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Effect of rosuvastatin on atherosclerotic plaque stability: An intravascular ultrasound elastography study

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ABSTRACT

Objectives: The present study aimed to investigate the effect of potent rosuvastatin therapy on plaque mechanical stabilization as seen on IVUSE.

Methods: 14 purebred New Zealand rabbits were fed a high-cholesterol diet; the abdominal aorta endothelium was balloon-injured after 2 weeks; at week 13, 7 rabbits received rosuvastatin (1.5 mg/kg/day), and the other 7 received an equal volume of saline. IVUS images of abdominal aortas were acquired, and 2 consecutive frames near the end-diastole images in situ were used to construct an IVUS elastogram.

Results: Control rabbits showed a significant increase in shear strain (SS) and area strain (AS) in total plaques. The rosuvastatin group showed no change in SS and AS, but serum TG and LDL-C levels were reduced, with less lipid deposition, macrophage infiltration, production of proinflammatory cytokines and apoptosis in plaques. The changes in SS and AS from baseline between groups significantly differed (SS: 1.15 (1.96) % *vs.* −0.99 ± 2.83%, p = 0.013; AS: 1.25 (2.29) % *vs.* −1.67 ± 5.05%, p = 0.022). At follow-up, for controls, strain values were increased in the shoulder of eccentric plaques (SS: 2.66 ± 1.31% *vs.* 4.86 ± 1.93%, p = 0.016; AS: 4.45 ± 2.33% *vs.* 7.91 ± 2.74%, p = 0.009) but not the plaque body. Changes in SS and AS in the plaque shoulder differed between the control and rosuvastatin groups (SS: 2.20 ± 2.17% *vs.* −0.87 ± 3.31%, p = 0.028; AS: 2.10 (4.61) % *vs.* −2.75 ± 5.97%, p = 0.009).

Conclusion: Rosuvastatin therapy in rabbits with atherosclerotic plaques led to less vulnerable plaque features. IVUSE is a very sensitive technique for detecting pharmacologically-induced mechanical changes in rabbit atherosclerotic plaques.

1. Introduction

Cardiovascular disease is a leading cause of deaths worldwide, and atherosclerosis is the main pathological change in cardiovascular disease. 3-hydroxy-3-methylglutaryl CoA reductase inhibitors (statins) are among the first-line pharmacotherapeutic agents for atherosclerosis treatment. Statins reduce the progression of atherosclerosis or even decrease the volume of atherosclerotic plaques [1–4]. Plaque volume or the consequent severity of stenosis produced might be a predictor of acute cardiovascular events, but the occurrence of events is affected simultaneously by many elements [5]. Plaque vulnerability is considered the most important determinant of acute cardiovascular events [6–8].

Atherosclerotic vulnerable plaque disruption with superimposed thrombosis is considered the main cause of acute cardiovascular events. Therefore, treatment of vulnerable plaque is important in preventing and managing acute cardiovascular events. Vulnerable plaque is characterized as a large lipid pool, a thin fibrous cap (<65 μm), a high content of inflammatory cells and reduced number of smooth muscle cells [8–11]. Therefore, morphological features (such as proportion of stenosis or plaque size) are insufficient for evaluating the effect of statins on plaque stability. A precise functional measure that can represent a diagnostic technology for plaque vulnerability in vivo is needed.

Intravascular ultrasound elastography (IVUSE) is an effective

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technique for assessing the mechanical characteristics of atherosclerotic plaque. Compared to conventional intravascular ultrasound (IVUS), IVUSE can be used to qualify or quantify specific histologic components of atherosclerotic plaques by measuring tissue elastic property. Because it is based on IVUS, IVUSE is similarly restricted by its weakness, such as invasiveness. Numerous studies have shown that IVUSE could be used in the tissue characterization of atherosclerotic plaque and identification of vulnerable plaque [12–16]. Our previous studies showed a good association of plaque deformation and its tissue features by means of an in-house–designed IVUSE software system [17,18]. However, IVUSE has not been used for assessing the effect of statins on the mechanical properties of atherosclerotic plaques.

Here, we used IVUSE to investigate the mechanical properties of atherosclerotic plaques in a rabbit model with rosuvastatin treatment.

2. Materials and methods

2.1. Animal preparation

A total of 20 purebred male New Zealand rabbits (mean weight 2.04 ± 0.14 kg) underwent abdominal aortic endothelium denudation with an intravascular balloon catheter (4.0 × 15 mm) after 2 weeks of an atherogenic diet (1% cholesterol, essential nutrients and vitamins) as described [17]. Rabbits received the diet and had free access to water and food during the whole experiment. At week 13, rabbits were randomly divided into 2 groups (n = 10 each) for treatment: rosuvastatin (1.5 mg/kg/day, Astra-Zeneca, London, UK) or equal volume of saline (control). Rosuvastatin calcium was dissolved in normal saline and infused via a stomach tube for 8 weeks. Normal saline was infused by the same means. At the end of week 20, animals were killed and abdominal aortas were harvested. Protocols for all animal experiments conformed to the Guide for the Institutional Animal Care and Use Committee of Shandong University and were approved by the Institutional Animal Care and Use Committee of Shandong University.

