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Polydatin prevents hypertrophy in phenylephrine induced neonatal mouse cardiomyocytes and pressure-overload mouse models

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ABSTRACT

Recent evidence suggests that polydatin (PD), a resveratrol glucoside, may have beneficial actions on the cardiac hypertrophy. Therefore, the current study focused on the underlying mechanism of the PD anti-hypertrophic effect in cultured cardiomyocytes and in progression from cardiac hypertrophy to heart failure in vivo.

Experiments were performed on cultured neonatal rat, ventricular myocytes as well as adult mice subjected to transverse aortic constriction (TAC). Treatment of cardiomyocytes with phenylephrine for three days produced a marked hypertrophic effect as evidenced by significantly increased cell surface area and atrial natriuretic peptide (ANP) protein expression. These effects were attenuated by PD in a concentration-dependent manner with a complete inhibition of hypertrophy at the concentration of 50 μM. Phenylephrine increased ROCK activity, as well as intracellular reactive oxygen species production and lipid peroxidation. The oxidizing agent DTDP similarly increased Rho kinase (ROCK) activity and induced hypertrophic remodeling. PD treatment inhibited phenylephrine-induced oxidative stress and consequently suppressed ROCK activation in cardiomyocytes. Hypertrophic remodeling and heart failure were demonstrated in mice subjected to 13 weeks of TAC. Upregulation of ROCK signaling pathway was also evident in TAC mice. PD treatment significantly attenuated the increased ROCK activity, associated with a markedly reduced hypertrophic response and improved cardiac function.

Our results demonstrated a robust anti-hypertrophic remodeling effect of polydatin, which is mediated by inhibition of reactive oxygen species dependent ROCK activation.

1. Introduction

Cardiac hypertrophy is one of the leading causes of increased cardiac morbidity and mortality since it can result in myocardial ischemia, arrhythmia, sudden death and, eventually, heart failure (Levy et al., 1990). Many natural products have been used on heart failure therapy. Polydatin (PD) is a monocrystalline drug that can be isolated from a traditional Chinese herb (*Polygonum cuspidatum*). The molecular composition of PD is 3, 4′, 5-trihydroxystibene-3-monoglucoside, which is akin to the polyphenol resveratrol (Res) (3, 4′, 5-trihydroxystibene). PD has also been found to be one of the major stilbenoid compounds in red wine. Similar to its analog resveratrol, PD has multiple biological activities in a cardiovascular and hematological system. The cardioprotection of PD may be related to activating cNOS, leading to an increase in NO production (Zhang et al., 2008) and decrease in apoptosis (Zhang et al., 2009). It can enhance heart function, improve microcirculatory perfusion in shock (Zhao et al., 2003) and survival rate in severe shock (Wang et al., 2013). It has shown that PD can cross the blood–brain barrier to protect brain tissues from ischemia–reperfusion injury or cerebral ischemia (Cheng et al., 2006; Su and Hsieh, 2011). Most of the studies thus far have focused on the beneficial effects of PD in the prevention of ischemia–reperfusion injuries and few concerns of its possible use as a therapeutic drug in hypertrophy. The only study of Gao et al. (2010) has firstly studied polydatin on pressure-overload rat models. However, the underlying mechanism of the beneficial effect is not completely clear.

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Rho-kinase (ROCK), a target protein of the small GTP-binding protein Rho, has been shown to be involved in a number of models of cardiac hypertrophy (Hamid et al., 2007; Phrommintikul et al., 2008; Zeidan et al., 2006) and it can be activated by a variety of stimuli including angiotensin II (Aikawa et al., 2000), leptin (Zeidan et al., 2006), and stretch (Pan et al., 2005). Upon activation by hypertrophic stimuli, RhoA signals through downstream kinases (ROCK) to phosphorylate and inhibit the actin depolymerizing protein cofilin, thereby increasing filamentous actin. Of interest, pharmacological inhibition of RhoA (C3 exoenzyme), ROCK (Y-27632), or actin polymerization (latrunculin B) is sufficient to prevent cardiac hypertrophy induced by a variety of stimuli (Aikawa et al., 2000; Zeidan et al., 2006). Although the pro-hypertrophic mechanism of RhoA/ROCK pathway signaling remains unclear, it has been suggested that actin reorganization may signal via p38 MAPK and serum response factor to mediate hypertrophy (Kuwahara et al., 2007; Zeidan et al., 2008). Thus, these data suggest that the RhoA/ROCK signaling cascade may be a final common pathway to mediate the development of cardiac hypertrophy and heart failure and as such represents a logical target for therapeutic intervention.

Evidences indicate that ROCK signaling pathway is activated by reactive oxygen species. Given that PD has prominent antioxidant activity, we speculated that PD may prevent the pathogenesis of hypertrophy by inhibiting ROCK activity via suppressing intracellular oxidative stress. The goal of the present study was to investigate the possible mechanisms of polydatin on ventricular remodeling induced by phenylephrine in neonatal rat cardiomyocytes and by transverse aortic constriction in mice. Here, we demonstrated a robust antihypertrophic and antiremodeling effect of polydatin, which is mediated by inhibition of reactive oxygen species dependent ROCK activation.

