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PPAR-γ is involved in the protective effect of 2,3,4',5-tetrahydroxystilbene-2-O-beta-D-glucoside against cardiac fibrosis in pressure-overloaded rats

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ABSTRACT

2, 3, 4′, 5-tetrahydroxystilbene-2–0-β-D glucoside (TSG) could inhibit cardiac remodeling in response to pressure overload. Peroxisome proliferator-activated receptor gamma (PPAR-γ) has been recognized as a potent, endogenous antifibrotic factor and maintaining a proper expression level in myocardium is necessary for assuring that structure and function of heart adapt to pressure overload stress. The aim of the present study was to investigate whether PPAR-γ is involved in the beneficial effect of TSG on pressure overload-induced cardiac fibrosis. TSG (120 mg/kg/day) or TSG (120 mg/kg/day) plus the PPAR-γ antagonist GW9662 (1 mg/kg/day) was administered to rats with pressure overload induced by abdominal aortic banding. 30 days later, pressure overload-induced *hypertension,* cardiac dysfunction and fibrosis were significantly inhibited by TSG. TSG also significantly reduced collagen I, collagen III, *fibronectin* and plasminogen activator inhibitor (PAI) – 1 expression, as makers of myocardial fibrosis. Theses anti-fibrotic effects of TSG in pressure overloaded hearts could be abrogated by co-treatment with GW9662. Accordingly, upregulated PPAR-γ protein expression by TSG in pressure overloaded hearts was also reversed by co-treatment with GW9662. Additionally, the inhibitory effects of TSG on angiotensin II induced cardiac fibroblasts proliferation, differentiation and expression of collagen I and III, *fibronectin* and PAI-1 were abrogated by PPAR-γ antagonist GW9662 and PPAR-γ silencing. Furthermore, TSG directly increased PPAR-γ gene expression at gene promoter, mRNA and protein level in angiotensin II-treated cardiac fibroblats in vitro. Our results suggested that upregualtion of endogenous PPAR-γ expression by TSG may be involved in its beneficial effect on pressure overload-induced cardiac fibrosis.

1. Introduction

As a common pathogenic factor, cardiac fibrosis occurs in a wide variety of chronic and acute cardiovascular disorders that are associated with impaired cardiac function and myocardial stiffness. It is characterized by activation of cardiac fibroblasts and abnormal extracellular matrix (ECM) proteins production and deposition, resulting in cardiac stiffness, cardiac remodeling, and eventual heart failure (Kong et al., 2014). Therefore, inhibiting cardiac fibrosis is an important strategy for prevention of heart disease in clinical practice.

Peroxisome proliferator-activated receptor gamma (PPAR-γ) belongs to the nuclear hormone receptor superfamily. It has been implicated in the regulation of a variety of biological processes within the cardiovascular system, including cardiac hypertrophic, fibrotic and inflammatory responses to hemodynamic stress (Takano and Komuro, 2009). For example, compared with wild-type mice, pressure overload induced greater cardiac hypertrophy in heterozygous PPAR-γ-deficient mice (Asakawa et al., 2002). Many preclinical and clinical studies have demonstrated that PPAR-γ agonist such as the thiazolidinediones has been shown to prevent cardiac hypertrophy and fibrosis caused by myocardial infarction, hypertension, pressure overload or diabetes in addition to their well described insulin-sensitizing properties (Geng et al., 2006; Qi et al., 2015; Sakamoto et al., 2013; Zhao et al., 2010). Both overexpression of PPAR-γ and pharmacological activation were also proved to inhibit proliferation, myofibroblast transformation and ECM overproduction in isolated cardiac fibroblasts in response to angiotensin II or transforming growth factor-β (TGF-β) treatment (Gong et al., 2011; Hao et al., 2008; Hou et al., 2013). Collagen synthesis was significantly increased in isolated mouse embryonic fibroblasts lacking PPAR-gamma (Ghosh et al., 2008). Taken together, these findings indicate that PPAR-γ has an antifibrotic effect and maximizing the potential benefits of endogenous PPAR-γ

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activation in heart could be an effective *approach* for prevention of cardiac fibrosis (Liu et al., 2016).

2,3,4',5-Tetrahydroxystilbene-2-O-beta-D-glucoside (TSG), the main active component extracted from *Polygonum multiflorum,* a widely used traditional Chinese herbal medicine for the prevention and treatment of various diseases, has received a great deal of attention for its beneficial effects on the cardiovascular diseases, such as inhibiting the atherosclerotic process (Yao et al., 2013; Yao et al., 2015), suppression of doxorubicin-induced cardiotoxicity (Zhang et al., 2009), improvement of aortic remodeling in spontaneously hypertensive rats (Duan et al., 2015). Additionally, our previous study revealed that TSG could prevent pressure overload induced cardiac remodeling in rats (Xu et al., 2014). Recently, several documents reported that upregulation of PPAR-γ expression by TSG contributes to its protective effects on acetic acid-induced experimental colitis and diabetic gastrointestinal disorders (Chang et al., 2012; Zeng et al., 2011). Given the important role of PPAR-γ in process of cardiac fibrosis, we hypothesize that PPAR-γ may be involved in the beneficial effect of TSG on cardiac remodeling. Therefore, the aim of the present study was to investigate the relationship between TSG and PPAR-γ in terms of antifibrotic effect in pressure overloaded rat hearts in vivo and angiotensin II-induced cardiac fibrosis in vitro.