2.2. Plasma assays

Before treatment and at sacrifice, a blood sample in fasting state was drawn from the rabbit ear margin vein. The supernatant was collected after 8-min centrifugation at 4000 rpm. Plasma levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured by an enzymatic method on fully automatic clinical biochemical analyzer (Thermo Scientific, USA).

2.3. IVUS examination and IVUSE acquisition

Abdominal aortas were investigated by use of a single-element rotating IVUS catheter (Atlantis SR 40-MHz probe) at a frame rate of 30/s and the iLab IVUS system (Boston Scientific, USA) before and after rosuvastatin or saline administration.

Before administration, abdominal aortas were first scanned by use of the IVUS system with the assistance of an automatic pullback device at 0.5 mm/s. We chose 2 plaques in at least 1-cm intervals from each rabbit for in situ imaging for at least 3 cardiac cycles. The distance of the plaques of interest from the end of the abdominal aorta identified by the joint of the left and right common iliac arteries was calculated according to the first IVUS pullback images. During IVUS examination, electrocardiography and blood pressure were simultaneously recorded. After 8 weeks, IVUS pullback scans were investigated again. The IVUS cines in situ of the 2 plaques were obtained at approximately the same axial position identified by the distance from the end of the abdominal aorta.

IVUSE involved an in-house-designed software system. We previously described the IVUSE construction [17]. Briefly, two consecutive frames near end-diastole from the IVUS cine in situ recognized as the pre- and post-deformed image, respectively, were used to generate the IVUSE. Then, the lumen and adventitia boundaries of aortas were semi-automatically delineated [19]. A search algorithm based on block-matching was used to identify the best-matched points on the post-deformed image. Finally, the points with matching correlation >0.9 were automatically chosen to construct the triangular meshes with the Delaunay triangulation technique. Then, the deformation of every triangular mesh could be easily calculated, and the strain (a geometrical description of deformation) of the total objective was color-coded and superimposed on the grayscale IVUS image.

2.4. Measurement of conventional and IVUSE variables

The IVUS pullback images and cross-sectional views in situ were used to determine the contours of atherosclerotic plaques. Conventional variables could be measured after the lumen and adventitia boundaries of aortas were delineated. Cross-sectional plaque area (PA) and plaque volume (PV) were defined as external elastic membrane area (EEM_{are}) minus lumen area ($Lumen_{area}$) and external elastic membrane volume (EEM_{volume}) minus lumen volume ($Lumen_{volume}$), respectively. Plaque burden (PB) and plaque volume burden (PVB) were calculated as (PA/EEM_{area}) × 100% and (PV/EEM_{volume}) × 100%, respectively. The eccentric index (EI) was defined as $(PT_{max} - PT_{min})/PT_{max}$, where PT_{max} = maximal plaque thickness and PT_{min} = minimal plaque thickness. The remodeling index (RI) was defined as EEM_{area} at follow-up/EEM_{area} at baseline [20]. IVUSE variables were measured after the construction of triangular meshes. Shear strain (SS) and area strain (AS) of each triangle were calculated as $SS = (L - L_0)/L_0$ and $AS = (S - S_0)/S_0$, where L and L_0 were the length of the corresponding triangles post- and pre-deformation, respectively, in the direction of shear stress, and S and S_0 the areas of the corresponding triangles post- and pre-deformation, respectively. Eccentric plaque was defined as plaque with eccentric index >0.5. By delineating the shoulder and the body region of the eccentric plaques on the IVUS elastogram, the regional SS and AS could be measured. The shoulder of the eccentric plaque was defined as the region of the thickness less than half of the maximum thickness of the plaque, and the region of the thickness greater than half of the maximum thickness of the plaque was considered the plaque body.

2.5. Histopathology and immunohistochemistry

The perfusion circuit was established with the ascending aorta and the right atrium of the animal cannulated. Rabbits were perfused with 500–1000 ml saline at approximately 100 mmHg until the outflow from the right atrium was clear, followed by 10% Ringer formaldehyde solution at the same pressure for 1 h. After the perfusion, abdominal aortas were segmented according to the distance from the end of the abdominal aorta recorded during the IVUS detection. All aorta segments were soaked in 10% Ringer formaldehyde solution for 48 h, then each segment was divided into two parts. One part was frozen, embedded and cut into serial slices of 6 μm for Oil-red O staining, and the other part was embedded in paraffin and cut into serial slices of 4 μm for picrosirius red staining and immunohistochemistry. General morphology was evaluated by haematoxylin & eosin staining. Collagen stained with picrosirius red was imaged by polarized light microscopy. Lipids were identified by Oil-red O staining. Macrophages and smooth muscle cells (SMCs) were stained