2. Materials and methods

2.1. Drugs and reagents

Polydatin (with a purity of 98.87%) was supplied by Haiwang Co. (Shenzhen, Guangdong, China). The antioxidant MnTBAP (Manganese (III) Tetrakis (4-Benzoic Acid) Porphyrin) was from Biochemical Santa Cruz (MnTBAP chloride sc-221954A) and oxidizing agent DTDP (2,2′-dithiodipyridine) was from Sigma (Sigma 43791). L-NAME (NO inhibitor) was from Sigma. Because ROCK inactivates myosin phosphatase through the specific phosphorylation of myosin phosphatase target subunit 1 (MBS or MYPT1) atThr-696, which results in an increase in the phosphorylated content of the 20-kDa myosin light chain (MLC20) in this study, we used the ratio of pMBS to MBS to represent the ROCK activity which is the most widely used method to test ROCK activity. Primary monoclonal antibodies for western blots and immunostaining were anti-myosin phosphatase target subunit-1 (MYPT-1), phosphor T853-MYPT1 and atrial natriuretic peptide (ANP) from Santa Cruz Biotechnology (USA, all diluted: 1:1000) and β-actin from Sigma. Masson's trichrome kit was from Sigma HT15-1KT. RhoA activity assay kit (ab173237) was from USA. Briefly, the anti-active RhoA mouse monoclonal antibody was incubated with cell lysates containing RhoA-GTP. The bound active RhoA was pulled down by protein A/G agarose and the precipitated active RhoA was detected by immunoblot analysis using anti-RhoA rabbit polyclonal antibody. The ratio of active RhoA/total RhoA protein represented the activation of RhoA (Liu et al., 2007).

2.2. Cell culture

All animal experiments were performed in accordance with ethical standards as formulated in the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication 85-23, revised 1996) and approved by the Institutional Animal Care and Use Committee of Shenzhen University. Neonatal cardiomyocytes were isolated and cultured using previously described methods (Liao et al., 2012). The ventricular heart from 1-day-old neonatal rats was removed, cut into small chunks and washed with Hanks' balanced salt solution (HBSS). Then, the tissue was incubated in 4 ml trypsin/EDTA solution (GIBCO, Carlsbad, CA) at 4 °C for 30 min with rotation. The digestion was stopped by addition of 6 ml DMEM containing 10% fetal calf serum (FCS, GIBCO, Carlsbad, CA). After centrifugation at 200*g* for 5 min, the supernatant was removed, and the tissues were incubated in 4 ml Liberase TH (0.1 U/ml in HBSS, Roche Diagnostics GmbH, Mannheim, Germany) at 37 °C for 15 min. The supernatant containing the released cells to DMEM-10% FCS was removed, and fresh Liberase TH was added to the undigested tissues, which were then incubated for a further 15 min. This digestion procedure was repeated until most of the cells had been released from ventricular tissue and the obtained cells were resuspended in DMEM. All collected cells were seeded into fibronectin-coated 12-well tissue culture plates (Costar; Corning, NY). After 1.5 h of incubation with 5% CO_2 at 37 °C, the attached fibroblasts were discarded and cardiomyocytes in the supernatant were enriched and seeded into fibronectin-coated tissue culture plates after cell concentration was adjusted. Cardiomyocytes were used in experiments when they had formed a confluent monolayer and beat in synchrony at 72 h. To initiate hypertrophy, myocytes were then treated with10 μM adrenoceptor agonist phenylephrine and then in the absence or presence of polydatin (30, 40, or 50 μM).

2.3. Measurement of cell surface area

Myocytes were visualized using a Leica DMIL inverted microscope (Leica, Wetzlar, Germany) equipped with an Infinity 1 camera. At least 10 random photographs were taken from each dish, and the cell surface area of a minimum of 30 cells from each treatment was measured using SigmaScan Software (Systat, Richmond, CA).

2.4. Western blotting

Total protein was measured with a bicinchoninic acid assay kit (Pierce) after sonication of the harvested cells. Various protein components were separated in 7.5% polyacrylamide gels, transferred onto nitrocellulose paper, and stained with primary and secondary antibodies. Reactive bands were visualized by the Supersignal ECL Western blotting detection kit (Pierce), and densitometry was obtained in ImageJ software.

2.5. Fluorescence of intracellular reactive oxygen species and measurement of malondialdehyde (MDA)

In neonatal rat cardiomyocytes a fluorescentprobe,5-(and-6)-chloro-methyldichlorodihydrofluorescein diacetate (DCFDA; Molecular Probes) were used for the assessment of intracellular reactive oxygen species formation as described previously (Jiang et al., 2013). Briefly, cells were loaded with10 μM DCFDA for 30 min at room temperature followed by 15 min for de-esterification. Frame fluorescence images (excitation at 488 nm and emission at 505–530 nm, laser intensity 4%, 6.6 s/frame) were acquired with a Zeiss510 inverted confocal microscope with 20 × lens. Each experiment was performed within 5 min after15 min for de-esterification of DCFDA. The MDA levels were determined using colorimetric assays with commercial kits following the manufacturer's instruction (Nanjing Jiancheng Biotechnology Institute, China). L-NAME was treated as 100 μM for 30 min before MDA testing.