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Atorvastatin prevents advanced glycation end products (AGEs)-induced cardiac fibrosis via activating peroxisome proliferator-activated receptor gamma (PPAR-γ)☆

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ABSTRACT

Background. Previous studies have shown that the activation of advanced glycation end products (AGEs) contributed to the cardiac fibrosis in diabetic patients. Although it had been reported that statins have beneficial effects on cardiac fibrosis in hypertension and myocardial ischemia models, their effects on AGEs models have not been studied. We aimed to investigate the effects of atorvastatin (Ator) on the AGEs-induced cardiac fibrosis both *in vitro* and *vivo*.

Methods. Male Sprague-Dawley rats were randomly divided into four groups: Control, AGEs, Ator or AGEs + Ator. The cardiac function was evaluated with the echocardiography at the second and the third month. Fibrosis area, α-SMA and RAGE expression in cardiac tissue were measured. For in vitro study, rat cardiac fibroblasts were treated with PD98059 (ERK inhibitor), Ator or Ator + GW9662 (PPAR-γ antagonist), and then were stimulated with AGEs. Fibroblasts proliferation, ERK1/2, phosphorylated ERK1/2, α-SMA, and RAGE expression were studied.

Results. Compared with the control group, in vivo treatment with Ator significantly retarded the AGEs-induced diastolic function and attenuated cardiac fibrosis, α-SMA, and RAGE over expression induced by AGEs. Consistently, Ator prominently downregulated RAGE and α-SMA, while inhibited phosphorylation of ERK1/2 and fibroblast proliferation induced by AGEs in vitro. The GW9662 neutralized these effects of Ator on cardiac fibroblasts stimulated by AGEs.

Conclusion. In this study, we demonstrated that AGEs-induced fibroblast proliferation and differentiation were dependent on AGEs-RAGE-ERK1/2 pathway and that atorvastatin could block this pathway via activating PPAR-γ.

1. Introduction

Cardiac fibrosis is a major pathological change in diabetic cardiomyopathy, which impairs cardiac elasticity and contractile function [1]. One of its principal mechanisms is myocardial fibroblast proliferation and differentiation, which are characterized by overexpression of alpha-smooth muscle actin (α-SMA) [2]. A considerable amount of evidence support that advanced

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glycation end products (AGEs), which are produced by non-enzymatic reaction between proteins and sugar residues and accumulated in the cardiovascular tissue, play an important role in the development of myocardial fibrosis in diabetes [3–5]. AGEs mainly bind to the receptor for AGEs (RAGE) to induce fibroblast proliferation [6]. As a member of the immunoglobulin superfamily of cell-surface molecules, RAGE unlike other receptors which are downregulated by negative feedback sent from their increased ligands. The RAGE-AGEs binding leads to positive feedback, which further enhance receptor expression [7]. Besides, there are increasing evidences showing that in diabetic patients, the activation of extracellular signal-regulated kinase (ERK1/2) via AGE-RAGE pathway was an important mechanism involved in the cardiac fibrosis [8].

Peroxisome proliferator-activated receptor gamma (PPAR-γ), a member of the nuclear hormone receptor superfamily, is involved in many biological processes such as lipid homeostasis, energy metabolism, and cellular proliferation. PPAR-γ is widely expressed in the cardiovascular system and is an important inhibitor of RAGE [9,10]. Studies demonstrated that pretreatment of pioglitazone, the agonist of PPAR-γ, could significantly attenuate RAGE over expression and suppress various cell proliferations induced by AGEs [11,12].

In addition to inhibiting the synthesis of cholesterol, atorvastatin (Ator) possess a pleiotropic effect [13,14]. Several studies have suggested that these pleiotropic effects were mediated by PPAR-γ [15–17]. Since there is a close correlation between PPAR-γ and AGEs-RAGE axis, we then hypothesized that Ator, a commonly used statin in clinic, may suppress AGEs-induced cardiac fibrosis. Besides, there are many mechanisms involved in the diabetic cardiomyopathy except AGEs accumulation; therefore, we established an AGEs models rather than DM models to avoid the disturbance of high blood glucose and thus focus on the effects of AGEs on cardiac fibrosis.

