molecular Biochemicals). β-Actin cDNA was amplified and quantitated in each cDNA preparation in order to normalize the relative amounts of the target genes. Primer sequences are listed in Table 1.

Table1 Primer oligonucleotide sequences

Gene	Primers
TNF-α	F:5′-TCTCATGCACCACCATCAAGGACT-3′ R:5′-ACCACTCTCCCTTTGCAGAACTCA-3′
IL-6	F:5′-TACCAGTTGCCTTCTTGGGACTGA-3′ R:5′-TAAGCCTCCGACTTGTGAAGTGGT-3′
IL-1β	F: 5′-TGCCACCTTTTGACAGTGAT-3′ R: 5′-TGTGCTGCTGCGAGATTTGA-3′
β-actin	F:5′-CGATGCCCTGAGGGTCTTT-3′ R:5′-TGGATGCCACAGG ATTCCAT-3′

Abbreviations: TNF-α, tumor necrosis factor-α; IL-6, interleukin- 6; IL-1β, interleukin- 1β

Western blotting for aortic tissue

Proteins were extracted from renal cortical tissues using radio immunoprecipitation assay buffer (P0013B; Beyotime, Shanghai, China). Samples were electrophoresed on 10% SDS-PAGE gel, and proteins were transferred to polyvinylidene fluoride membrane (Immobilon, Millipore, Billerica, MA, USA). Membranes were blocked in Tris-buffered saline with 0.1% Tween-20 (TBS-T) containing 5% skim milk, and then were incubated in primary antibody diluent (P0023A; Beyotime) and gently shaken overnight at 4°C. Primary antibodies against CD36 (rabbit anti-CD36 antibody, 1:1000; Proteintech), Phospho-Erk (Rabbit anti-phospho-Erk,1:1000; Proteintech), anti-β-actin (1:1000; Proteintech). Membranes were then incubated with secondary antibody (anti-rabbit Ig-G, 1:1000; Cell Signaling Technology for 1 hour). This analysis was carried out independently three times. Protein levels are expressed as protein/β-actin ratios to minimize loading differences. The relative signal intensity was quantified using NIH ImageJ software.

Statistical Analysis

All data are presented as the mean ± SEM. Statistical analysis was performed using SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA). Inter-group variation was measured by one-way ANOVA and subsequent Tukey's test. The minimal level for significance was $P< 0.05$.

Results

Metabolic characterisation

According to the metabolic characteristics, we found the results of serum lipid measurements (Figure1) indicated that a

hyperlipidemia mouse model had been successfully established. Body weights (BWs) and TG did not differ among the four groups. LDL-R$^{-/-}$ mice in the H group showed a marked increase in TC and LDL-c levels. TC levels decreased in HA and HAS groups, compared to those in the H group. Treatment with SP plus atorvastatin were more effective in reducing TC and LDL-c levels, compared to treatment with atorvastatin alone.

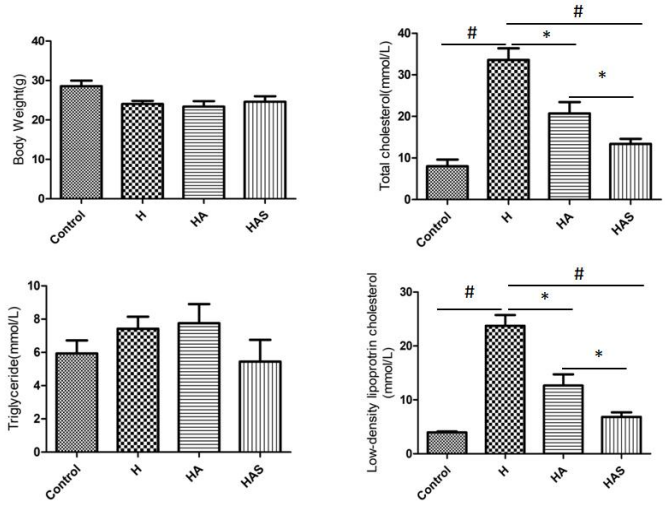

Fig 1. Metabolic data from the *LDL-R$^{-/-}$* mice of the four groups after treatment. Body weights, TC, TG and LDL-c expressions of the four groups after treatment are presented. Data are mean ± SEM; n = 7–8 per group. $^{*}P < 0.05$, $^{\#}P<0.01$.

