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Atorvastatin reverses cardiac remodeling possibly through regulation of Protein Kinase D/Myocyte Enhancer Factor 2D activation in spontaneously hypertensive rats

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ABSTRACT

The present study was designed to determine whether atorvastatin reduced hypertension-induced cardiac remodeling and whether these effects involved Protein Kinase D (PKD) and Myocyte Enhancer Factor 2D (MEF2D), factors known to be implicated in cardiac hypertrophy and fibrosis. 16-Week-old spontaneously hypertensive rats (SHRs) and age-matched Wistar-Kyoto (WKY) rats were included. Blood pressure and serum lipid concentration were measured. H–E staining, myocardial transverse diameter, and echocardiography were examined to evaluate cardiac hypertrophy. Hydroxyproline content assay and Masson's trichrome staining were used to estimate cardiac fibrosis. Atorvastatin (10, 25 and 50 mg/kg/day) was administered for 8 weeks. Increased blood pressure and cardiac remodeling were prominent in SHRs compared with WKY rats. SHRs also had elevated PKD and MEF2D activation. The systolic blood pressure, myocardial transverse diameter and hydroxyproline content were positively correlated with the activation level of PKD and MEF2D in SHRs. Atorvastatin significantly attenuated the activation of PKD and MEF2D. It may be concluded that atorvastatin reverses hypertension-induced cardiac remodeling partially through down-regulation of PKD/MEF2D activation. Our results predict novel therapeutic targets for atorvastatin in treating hypertensive patients.

1. Introduction

Cardiac remodeling is a fundamental process of adaptation to hypertension, however, long-term remodeling is associated with a significant increase in risks of heart failure, dilated cardiomyopathy, ischemic heart disease, and sudden death [1]. Hypertensive myocardial remodeling is an independent risk factor for lethal cardiovascular events, and is the key pathological manifestations during the transition of heart function from compensate to decompensate.

PKD is a recent addition to the calcium/calmodulin-dependent serine/threonine protein kinase, accumulating evidence demonstrating that PKD plays an important role in the cardiovascular system, particularly in the regulation of myocardial contraction, hypertrophy and remodeling [2–5]. Studies also showed that PKD expression was increased in human heart failure [6]. The PKD signaling can be transmitted through transcriptional factor MEF2, in which MEF2D plays an important role in stress-dependent pathological remodeling of the adult heart [7]. These exciting data necessitate further investigation of the role of PKD in the development of cardiac remodeling in vivo in response to clinically relevant stresses such as pressure overload and myocardial infarction.

As hypertension is often accompanied by dyslipidemia, the treatment frequently involves statins. Statins reduce cardiovascular morbidity and mortality in patients with high and moderate hypercholesterolemia or even normal cholesterol levels [8]. Recently, statins have been shown to inhibit cardiac hypertrophy and fibrosis. For example, simvastatin reduces human myofibroblast proliferation [9]. Atorvastatin attenuates the angiotensin II-induced collagen synthesis by rat or human cardiac fibroblasts [10]. Simvastatin also induces regression of cardiac hypertrophy and fibrosis and improves cardiac function in a transgenic rabbit model of human hypertrophic cardiomyopathy [11]. However, the molecular mechanism of statins in reversing cardiac remodeling is not explored yet. Therefore, the present study was designed to investigate whether these effects of atorvastatin were mediated by PKD and MEF2D.

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2. Materials and methods

2.1. Animals and drug treatment

16-Week-old male SHRs were obtained from Genetic Models Inc. (Indianapolis, IN). Age-matched normotensive male WKY rats were purchased from animal center of Shandong University as the control. Animals were given rodent diet and tap water ad libitum throughout the experiments. They were maintained under conditions of standard lighting (alternating 12 h light/dark cycle), temperature (20–22 °C) and humidity (50–60%).

Rats were divided into the following groups: WKY-vehicle, SHR-vehicle, SHR-atorvastatin 10 mg/kg/day, SHR-atorvastatin 25 mg/kg/day, and SHR-atorvastatin 50 mg/kg/day. Each group has 10 rats for 8 weeks treatment. Atorvastatin (Novartis Pharmaceutical Company, China) and vehicle were administered orally by gavage once a day. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, Revised 1985), and was approved by the Animal Care and Use Committee, Shandong University.

2.2. Measurement of systolic blood pressure (SBP)

SBP was measured by the non-invasive tail-cuff method. Ambient temperature was maintained at 30 °C. The animals were acclimated to the restraining cages and tail-cuff apparatus before SBP was determined.

