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Statin treatment decreases serum angiostatin levels in patients with ischemic heart disease

Artem A. Tykhomyrov [a,*], Victor S. Nedzvetsky [b], Nataliia I. Bardachenko [c], Tatiana V. Grinenko [a], Olexandr V. Kuryata [c]

[a] Department of Enzyme Chemistry & Biochemistry, Palladin Institute of Biochemistry of National Academy of Sciences of Ukraine, Kyiv, Ukraine
[b] Department of Biophysics & Biochemistry, Dnipropetrovsk National University, Dnipropetrovsk, Ukraine
[c] Department of Hospital Therapy No. 1 and Occupational Diseases, State Establishment "Dnipropetrovsk Medical Academy of Health Ministry of Ukraine", Dnipropetrovsk, Ukraine

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ABSTRACT

Aim: Angiogenesis and chronic inflammation are known to be co-dependent in atherosclerosis and cardiovascular diseases. This study was undertaken to investigate whether simvastatin could affect serum levels of angiostatin, a potent endogenous inhibitor of neovascularization, in patients with ischemic heart disease (IHD).
Main methods: Twenty-six patients with clinically confirmed IHD and hypercholesterolemia were assigned 40 mg/day of simvastatin for 8 weeks. Levels of lipid metabolism, C-reactive protein (C-RP) and other biochemical parameters in serum samples were measured using biochemical analyzer. Serum angiostatin levels were determined by Western blot. Association of serum angiostatin levels with total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and C-RP levels was evaluated.
Key findings: Simvastatin therapy improved the main parameters of lipid metabolism, including statistically significant ($P < 0.05$) reductions in TC (by 46%) and LDL-C (by 42%), and decreased inflammatory marker C-RP (by 32%), as compared with the baseline. Simvastatin treatment resulted in marked reduction of serum angiostatin level (by 80% in comparison with baseline, $P < 0.05$). Strong positive correlations between serum angiostatin level versus concentrations of TC, LDL-C, and C-RP were demonstrated before onset of the study ($r = 0.48311$, 0.6252, and 0.653, respectively) and after simvastatin therapy ($r = 0.67752$, 0.6485, and 0.8244, respectively).
Significance: We describe for the first time novel pleiotropic effect of statin therapy associated with decrease of serum angiostatin levels. Thus, circulating angiostatin represents an independent additional risk marker for cardiovascular events and could be applied as potential supplementary indicator for evaluation of statin therapy efficacy.

1. Introduction

Atherosclerosis and its complications are considered to be the most common causes of mortality and morbidity worldwide [47]. It is generally accepted that atherosclerosis, which underlies most ischemic vascular disease, is a multifactorial disease with a number of risk factors, including dyslipidemia, hypertension, diabetes and smoking [39]. All these factors provide potential targets for reducing the risk of the disease, however, cholesterol and lipid levels are considered to be the major targets for reducing the risk of the ischemic heart disease (IHD) [18]. Statins are a chemically and pharmacologically diverse group of drugs that share the ability to inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme that controls the rate-limiting step of cholesterol synthesis, but this inhibition is followed by other subsequences associated with the mevalonate pathway [1,45]. Simvastatin (Fig. 1) belongs to the statin family of drugs that effectively inhibit the metabolic pathway responsible for the endogenous production of cholesterol [35].

A number of outlying clinical observations suggest that simvastatin and other statins can work in another way than the lowering of cholesterol and induces so-called "pleiotropic effects" that are independent of their effects on lipids and lipoproteins [10,22]. It has been explored that some of the cholesterol-independent effects of statins involve improving endothelial function, enhancing the stability of atherosclerotic plaques, reducing oxidative stress and inflammation, decreasing activation of the blood coagulation cascade, and inhibition of platelet aggregation [2,6,25,38]. The variety of pleiotropic effects of statins encourages further research activity [27]. However, there is still little experimental evidence and solid theory, which can describe systemic influence of statins on an organism.

Endothelial dysfunction and chronic inflammation of the arterial wall continuously drive the development of atherosclerotic lesions [20,37]. A number of clinical trials have shown that statins improve

* Corresponding author at: Department of Enzyme Chemistry & Biochemistry, Palladin Institute of Biochemistry of NASU, 9 Leontovicha str., Kyiv 01601, Ukraine. Tel.: +380 44 2349056; fax: +380 44 2796365.
E-mail address: artem_tykhomyrov@ukr.net (A.A. Tykhomyrov).

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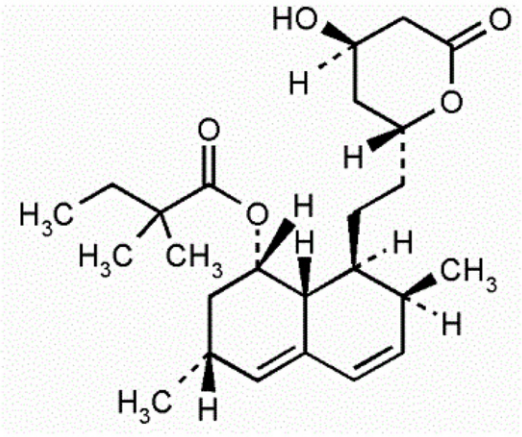

Fig. 1. Chemical structure of simvastatin, or (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate.