Histopathological changes in aortic tissues

To evaluate aortic tissue damage, we used the HE, CD68 staining (Figure2) facilitated the visualisation of aortic structural disorder, inflammatory cell infiltration, macrophage infiltration with aortic damage as seen in hyperlipidemia. Mice in the HA and HAS groups showed a marked reduction in aortic damage compared to that in the H group. In addition, HE and CD68-positive staining were significantly lower in the HAS group than in the HA group. Those results indicate that SP plus atorvastatin was more effective in reducing inflammatory cell infiltration and macrophage infiltration in LDL-R$^{-/-}$ mice with hyperlipidemia, compared to LDL-R$^{-/-}$ alone.

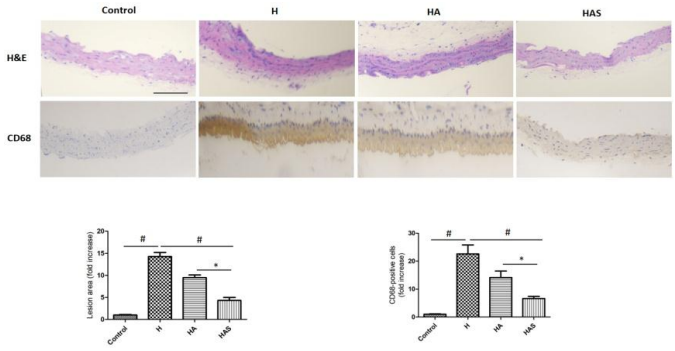

Fig 2. Sarpogrelate and atorvastatin reduce the plaque lesion areas and macrophages. H&E staining shows the plaque lesions and bar graph presents the lesion area. Macrophages （CD68） stained brown by immunohistological staining and bar graph presents the quantification of macrophages. Scale bar=100 μm. *Data are mean ± SEM; n = 5 per group.* $^{*}P < 0.05$, $^{\#}P<0.01$.

mRNA expression of IL-1β, IL-6, and TNF-α in aortic tissues

Pro-inflammatory cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), were upregulated in the H group, while attenuated in the HR and HAS groups. IL-1β, IL-6 and TNF-α expression was significantly lower in the HRS group than in the HR group (Figure 3).

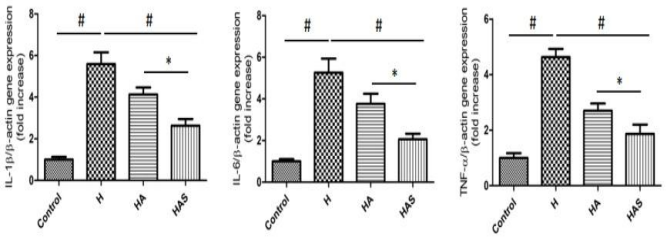

Fig3. *Expression of pro-inflammatory genes in the aortic tissues from the* LDL-R$^{-/-}$ *mice of the four groups after treatment. Relative mRNA expression of* IL-1β, *IL-6 and TNF-α in the aortic tissues of the four groups after treatment. Data are given as mean ± SEM; n = 6 in each group.* $^{*}P < 0.05$, $^{\#}P<0.01$.

CD36 protein expression in the aortic tissue of LDL-R$^{-/-}$ mice

Immunoblotting was performed to measure CD36 protein expression. It was found that CD36 protein expression in the aortic of mice in the H group increased, compared to that in the control group. However, mice in HA and HAS groups exhibited markedly reduced CD36 expression in aortic tissues, compared to that in the H group. In addition, CD36 protein expression was significantly suppressed in the HAS group, compared to that in the HA group. Results indicate that PS plus atorvastatin was more effective in reducing CD36 expression in LDL-R$^{-/-}$ mice with hyperlipidemia (Figure 4).