2.3. Lipid measurement

Before and after the studies, a 2 ml blood sample was taken and the levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL-C) were measured with commercially available enzymatic kits.

2.4. Echocardiographic evaluation

Before killing, each rat was anesthetized with ketamine HCl (50 mg/kg) and xylazine (10 mg/kg), and was placed in the left lateral decubitus position. The chest of the rat was shaved and a layer of acoustic coupling gel was applied to the thorax. Two-dimensional and M-mode echocardiography was performed using a commercially available 12-MHz linear array transducer system and echocardiogram machine (Sonos 5500, HP, Boston, MA, USA). The M-mode recordings obtained were of the left ventricle at the level of the mitral valve in the parasternal view using two-dimensional echocardiographic guidance in both the short- and long-axis views. Pulsed wave Doppler was used to examine mitral diastolic inflow from the apical four-chamber view. For each measurement, data from three consecutive cardiac cycles were averaged. All measurements were made from digital images captured at the time of the study by use of inherent analysis software (Sonos 5500 software package).

2.5. Left ventricular mass index analysis

Animals were killed by decapitation. The hearts were immediately resected and weighed. Body weight and left ventricular mass were measured. The left ventricular mass index (LVMI) is the ratio of left ventricular weight (in milligrams) to body weight (in grams) (LVW/BW).

2.6. Hydroxyproline content assay

Hydroxyproline was assayed using a procedure described previously [12]. The apex of left ventricle was defatted and lysed. The sample was centrifuged at 4000 rpm for 10 min. The supernatant was mixed with fresh chloramine T for 10 min and then with Ehrlich's reagent at 75 °C for 20 min. After samples were cooled, optical density was read at 560 nm with a spectrophotometer that was adjusted by a blank. The blank was prepared by the same procedure but without cardiac tissues in the reaction mixture. Hydroxyproline concentration, expressed as micrograms per milligram of dry heart weight, was then calculated as described before [12].

2.7. Histopathology staining

Heart tissues were fixed in buffered formaldehyde, embedded in paraffin, and sectioned on a microtome. Every 5-μm thick sections through the left ventricle was stained with H–E [13]. Myocardial transverse diameter (TDM) was measured at the nuclear level with H–E staining. For the detection of collagen, the ventricular tissue was stained with Masson's trichrome staining [14]. The collagen volume fraction (CVF) was determined as the ratio of interstitial collagen area to myocardial area.

2.8. Western blot analysis

Tissue samples from the hearts were homogenized in a buffer containing 20 mM Tris–HCl, pH 6.8, 1 mM EDTA, 1% SDS, 1 mM PMSF, and 1× protease inhibitor cocktail (Roche, Germany). Equal amounts of protein from each sample were separated with a 10% SDS-PAGE as described previously. The separated proteins were transferred onto nitrocellulose membranes, and the membranes were blocked for 2 h with 5% defatting milk, then incubated overnight at 4 °C with one of the following antibodies: rabbit polyclonal anti-PKD, anti-p-PKD, anti-MEF2D, anti-p-MEF2D diluted 1:1000 (Cell Signal Technology, CST, USA), and anti-nitrotyrosine diluted 1:800 (Cayman Chemicals, Ann Arbor, USA). After being washed, the membrane was incubated with the corresponding secondary antibody conjugated to horseradish peroxidase. Immunoreactive bands were visualized with the SuperSignal West Pico enhanced chemiluminescence kit (Pierce, Rockford, IL) according to the manufacturer's instructions. Band intensities were quantified using a densitometer analysis system Flurochem 9900-50 (Alpha Innotech, USA). Equal protein loading was confirmed by staining the gel with Coomassie blue and probing with β-actin antibody (Sigma, USA).

2.9. Determination of tissue concentrations of IL-6 and TNF-α

For cytokine assays, LV myocardium was homogenized in a buffer containing 10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton X-100, 10% glycerol, 1.0% SDS, 0.5% deoxycholate, 1 mM PMSF, and a protease inhibitor cocktail. The homogenate was centrifuged at 14,000 rpm for 15 min, and the supernatant was used for measurement of IL-6 and TNF-α levels with commercially available enzyme-linked immunosorbent assay kits (BioSource International, Inc., USA) according to the manufacturer's instructions. Final results were expressed as pg/ml.

2.10. Statistical analysis

Data were analyzed by using SPSS 11.5 software under Windows XP. All statistics were analyzed by ANOVA analysis, with a