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Effect of farnesyltransferase inhibition on cardiac remodeling in spontaneous hypertensive rats

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ABSTRACT

Background: Farnesyltransferase (FT), an essential enzyme at the downstream of mevalonate pathway, was reported to be upregulated in hypertrophic cardiomyocytes of spontaneously hypertensive rats (SHRs) compared with myocardium of Wistar-Kyoto rats (WKYs). This upregulation was accompanied with cardiac remodeling. This study was designed to determine whether FT inhibition can alter cardiac remodeling in SHRs.
Methods: Twelve-week-old SHRs were randomized to receive infusion of either NS or FTI-276 (307 μg/kg/d i.v. each n = 10). WKY rats served as normal controls (n = 6). Echocardiography was performed before and after intervention. SHR hearts were perfused *ex vivo* for the evaluation of cardiac performance, collagen deposition and biochemical changes (activation of Ras, extracellular-signal regulated kinases/ERK1/2, procollagen type I/III, TGF-β1, connective tissue growth factor/CTGF, and bone morphogenetic protein-7/BMP-7 expression).
Results: FTI-276 intervention decreased interventricular septum wall thickness at end- diastole (IVSd) and relative wall thickness (RWT) of SHRs ($P<0.05$). Three week intervention with FTI-276 attenuated hydroxyproline content ($P < 0.05$), collagen deposition ($P < 0.01$), Ras activation, ERK1/2 phosphorylation ($P < 0.01$) and mRNA expression of procollagen type I, TGF-β1 and CTGF and elevated mRNA expression of BMP-7 ($P < 0.05$) in left ventricle of SHRs.
Conclusion: The present study indicated that FT inhibition could attenuate myocardial fibrosis and partly improve cardiac remodeling in SHRs. The beneficial effects might be mediated through suppression of the activation of Ras and ERK1/2 phosphorylation pathway. The enhanced mRNA expression of BMP-7 with inhibition of TGF-β1 and CTGF mRNA expression might be an important mechanism.

1. Introduction

Cardiac remodeling is defined as genome expression resulting in molecular, cellular and interstitial changes and manifested clinically as changes in size, shape and function of heart resulting from cardiac load or injury [1]. It is a long process from genome expression and molecular changes to myocardial hypertrophy, fibrosis and cardiac decompensation, which is manifested clinically as chronic heart failure.

Farnesyltransferase (FT) is an essential enzyme at downstream of mevalonate (MVA) pathway (Fig. 1). FT catalyzes the farnesylmoieties of farnesyl pyrophosphate (FPP) coupled to Ras protein, which is dependent on farnesylation anchoring itself in membrane for its activity [2,3]. FT regulates the activation of Ras protein and consequently affects intracellular signal transduction, cell growth and proliferation [4]. Our previous study showed that FT expression was significantly upregulated in left ventricular myocardium of spontaneously hypertensive rats (SHR) compared with Wistar-Kyoto rats (WKY) [5]. In addition, this upregulation was accompanied with cardiac remodeling [5]. However, it has not been demonstrated whether inhibition of FT exerts direct anti-remodeling effects on myocardium of SHRs.

Abbreviations: FT, farnesyltransferase; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto rat; FTI, farnesyltransferase inhibitor; ERK1/2, extracellular-signal regulated kinases 1/2; FPP, farnesyl pyrophosphate; MVA, mevalonate; TGF-β1, transforming growth factor-beta 1; CTGF, connective tissue growth factor; BMP, bone morphogenetic protein; HMG-CoA, 3-hydroxy-3-methyl-glutaryl coenzyme A; NS, normal saline; SNP, sodium nitroprusside; SBP, systolic blood pressure; MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; HR, heart rate; IVS, interventricular septum; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall thickness; RWT, relative wall thickness; EF, ejection fraction; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; WHM, the mass of the whole heart; LVM, the mass of left ventricle; HMI, heart mass index; LVMI, left ventricular mass index; PE, phenylephrine; ACh, acetylcholine; I/R, ischemia/reperfusion; Hyp, hydroxyproline; CVF, collagen volume fraction; RBD, Ras binding domain; RT-PCR, real-time polymerase chain reaction; GADPH, glyceraldehyde-3-phosphate dehydrogenase; Smad, small mother against decapentaplegic; BNP, B-type natriuretic peptide.