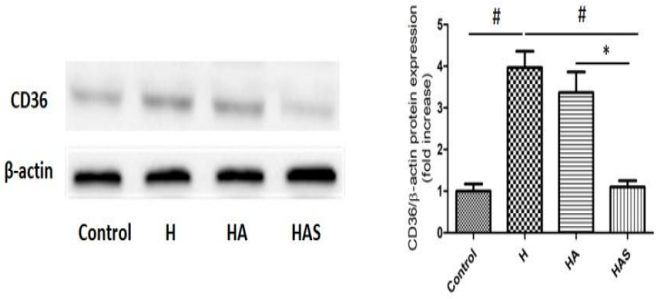

Fig4. CD36 protein expression in heart tissues of four groups. Immunoblotting for CD36 in aortic tissues. Bar graph showing quantification of CD36 protein expression. *Data are given as mean ± SEM; n = 6 in each group.* $^{*}P < 0.05$, $^{\#}P<0.01$.

Phosphor-ERK expression in the aortic tissue of LDL-R$^{-/-}$ mice

Protein kinases play a role in lipid deposition, thus phosphor-ERK protein immunoblotting was performed (Figure 5). We found that phosphor-ERK protein expression in the aortic of mice in the H group increased, compared to that in the control group. However, mice in HA and HAS groups exhibited markedly reduced phosphor-ERK protein expression in aortic

tissues, compared to that in the H group. In addition, CD36 protein expression was significantly suppressed in the HAS group, compared to that in the HA group.

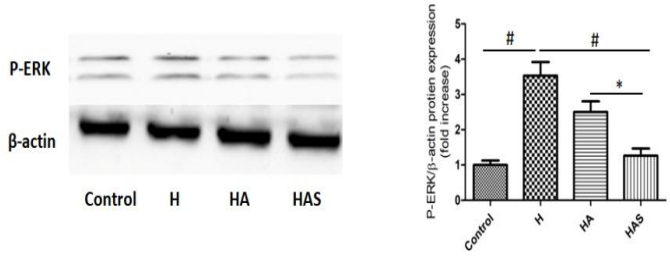

Fig5. P-ERK protein expression in heart tissues of four groups. Immunoblotting for P-ERK in aortic tissues. Bar graph showing quantification of P-ERK protein expression. *Data are given as mean ± SEM; n = 6 in each group.* $^{*}P < 0.05$, $^{\#}P<0.01$.

Discussion

Most importantly, results of the current study suggested that SP plus atorvastatin was more effective in ameloriating aortic damage in LDL-R$^{-/-}$ mice with hyperlipidemia, compared to atorvastatin alone. In particular, SP plus atorvastatin significantly attenuated lipid deposition, inflammatory cytokine levels, macrophage infiltration in hyperlipidemia-induced aortic damage.

TC and LDL-c levels increased in the H group, compared to those in the control group. Interestingly, TC and LDL-c levels were significantly suppressed in both the HA and HAS groups, compared to that in the H group. There was a significant difference between HA and HAS groups. Results indicate that atorvastatin plus SP synergistically lowered TC and LDL-c levels in LDL-R$^{-/-}$ mice with hyperlipidemia. Present results are consistent with those of Park et al[19]. The present study did not include a group of LDL-R$^{-/-}$ mice fed a high-cholesterol diet + SP alone. Thus, this study could not compare the effects of SP and atorvastatin in hyperlipidemic LDL-R$^{-/-}$ mice.

