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Statins exert differential effects on angiotensin II-induced atherosclerosis, but no benefit for abdominal aortic aneurysms

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ABSTRACT

Objective: Statins reduce atherosclerosis, but it is controversial whether they suppress abdominal aortic aneurysm (AAA) expansion. We hypothesized that statins (rosuvastatin and atorvastatin) would attenuate angiotensin II (AngII)-induced atherosclerosis and AAA.
Methods and results: Sixty apoE−/− male mice fed a normal diet were administered with either rosuvastatin (10 mg/kg/day) or atorvastatin (20 mg/kg/day) through drinking water for 1 week prior to initiating 28-day AngII infusion (1000 ng/kg/min). Statins administration led to therapeutic serum concentrations of drugs. Administration of either rosuvastatin or atorvastatin exerted no significant effect on AngII-induced expansion of suprarenal diameter or area. However, atorvastatin significantly reduced AngII-augmented atherosclerotic lesion areas in intimas of both aortic arches and cross-sections of aortic roots ($P < 0.001$). Atherosclerosis was attenuated independent of reductions in serum total cholesterol concentrations. Although serum MCP-1 and MIF concentrations were not changed by either statins, atorvastatin administration increased PPAR-α and -γ mRNA abundances and decreased NF-κB p50, p65, MCP-1 and TNF-α mRNA abundances in atherosclerotic lesions.
Conclusions: This study demonstrated both statins failed to suppress AngII-induced AAA. In contrast, atorvastatin reduced AngII-induced atherosclerosis associated with no change in serum inflammatory markers but a shift to upregulation of anti-inflammatory status in lesions.

1. Introduction

Although abdominal aortic aneurysm (AAA) and atherosclerosis are chronic inflammatory vascular diseases, the pathogenetic mechanisms are disparate. However, the renin-angiotensin system (RAS) is involved in both the development of AAA and the pathogenesis of atherosclerosis by stimulating a series of coordinated cellular and molecular events [1,2]. Angiotensin II (AngII) contributes to acceleration of atherosclerosis and induction of aneurysm by promoting complex changes of arteries that are independent of blood pressure [3]. Subcutaneous infusion of AngII into hypercholesterolemic mice both induces AAA formation and augments atherosclerosis [1,2]. In contrast, blockade of AngII suppresses development of AAAs associated with decreased macrophage accumulation and matrix metalloproteases (MMPs) expression [4].

Administration of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) inhibitor (statin) is a highly validated therapeutic approach for patients with coronary heart disease [5]. Reductions of cardiovascular morbidity and mortality with statin therapy have been attributed to lowering of plasma cholesterol concentrations and other non-lipid-based mechanisms, such as improved endothelial function and anti-inflammatory effects [6]. Rosuvastatin and atorvastatin are two of the most widely prescribed statins that profoundly reduce plasma concentrations of total cholesterol and low-density lipoprotein cholesterol (LDL-C). It has been suggested that administration of rosuvastatin can reach the recommended lipid targets more expeditiously than atorvastatin at equivalent doses [7]. Several anti-atherogenic mechanisms have been proposed for atorvastatin, such as reducing inflammatory chemokines expression by inhibition of NF-κB signaling and activation of inducible NO synthase expression [8,9], decreasing

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MMP-9 content, and increasing collagen and fibrous cap thickness in atherosclerotic lesions [10]. Recent studies have shown the anti-atherogenic effects of rosuvastatin administration associated with anti-inflammatory and anti-atherothrombotic properties [11].

However, the effects of statins on AAAs have not been fully defined. Most reports focus on the beneficial effects of statins for AAA therapy on protease and inflammatory markers in plasma or aneurysmal wall [12,13]. It is controversial whether statins can prevent the expansion and rupture of human AAAs [14]. Atorvastatin has been demonstrated to suppress the development of AAAs in elastase-induced rats through inhibition of macrophage migration [15]. No studies have defined the effects of rosuvastatin on AAA.

We hypothesized that both statins would suppress development of AngII-induced AAAs and atherosclerosis. Rosuvastatin and atorvastatin, as the representatives of hydrophilic and lipophilic statins, respectively, were administered into AngII-infused apoE−/− male mice. These drugs were administered in drinking water at concentrations that achieved serum concentrations that were sufficient to inhibit mouse HMG-CoA reductase. AngII (1000 ng/kg/min) was infused subcutaneously to promote development of AAAs and augment atherosclerotic lesions [1]. Serum inflammatory markers and associated transcription factors in lesions were analyzed to provide a basis for potential mechanisms.

2. Materials and methods

2.1. Animals

ApoE-deficient (apoE−/−) mice on C57BL/6 background ($n = 60$) were originally purchased from the Beijing Vital River Laboratory Animal Technology Corporation and bred in-house. Mice were maintained under specific pathogen-free (SPF) conditions and fed normal laboratory diet. All studies were performed with the approval of Zhejiang University Institutional Animal Care and Use Committee.