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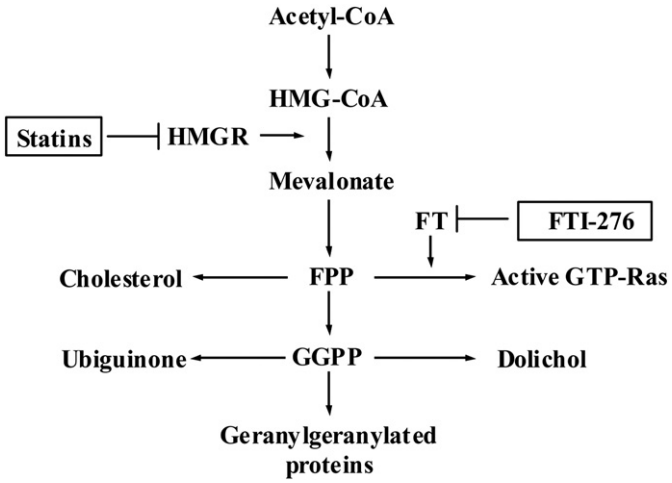

Fig. 1. Mevalonate (MVA) pathway. HMG-CoA, 3-hydroxy-3-methyl-glutaric acid coenzyme A; HMGR, 3-hydroxy-3-methyl-glutaric acid coenzyme A reductase; FT, farnesyltransferase; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate.

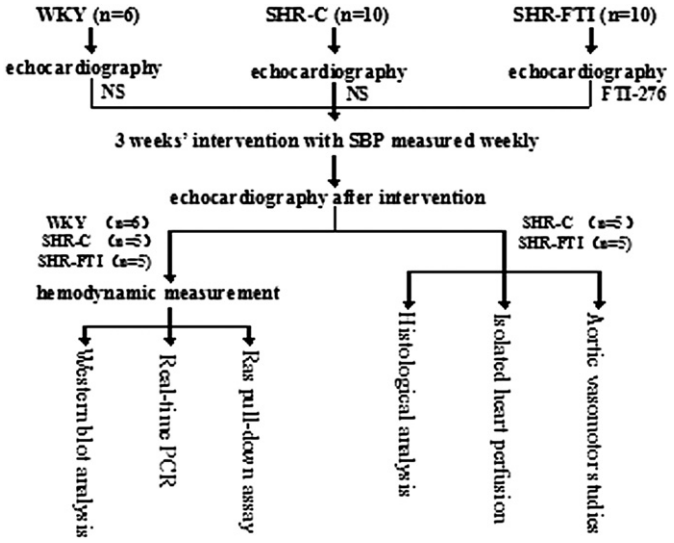

Fig. 2. Flow-chart of the methodology. All rats received lipid measurement, heart mass index, left ventricular mass index and hydroxyproline content assay.

Calderone's study indicated that FT inhibition attenuated cardiomyocyte hypertrophy induced by NE and ET-1[6]. *In vivo* studies revealed that FT inhibition reduced hypertension induced by deoxycorticosterone-salt and angiotensin II [7,8].

Recent study revealed that the activation of Ras and extracellular-signal regulated kinases 1/2 (ERK1/2)/transforming growth factor-β1 (TGF-β1) pathway involved in cardiac fibrosis [9]. SHR has served as one of the preferred animal models of myocardial fibrosis for decades [10]. Thus, we can assume that upregulation of FT is involved in cardiac remodeling in SHRs *via* activation of Ras pathway. The present study was designed to determine whether FT inhibition can alter cardiac remodeling in SHRs.

2. Methods

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

2.1. Animals

Experiments were carried out using twelve-week-old male SHRs and WKY rats (clean grade, purchased from the Laboratory Animal Center of Chinese Academy of Sciences, Shanghai, China). The investigation followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996) and the guidelines of the Animal Care and Use Committee of Zhejiang University. Animals were housed in conventional cages with free access to standard pellet diet and water *ad libitum* at the controlled temperature (23 ± 2 °C) and humidity (55% ± 5%) with a 12:12 h light/dark cycle.