Recently, statins have been shown to exhibit immunomodulatory effects, reducing inflammatory cytokine secretion, T lymphocyte activation, mononuclear cell proliferation, and antigen-presenting capacity [20-22]. Previous studies have shown that statins reduce the production of proinflammatory cytokines, such as IL-1β, IL-6, IL-10, interferon-γ, and TNF-α, and stabilize vulnerable atherosclerotic plaques, which might be partially attributed to their immunomodulatory effects [19, 23-25]. Present findings are consistent with the results of a previous study. Atorvastatin plus SP reduced the progression of plaque lesions due to its anti-inflammatory properties, including suppression of IL-1β, IL-6 and TNF-α levels. The present study showed that IL-1β, IL-6 and TNF-α expression were upregulated in the H group. However, these increase in expression of pro-inflammatory cytokines was attenuated in HA and HAS groups. In addition, IL-1β, IL-6 and TNF-α expression significantly decreased in the HAS group, compared to that in the HA group. Hyperlipidemia-induced aortic damage is usually associated with an increase in macrophages. Macrophages are major innate immune cells that play a principal role in the transition from inflammatory response to regeneration. CD68, an important macrophage biomarker, reflects macrophage burden [26, 27]. In the present study, immunohistochemical staining of CD68 showed that mice in the HA and HAS groups exhibited obviously reduced CD68-positive staining in the aortic tissues, compared to those in the H group. In addition, there was a significant difference between HAS and HA groups. Results suggest that SP plus atorvastatin was more effective in reducing macrophage infiltration in LDL-R$^{-/-}$ mice with hyperlipidemia, compared to atorvastatin alone.

Cellular lipid homeostasis involves the regulation of the influx, synthesis, catabolism, and efflux of lipids. This pathway is mediated by several independent pathways including class B (CD36) [28]. CD36 has been reported to mediate oxLDL internalization in macrophages, and have been implicated in the pathogenesis of atherosclerosis[29, 30]. The present study measured CD protein expression significantly increased in the aortic of mice in the H group, compared to that in the control group. This increase in expression of CD36 was suppressed in HA and HAS groups. There was a significant difference in CD36 expression between HA and HAS groups. These findings indicate that CD36 might be a critical factor involved in lipid accumulation in the aortic of LDL-R$^{-/-}$ mice. Atorvastatin plus SP effectively ameliorated lipid deposition. Hu et al reported that compared with normal vessal tissue, or the aortic media of cholesterol-fed rabbits, there was a marked increase in the amount of ERK1/2 proteins from atherosclerotic lesions[31]. In the present study, we found that P-ERK protein expression significantly increased in the aortic of mice in the H group, compared to that in the control group. This increase in expression of P-ERK was suppressed in HA and HAS groups. There was a significant difference in P-ERK expression between HA and HAS groups. In conclusion, present results suggest that TC and LDL-c levels substantially decreased after atorvastatin and SP combined therapy, compared to atorvastatin alone treatment. Treatment with SP plus atorvastatin demonstrated superior effects to atorvastatin alone in terms of attenuation of inflammatory cytokine expression, macrophage infiltration, lipid deposition. The findings of this study may be beneficial in developing novel strategies for prevention and treatment of hyperlipidemia induced cardiovascular disease.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Zuowei Pei designed this study; Zhipeng Zhang and Fan Yang helped in performing experiments; Zuowei Pei and Rongmei Na analyzed data and interpreted the results of experiments; Rongmei Na and Hongyang Liu prepared figures; Zuowei Pei drafted the manuscript. All authors read and approved the final manuscript.

References

[1] Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammer RE, Herz J. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediatedgene delivery. J Clin Invest. 1993;92:883–893.

[2] Wijeysundera DN, Duncan D, Nkonde-Price C, Virani SS, Washam JB, Fleischmann KE, Fleisher LA: American College of Cardiology American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2014; 22: 2406-2425.

[3] Arsenault BJ, Kritikou EA, Tardif JC: Regression of atherosclerosis. Curr Cardiol Rep. 2012; 4: 443-449.