2.2. Study design

Sixty male apoE−/− mice (12–20 weeks old) were randomly divided into 3 groups, given rosuvastatin, atorvastatin (kindly provided by AstraZeneca and Pfizer Inc., respectively), or vehicle orally 7 days prior to initiation of AngII infusions and throughout the infusion period. Mice were visually inspected daily and weighed weekly. Rosuvastatin and atorvastatin were dissolved in drinking water at concentrations that gave approximate doses of 10 mg/kg/day and 20 mg/kg/day, respectively. Drug solutions were covered with foil to protect from light and freshly prepared every another day. Osmotic minipumps (Alzet Model 2004, Durect Corp) were implanted subcutaneously to deliver AngII (1000 ng/kg/min, Bachem) for 28 days. Suprarenal lumen dimensions were measured at selected intervals (Days 0 and 28) by a high frequency ultrasound imaging system (Visualsonics; Toronto, Canada) as described previously [16]. AAA was defined as an increase of 50% or greater in the maximal suprarenal diameter compared to the baseline.

2.3. Blood pressure measurement

Systolic blood pressure was measured in conscious mice using a computerized tail cuff (CODA 6+, Kent Scientific Corp, CT) [17]. All mice were acclimated to the system for 1 week prior to the start of the study.

2.4. AAA and atherosclerosis analyses

Mice were terminated after 28 days of AngII infusion, with blood harvested from left ventricles and aortic arches fixed with 10% neutral buffer formalin. Quantification of AAAs was based on luminal diameter and area as described above. Aneurysm severity was scored as described previously: Type I, dilated lumen without thrombus; Type II, remodeled tissue with little thrombus; Type III, a pronounced bulbous form of Type II with thrombus; Type IV, multiple, often overlapping aneurysms containing thrombus [17].

Aortic roots were embedded in OCT and frozen at −20 °C. Atherosclerosis was assessed on the intimas of both aortic arches by an en face technique and also using cross-sections (10 μm thick) of aortic roots as described previously [18]. Oil Red O staining was used to assist in visualization of lesions. Quantitative analysis of atherosclerosis was performed using Image-Pro software (Media Cybernetics) as described previously [1]. Cellular components of atherosclerotic plaques on aortic root were detected by immunostaining with rabbit antisera against mouse macrophage (Accurate Chemical Company), rabbit polyclonal to α-smooth muscle actin (α-SMA, Abcam), rat monoclonal to mouse CD19 for B lymphocyte (BD Pharmingen) and rat monoclonal to mouse CD90.2 for T lymphocyte (BD Pharmingen) [19]. Immunostaining was performed with a commercially available system (Fisher Microprobe). A peroxidase-based ABC system and the red chromogen, AEC, were used to visualize the antigen–antibody reaction. Cellular components consisting of atherosclerotic lesions were graded as follows: 0 = no staining, 1 = slight staining, 2 = mild staining, 3 = moderate staining and 4 = abundant staining (Supplemental data II Fig. 1). Six visual fields (magnification ×400) of every lesion section were randomly included to count the numbers of T lymphocytes and get the average of cell numbers in lesions.

Location of mRNA for nuclear factor κB (NF-κB p50, p65), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor (TNF-α), peroxisome proliferator-activated receptor (PPAR-α and -γ) in atherosclerotic lesions was determined with in situ hybridization kits and visualized using DAB as chromogen (Boster, Wuhan, China). Briefly, after fixed with 4% formaldehyde/PBS contained 0.1% DEPC, sections were quenched in freshly prepared 0.5% hydrogen peroxide in methanol, digested for 120 s at 37 °C in 10% (w/v) pepsin dissolved in 3% (w/v) citric acid, prehybridized in hybridization mix without probe for 2 h at 37 °C, and then hybridized overnight at 37 °C (probe sequence available on Supplemental data I). Probe bound to the section was immunologically detected using anti-digoxigenin Fab fragment covalently coupled to peroxidase and DAB as chromogenic substrate. All the data were quantified by two observers that were blinded to the study design.

Full details of the experimental protocols used are given in the Online supplemental data.

2.5. Serum measurements

Serum total cholesterol concentrations were determined using enzymatic assay kits (Wako Chemical Co). Serum concentrations of monocyte chemoattractant protein-1 (MCP-1, Bender MedSystems) and macrophage migration inhibitory factor (MIF, R&D Systems) were measured with ELISA kits according to manufacturer's recommendation. Serum concentrations of rosuvastatin and atorvastatin were measured by liquid chromatography with electrospray ionization tandem mass spectrometry as described previously [20]. Blood was collected about 1 h after drug administration to measure serum atorvastatin concentrations.

2.6. Statistical analyses

Mean and standard error of mean (SEM) were calculated for each parameter. Data were tested for use of parametric or non-parametric post hoc analysis and then analyzed by One way ANOVA