2.2. Intervention

Twenty male SHRs were randomly divided into two groups: SHR-FTI, given an intravenous injection of FTI-276 (307 μg/kg/d) for three weeks; SHR-C, given an intravenous injection of normal saline (NS) in same volume as control. Six male age- and weight-matched WKY rats were housed as normal control (WKY), given an intravenous injection of NS in same volume, too.

2.3. Design and setting

All rats received echocardiography before and after three week intervention. Hemodynamic studies were performed in six WKY rats, five SHRs from SHR-FTI group and five SHRs from SHR-C group. Other SHRs received isolated heart perfusion, and three SHRs randomly selected among them in each SHR group received vasomotor studies by isolated aortic rings perfusion. The flow-chart of methodology was depicted in Fig. 2.

2.4. Reagents

Reagents and antibodies were purchased as follow: FTI-276, phenylephrine (PE), acetylcholine (ACh) and sodium nitroprusside (SNP) were from Sigma Chemical Co. (St. Louis, MO, USA). Rabbit anti-type I collagen polyclonal antibody and rabbit anti-type III collagen polyclonal antibody were from Boster Biological Engineering Co, Ltd (Wuhan, China). Rabbit anti-ERK1/2 polyclonal antibody and rabbit anti-phospho-ERK1/2 (Thr202/Tyr204) monoclonal antibody were from Cell Signaling Technology (CST, USA). EnVision two-step staining kit was from DAKO Co. (Denmark). Tissue Protein Extraction Reagent/T-PER was from Pierce Co. (USA). The Ras pull-down kit was from Cytoskeleton Co. (Germany). Trizol kit was from Life Technologies Invitrogen (USA). MasterAmp™ High Fidelity RT-PCR kit was from Epicentre Co. (USA).

2.5. Blood pressure and hemodynamic measurements

Systolic blood pressure (SBP) was measured weekly in all rats using tail-cuff method [11].

After three weeks of drug intervention, hemodynamic studies were performed using tube methods as previously described [12]. Briefly, after each rat was anesthetized by intraperitoneal injection of chloral hydrate (400 mg/kg body weight), a polyethylene tube (PE50, Becton-Dickinson) was introduced *via* the right carotid artery. After balancing with atmospheric pressure, the mean arterial pressure (MAP) was recorded with a biosignal system (MedLab 6.0 polygraph, Nanjing Medease, China) *via* a pressure transducer. Then the tube was further advanced into left ventricle to detect left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), heart rate (HR), the maximum velocity of ascending and descending in intraventricular pressure (±dp/dt max). Values were determined by averaging the measurements from stable cardiac cycles for at least 3 min.

2.6. Echocardiography

Rats received echocardiography before and at the end of the experiment.

A Sonos 5500 echocardiographic system (Philips Medical Systems, USA) equipped with a 10 MHz transducer was used to obtain images. Rats were lightly sedated with an intraperitoneal injection of chloral hydrate (400 mg/kg body weight) to achieve semiconscious sedation. Five minutes later, 2-D parasternal long-axis images were acquired with the rats in the left lateral decubitus position. M-mode was used for measurements of interventricular septum wall thickness at end-diastole (IVSd), left ventricular internal diameter at end-diastole (LVIDd), left ventricular posterior wall thickness at end-diastole (LVPWd), interventricular septum wall thickness at end-systole (IVSs), left ventricular internal diameter at end-systole (LVIDs) and left ventricular posterior wall thickness at end-systole (LVPWs). The left ventricular ejection fraction (EF) was calculated using the area-length method, and cardiac output (CO) was calculated by echocardiographic system. Relative wall thickness (RWT) was calculated according to the formula: 2 * (LVPWd / LVIDd) [13]. LV geometry was identified into four patterns based on RWT and LVMI according to Ganau's report [13]: normal (normal RWT and normal LVMI), concentric remodeling (increased RWT and normal LVMI), concentric LV hypertrophy (increased RWT and increased LVMI) and eccentric LV hypertrophy (normal RWT and increased LVMI).

Values were determined by averaging the measurements from three consecutive beats. The echo reader was blinded to the treatment groups. Intraobserver variability was expressed as mean percent error [14].

2.7. Lipid measurements

At the end of the hemodynamic studies, a 2 ml of blood samples was taken, the serum was isolated, and the levels of triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL), and high density lipoprotein (HDL) were detected by automatic