[4] Karshovska E, Zhao Z, Blanchet X, Schmitt MM, Bidzhekov K, Soehnlein O, von Hundelshausen P, Mattheij NJ, Cosemans JM, Megens RT, Koeppel TA, Schober A, Hackeng TM, Weber C, Koenen RR: Hyperreactivity of junctional adhesion molecule A-deficient platelets accelerates atherosclerosis in hyperlipidemic mice. Circ Res. 2015; 116: 587-599.

[5] Pei Z, Okura T, Nagao T, Enomoto D, Kukida M, Tanino A, Miyoshi K, Kurata M, Higaki J: Osteopontin deficiency reduces kidney damage from hypercholesterolemia in Apolipoprotein E-deficient mice. Sci Rep.2016; DOI: 10.1038/srep28882.

[6] Collaboration, C. Baigent, L. Blackwell, J. Emberson, L. E. Holland, C. Reith, N. Bhala, R. Peto, E. H. Barnes, A. Keech, J. Simes, R. Collins, Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376: 1670–1681.

[7] Pasternak RC, Smith SC Jr, Bairey-Merz CN, Grundy SM, Cleeman JI, Lenfant C. American College of, Cardiology, American Heart, Association, National Heart, Lung Blood, Institute, ACC/AHA/NHLBI clinical advisory on the use and safety of statins. Circulation. 2002; 106:1024-1028.

[8] Kim CH, An H, Kim SH, Shin D. Pharmacokinetic and pharmacodynamic interaction between ezetimibe and rosuvastatin in healthy male subjects. Drug Des Devel Ther. 2017; 11: 3461-3469.

[9] Waters DD, Brotons C, Chiang CW, Ferrières J, Foody J, Jukema JW, Santos RD, Verdejo J, Messig M, McPherson R, Seung KB, Tarasenko L. Lipid Treatment Assessment Project 2 Investigators. Lipid treatment assessment project 2: a multinational survey to evaluate the proportion of patients achieving low-density lipoprotein cholesterol goals. Circulation. 2009; 120: 28-34.

[10] Kostapanos MS, Milionis HJ, Elisaf MS. Rosuvastatin-associated adverse effects and drug-drug interactions in the clinical setting of dyslipidemia. Am J Cardiovasc Drugs. 2010; 10: 11-28.

[11] Duhamel TA, Xu YJ, Arneja AS, Dhalla NS. Targeting platelets for the prevention and treatment of cardiovascular disease. Expert Opin Ther Targets. 2007; 11: 1523-1533.

[12] Uchiyama S, Ozaki Y, Satoh K, Kondo K, Nishimaru K. Effect of sarpogrelate, a 5-HT(2A) antagonist, on platelet aggregation in patients with ischemicstroke: clinical-pharmacological dose-response study. Cerebrovasc Dis. 2007; 24: 264-270.

[13] Brault M, Ray J, Gomez YH, Mantzoros CS, Daskalopoulou SS. Statin treatment and new-onset diabetes: a review of proposed mechanisms. Metabolism. 2014; 63: 735-745.

[14] Hayashi T, Sumi D, Matsui-Hirai H, Fukatsu A, Arockia Rani PJ, Kano H, Tsunekawa T, Iguchi A. Sarpogrelate HCl, a selective 5-HT2A antagonist, retards the progression of atherosclerosis through a novel mechanism. Atherosclerosis. 2003; 168: 23-31.

[15] Xu YJ, Zhang M, Ji L, Elimban V, Chen L, Dhalla NS. Suppression of high lipid diet induced by atherosclerosis sarpogrelate. J Cell Mol Med. 2012; 16: 2394-2400.

[16] Lee DH, Chun EJ, Hur JH, Min SH, Lee JE, Oh TJ, Kim KM, Jang HC, Han SJ, Kang DK, Kim HJ, Lim S. Effect of sarpogrelate, a selective 5-HT2A receptor antagonist, on characteristics of coronary artery disease in patients with type 2 diabetes. Atherosclerosis. 2017; 257: 47-54.