2. Materials and methods

2.1. Materials

TSG (purity above 95%) was obtained from the Traditional Chinese Medicine Laboratory of Nantong University (Nantong, China). Angiotensin II was purchased from Sigma-Aldrich Co. (St. Louis, USA). GW9662 was obtained from Enzo Biochem (New York, USA). Antibodies against collagen I, III were obtained from Proteintech Group, Inc. (Chicago, USA). Antibodies against *fibronectin* and α-smooth muscle actin (α-SMA) were purchased from Abcam (MA, USA). The antibodies against plasminogen activator inhibitor (PAI)-1 and PPAR-γ were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody against histone H_2A was obtained from Cell Singnaling Technology (Beverly, MA, USA). Antibody against β-actin was obtained from Sunshine Biotechnology (Nanjing, China).

2.2. Pressure-overload rat model

Male Sprague-Dawley rats (weight range 180–220 g) were provided from Experimental Animal Center of Nantong University. All procedures in the present study were approved by the Animal Care and Use Committee of Nantong University. Pressure overload was produced by abdominal aortic banding as described previously (Xu et al., 2014) by tying a suture around the abdominal aorta above the renal artery over a 7-gauge needle causing occlusion of the aorta. Then the needle was quickly removed, resulting in a stenotic aortic lumen. Sham-operated rats without abdominal aorta binding served as controls. The pressure overload rats were randomly divided into three groups three days following surgery: model group, model+TSG group, model+TSG+GW9662 group. In model+TSG group, pressure overloaded rats (model rats) were orally administered TSG 120 mg/kg/day for 30 days. In the model+TSG+ GW9662 group, 1 mg/kg GW9662 was added to the model rats by intraperitoneal injection at 2 h prior to the administration of TSG 120 mg/kg/day for 30 days. Meanwhile, distilled water (vehicle of TSG) and 1%DMSO (vehicle of GW9662) were administered orally and intraperitoneally for 30 days both in sham group and model group. The dosage of TSG used in the present study was determined on the basis of our previous studies (Xu et al., 2013, 2014).

For all rats, the blood pressure was measured at 0, 3, 10, 20 and 33 days post-surgery with a tail-cuff system (BP-2000; *Visitech* Systems, USA). 30 days after drug administration, rats were anaesthetized with 40 mg/kg pentobarbital sodium intraperitoneally for cardiac echocardiography. Philips IE33 echocardiography system (20 Hz probe, Netherlands) was used to measure left ventricle internal diameter at end-systole and diastole (LVIDs and LVIDd), left ventricle end-systolic and diastolic volumes (ESV, EDV), thickness of interventricular septum (IVST) and left ventricular posterior wall (LVPWT), fractional shortening (FS) and ejection fraction (EF). Then hearts of the rats were harvested and weighed in order to calculate the heart to body weight ratio.

2.3. Masson's trichrome staining

Paraffin-embedded left ventricles were cut into 5 μm thick sections, subjected to Masson's trichrome to detect *blue collagen* deposition using a quantitative digital image analysis system (Image Pro-Plus, version 6.0, USA). Five vascular and non-vascular areas within each direction of slide were randomly selected. The percentage of collagen area in each field and the ratio of perivascular collagen to vessel lumen area were calculated as the myocardial interstitial collagen volume fraction (ICVF) and perivascular collagen volume fraction (PCVF) respectively.

2.4. Measurement of hydroxyproline

The *myocardial hydroxyproline content* was detected by a colorimetric method in accordance with the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

2.5. Preparation of cardiac fibroblasts

Cardiac fibroblasts were isolated from 1- to 3-D-old neonatal Sprague-Dawley rat hearts as described previously (Chen et al., 2014). Cardiac fibroblasts at the second or third passages were used in the present experiments. At 80% confluence, cardiac fibroblasts were placed in serum-free media for 24 h before use.

2.6. Cell proliferation assay

Cell proliferation was measured by 5-bromodeoxyuridine (BrdU)-incorporation assay using a cell proliferation elisa kit (Roche Diagnostics, Mannheim, Germany) to determined DNA synthesis as described previously (Xu et al., 2015).

2.7. Immunofluorescence staining

Immunofluorescent staining was performed on cardiac fibroblasts to detect α-SMA expression. Cultured cardiac fibroblasts were fixed for 10 min in 4% paraformaldehyde followed by permeabilization with ice-cold 0.3% Triton X100 for 10 min. After being blocked with goat serum, cells were incubated with primary antibody against α-SMA overnight at 4 °C followed by Cy3-conjugated anti-mouse IgG for 1 h. After washing with PBS three times, the cells were incubated with DAPI for another 1 h and then examined under a fluorescence microscope.

2.8. Transient transfection with siRNAs

PPARγ siRNA and scrambled control siRNA were obtained from GenePharma RNAi Company (Shanghai, China). The PPAR-γ siRNA were: sense 5′-CCAUCCGAUUGAAGCUUAUTT-3′ and antisense 5′-AUAAGCUUCAAUCGGAUGGTT -3′. siRNAs were reversely transfected into cardiac fibroblasts using Lipofectamine 2000 according to manufacturer's guidelines.