endothelial dysfunction in patients with coronary risk factors beyond what could be attributed to their impact on plasma lipids [16,25]. However, limited information is available on evaluating blood levels of angiogenic regulators in atherosclerosis, and the reports on their contribution to angiogenesis are controversial [12,48]. Angiogenesis impairments play a crucial role in atherosclerosis, thus it is important to elucidate possible effects of statins on blood levels of vital angiogenesis regulators in cardiovascular diseases. Angiostatin is known to be a potent endogenous inhibitor of vascular endothelial cell proliferation, migration, and tube formation, which is able to suppress capillary outgrowth and neovascularization. In 1994, O'Reilly et al. [32] described angiostatin and revealed that structurally it is an internal fragment of plasminogen/plasmin. Later, it was shown that angiostatin is derived through plasmin autoproteolysis or proteolytic action of some other endopeptidases and can consist of various numbers of plasminogen kringle (K) domains [43]. Thus, several angiostatin isoforms have been reported, differing in kringle content (K1–3, K1–4, K1–4.5, K1–5, etc.), tissue diversity and intensity of physiological effects. Angiostatin has long been known to be involved in regulation of tumor growth and metastasis via suppressing growth-factor induced migration and proliferation of endothelial cells and thus restricting blood vessel growth [7]. At present, there are strong lines of evidence that angiostatin has a wider range of physiological implications than previously believed. For example, angiostatin up-regulation in aortic wall and myocardial tissues has been negatively associated with coronary collateral development in patients with coronary artery disease (CAD) and type II diabetes mellitus [42]. Although it has been definitely demonstrated that exogenous angiostatin inhibits plaque angiogenesis and reduces atherosclerosis [9], it is still uncertain whether angiostatin blood levels reflect efficacy of anti-atherosclerosis therapy. In this study, we checked a hypothesis that simvastatin, reducing the major risk factors of atherosclerosis (cholesterol and lipid levels) and ameliorating inflammation, could alter levels of circulating angiostatin in patients with ischemic heart disease (IHD). If so, this regulator of angiogenesis could be considered as a novel supplementary indicator for evaluation of statin treatment efficacy in IHD.

2. Materials and methods

2.1. Subject selection

Twenty-six patients (20 females, 6 males), aged between 45 and 76 years (average age 58.7 ± 1.64 years) with clinically confirmed IHD and hypercholesterolemia were recruited for this study. This study was approved by the institutional review boards and local ethics committees. All patients provided written informed consent before enrolling in the study. Among them, 11 patients (42%) suffered an acute myocardial infarction no later as 6 months before the onset of the study, 5 patients (19%) had controlled diabetes mellitus. Exclusion criteria were as follows: (1) any oncology diseases; (2) acute or chronic liver diseases, including virus hepatitis; (3) severe disorders of cerebral circulation; (4) vascular thrombosis; (5) significant cardiac arrhythmia; (6) acute myocardial infarction within 6 months before study enrolment; and (7) receiving any lipid-lowering drugs within 6 months at study entry. Eligible patients were treated with 40 mg of simvastatin (Vabadin®, Berlin-Chemie Menarini Group, Germany) once daily for 8 weeks according with recommendations and guidelines of the European Atherosclerosis Society for moderate-intensity statin therapy [40]. Any pharmaceutical companies did not play a role in the study design, data collection and analysis, or preparation of the manuscript.

2.2. Lipid profile tests and other biochemical analysis

Peripheral blood samples were taken before initiation of therapy and then after 8 weeks of simvastatin treatment. All blood samples were obtained in the morning hours after at least 12 h of fasting. All specimens were centrifuged for 10 min at 1500 *g* to separate the serum from the clot. The sera taken from all individuals were stored at −70 °C until assay.

Total cholesterol (TC), very low density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured to evaluate effect of simvastatin on lipid status of IHD patient were measured using semi-automatic biochemical analyzer "RT-1904C" (Germany). Isolation of the low density lipoprotein (LDL) fraction requires ultracentrifugation, a technique not generally available in service laboratories, so the concentration of LDL-cholesterol (LDL-C) is usually calculated by the Friedewald's formula [13]:

$$[\text{LDL-C}] = [\text{TC}] - ([\text{HDL-C}] + [\text{TG}]/2.2),$$

where [TG] / 2.2 is equivalent of levels of VLDL-C.

For more detailed characteristics of lipid profile, non-HDL-C levels were additionally estimated as a difference between TC and HDL-C levels. Ratio TG/HDL-C was then calculated as an important characteristic of lipid profile [15].

Biomarkers of liver and kidney functions and other than lipid parameters, including aspartate aminotransferase (AST) alanine aminotransferase (AST) activities, urea, creatinine, glucose, and fibrinogen were examined in patients at baseline and over 8 weeks of simvastatin treatment. Additionally, serum levels of C-reactive protein (CRP), a highly sensitive marker of inflammation [5], were measured by latex agglutination test with use of Humatex CRP kit® (Human GmbH, Germany).

2.3. Antibody generation and angiostatin detection

Effects of simvastatin treatment on serum angiostatin levels were evaluated by Western blot with use of antibody produced in rabbits as described previously [46]. Briefly, plasminogen from fresh human plasma was purified on the Lys-sepharose column (Sigma, USA, cat. no. P-3391), and then processed with limited proteolysis by porcine pancreatic elastase (Sigma, USA, cat. no. E-1250) aimed to obtain angiostatin-like fragments. Isolation of plasminogen fragment containing first three plasminogen kringle domains (K1–3) was performed by affine chromatography on Lys-sepharose followed by gel-filtration. Purified angiostatin was used as an antigen for rabbit immunization. Antibodies recognizing parent molecule (plasminogen) as well as its fragments were purified from blood serum of immunized rabbits by chromatography on protein A-sepharose column followed by chromatography on angiostatin-containing immunoaffine sorbent.