[17] Fujita M, Mizuno K, Ho M, Tsukahara R, Miyamoto A, Miki O, Ishii K, Miwa K. Sarpogrelate treatment reduces restenosis after coronary stenting. Am Heart J. 2003; 145: E16.

[18] Hirotaka SHK. Effect of the 5HT2A receptor antagonist. Sarpogrelate hydrochloride, on the rate of restenosis after percutaneous old balloon angioplast vol. 74. Tokyo Women's Medical University. 2004; 140-146.

[19] Park KY, Oh E, Kwak MK, Jun HS, Heo TH. Pravastatin and sarpogrelate synergistically ameliorate atherosclerosis in LDLr-knockout mice. PLoS One. 2016; 11(3):e0150791.

[20] Meng X, Zhang K, Li J, Dong M, Yang J, An G, Qin W, Gao F, Zhang C, Zhang Y. Statins induce the accumulation of regulatory T cells in atherosclerotic plaque. Mol Med. 2012; 18: 598-605.

[21] Mausner-Fainberg K, Luboshits G, Mor A, Maysel-Auslender S, Rubinstein A, Keren G, George J. The effect of HMG-CoA reductase inhibitors on naturally occurring CD4+CD25+ T cells. Atherosclerosis. 2008; 197: 829-839.

[22] Peng X, Jin J, Giri S, Montes M, Sujkowski D, Tang Y, Smrtka J, Vollmer T, Singh I, Markovic-Plese S. Immunomodulatory effects of 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors, potential therapy for relapsing remitting multiple sclerosis. J Neuroimmunol. 2006; 178: 130-139.

[23] Zhang X, Jin J, Peng X, Ramgolam VS, Markovic-Plese S. Simvastatin inhibits IL-17 secretion by targeting multiple IL-17-regulatory cytokines and by inhibiting the expression of IL-17 transcription factor RORC in CD4+ lymphocytes. J Immunol. 2008; 180: 6988-6996.

[24] Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. Nat Rev

Drug Discov. 2005; 4: 977-987.

[25] Zhou X, Li D, Yan W, Li W. Pravastatin prevents aortic atherosclerosis via modulation of signal transduction and activation of transcription 3 (STAT3) to attenuate interleukin-6 (IL-6) action in ApoE knockout mice. Int J Mol Sci. 2008; 9: 2253-2264.

[26] Ryabov V, Gombozhapova A, Rogovskaya Y, Kzhyshkowska J, Rebenkova M, Karpov R. Cardiac CD68+ and stabilin-1+ macrophages in wound healing following myocardial infarction: From experiment to clinic. Immunobiology. 2017; 30213-30219.

[27] Ding Z, Mizeracki AM, Hu C, Mehta JL. LOX-1 deletion and macrophage trafficking in atherosclerosis. Biochem Biophys Res Commun 2013; 440: 210-214.

[28] Abrass CK: Cellular lipid metabolism and the role of lipids in progressive renal disease. Am J Nephrol. 2004; 24: 46-53.

[29] Kunjathoor VV, Febbraio M, Podrez EA, Moore KJ, Andersson L, Koehn S, Rhee JS, Silverstein R, Hoff HF, Freeman MW: Scavenger receptors class A-I/II and CD36 are the principal receptors responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. J Biol Chem.2002; 51: 49982-49988.

[30] Zeng Y, Tao N, Chung KN, Heuser JE, Lublin DM: Endocytosis of oxidized low density lipoprotein through scavenger receptor CD36 utilizes a lipid raft pathway that does not require caveolin-1. J Biol Chem.2003; 46: 45931-45936.

[31] Hu Y, Dietrich H, Metzler B, Wick G, Xu Q: Hyperexpression and activation of extracellular signal-regulated kinases (ERK1/2) in atherosclerotic lesions of cholesterol-fed rabbits. Arterioscler Thromb Vasc Biol. 2000; 20: 18-26.