2. Methods

2.1. Reagents

Rat serum albumin (RSA), Dulbecco's Modified Eagle's Medium (DMEM), antibiotics and other medium additives were obtained from Invitrogen Life Technologies (CA, USA). Antibodies against ERK1/2 and phosphorylated ERK1/2 (p-ERK1/2) were from Millipore Corporation (MA, USA). Anti-RAGE and anti-α-SMA antibody, GW9662, PD98059, and Ator were obtained from Sigma-Aldrich (MO, USA).

2.2. Preparation and Identification of AGEs

The synthesis of AGEs-RSA had been described previously [18]. Briefly, solutions of D-glucose (1.6 M) and RSA (100 mg/mL) were incubated in 10 mM phosphate-buffered saline (PBS, PH7.4) filtered by a 0.2 μm sterilization filter to prevent contamination by live bacteria, and then was kept in dark for ten weeks at 37 °C. Control non-glycated RSA was incubated without glucose using the above procedure. At the end of the incubation period, the extensive dialysis for 48 h at 4 °C against 10 mM PBS was applied to remove the unreacted glucose. The protein concentration was determined by a Bradford method. The AGE content was assessed by fluorescence with 360 nm excitation and 440 nm emission wavelength and expressed as fluorescence intensity at 1 mg/mL protein. AGEs contained 56.5 fluorescence U/mg protein, and control contained 0.7 fluorescence U/mg protein. The endotoxin level of AGEs-RSA, which was assayed by kinetic-turbidimetric ttachypleus amebocyte lysate kit (LONZA, USA), was 0.3 EU/ml, suggesting no obvious live bacteria infection existed.

2.3. In Vivo Study

Male Sprague-Dawley (SD) rats (Vital River, Beijing, China) weighing about 200 g were housed in an environmentally controlled room and were provided with tap water ad libitum. The rats (N = 7) were randomly divided into four groups: (1) normal control, receiving vehicle saline, (2) AGEs, (3) Ator, or (4) AGEs + Ator. Ator (10 mg/kg) was administered by oral gavage once daily, while AGEs (50 mg/kg, BW/day) was administered by infusion. After 90-day treatment, 12 h fasted rats were anesthetized by injecting pentobarbital, then weighed, evaluated with echocardiography and were sacrificed by cervical dislocation. Blood (serum) samples were collected to measure AGEs level, fasting blood glucose (FBG) and total cholesterol level. Hearts were weighed and fixed with Bouins' solution. This study was conformed to the guidelines of Capital Medical University for the care and use of laboratory animals.

2.4. Measurement of Blood Pressure

Blood pressure (BP) was recorded with tail-cuff blood pressure recorder at end of treatment. Rats were accustomed to 26 °C chamber for 30 min before the blood pressure was recorded between 9 and 11 AM. Measurement was repeated three times, and the average was calculated.

2.5. Echocardiography

After the anesthetization, rats were evaluated in the left lateral decubitus position by echocardiography studies with a VEVO 2100 high resolution *in vivo* imaging system (VisualSonics, Toronto, Canada) at the second and the third month. The systolic function evaluation contained LV end-systolic dimension (LVESD), LV end-diastolic dimension (LVEDD), and LV Fractional Shortening (FS). Using a pulsed wave Doppler, early diastolic peak velocity (E velocity) and late diastolic peak velocity (A velocity) of the LV inflow were measured as indices of LV diastolic function. To identify the pseudo normalization, early diastolic peak velocity of the mitral valve movement (E′) were measured with tissue Doppler imaging. Mean values of three measurements were used for analysis.

2.6. Sirius Red Staining and Histological Examination

After fixed with pre-warmed Bouins' solution for one hour, the heart tissues were washed in running tape water until yellow disappeared, then embedded in paraffin and cut into 5-mm