Rosuvastatin prevents pressure overload-induced myocardial hypertrophy via inactivation of the Akt, ERK1/2 and GATA4 signaling pathways in rats

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Received January 28, 2013; Accepted May 29, 2013

DOI: 10.3892/mmr.2013.1548

Abstract. Pressure overload-induced myocardial hypertrophy is associated with a poor prognosis in humans and contributes to the development of cardiac arrhythmias, diastolic dysfunction and ultimate congestive heart failure. 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, also known as statins, have been previously shown to induce regression of myocardial hypertrophy in aortic banding models. However, there is limited knowledge regarding the underlying molecular mechanisms. Therefore, we hypothesized that the myocardial hypertrophy-related signaling pathways protein kinase B (Akt), extracellular signal-regulated kinases 1 or 2 (ERK1/2) and GATA binding protein 4 (GATA4) activation pathways are involved in the pressure overload-induced myocardial hypertrophy treated by RSV. Twenty-eight Wistar rats were randomly allocated into 4 groups: the sham operation-vehicle (SH-V), abdominal aortic constriction-vehicle (AAC-V), abdominal aortic constriction-RSV 10 mg/kg/day (AAC-LO) and the abdominal aortic constriction-RSV 20 mg/kg/day (AAC-HI) group. Following the establishment of the abdominal aorta constriction model, we investigated the effect of RSV, a new hydrophilic statin, on abdominal aortic constriction-induced myocardial hypertrophy as well as the underlying intercellular signaling pathways after 5 days and 4 weeks of drug intervention. Moreover, echocardiographic features and the left ventricular weight to final body weight ratio (LVW/BW) were determined. Cross-sectional areas (CSAs) of cardiomyocytes were assessed by hematoxylin and eosin (H&E) staining. Atrial natriuretic factor (ANF), β-myosin heavy chain (β-MHC) and peroxisome proliferator-activated receptor α (PPARα) messenger RNA (mRNA) expression was assessed using RT-PCR. The phosphorylation of Akt, ERK1/2 and GATA4 were also examined using western blot analysis. Our results showed that RSV significantly attenuates pressure overload-induced myocardial hypertrophy by preventing myocardial hypertrophy-related activation of Akt, ERK1/2 and GATA4 signaling pathways.

Introduction

Chronic pressure overload induces myocardial hypertrophy, also called ‘pathological’ cardiac hypertrophy, which is compensatory. Excessive hypertrophy, however, is associated with significantly poor prognosis including increased cardiac morbidity, mortality and ultimate heart failure in humans (1,2). 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, also known as statins, exert a lipid-lowering effect in the blood which has been extensively investigated (3). Additionally, recent experimental evidence indicates that certain statins restore or improve endothelial function (4), decrease smooth muscle cell (SMC) content and collagen accumulation in atherosclerotic plaques (5), play an anti-inflammatory role associated with acute coronary events (6) and activate the expression of peroxisome proliferator-activated receptors (PPARs) (7,8). Moreover, according to previous studies, statins have been shown to attenuate cardiac hypertrophy (9) and to delay the progression from cardiac hypertrophy to failure under pressure overload in aortic banding models (4,10). Additional evidence suggests that phosphorylated Akt/glycogen synthase kinase 3β (GSK3β), extracellular signal-regulated kinases 1 or 2 (ERK1/2) and GATA binding protein 4 (GATA4) pathways play important roles in the process of cardiac hypertrophy and ventricular remodeling induced by pressure overload (11-13). However, the molecular mechanisms of action of rosuvastatin (RSV), a hydrophilic statin which is involved in the hypertrophic response to pressure overload, remains to be fully elucidated in vivo. Therefore, we hypothesized that statins inhibit cardiac hypertrophy through the inhibition of numerous activation signaling pathways differentially associated to myocardial hypertrophy.

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Key words: rosuvastatin, myocardial hypertrophy, pressure overload, Akt, extracellular signal-regulated kinase 1/2, GATA binding protein 4
hypertrophy, such as the Akt, ERK1/2 and GATA4 signaling pathways.

The present study was divided into two major parts. We firstly investigated the transition from normal to hypertrophic myocardium and evaluated the effects of RSV on rats subjected to abdominal aortic constriction. In the second part of this study, we investigated the variation of activation pathways including Akt, ERK1/2 and GATA4 in four groups. We also explored whether RSV could reverse myocardial hypertrophy of the remodeling process following long-term treatment.

Materials and methods

Reagents. RSV was kindly provided by AstraZeneca (London, UK). Primary antibodies for total ERK1/2, AKT and their phosphorylated forms were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). GATA4 and its phosphorylated forms were purchased from Abcam (Cambridge, MA, USA). Rabbit anti-glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH) antibody was provided by Hangzhou Goodhere Biotechnology Co., Ltd. (Hangzhou, China). Secondary horseradish peroxidase (HRP)-conjugated anti-rabbit antibody was ordered from Multisciences Biotech Co., Ltd. (Hangzhou, China). Enhanced chemiluminescence (ECL) reagent was obtained from Millipore (Billerica, MA, USA). All the reagents were used according to the manufacturer’s instructions.

Animals. Male Wistar rats were supplied by the Novel Pharmaceuticals Research Center of Shandong University (Jinan, Shandong, China). The study was approved by the ethics committee of Shandong Provincial Qianfoshan Hospital, Affiliated Hospital of Shandong University, Jinan, China and the protocols were in compliance with the Guidelines for the Care and Use of Laboratory Animals published by the National Academy Press (NIH publication no. 85-23, revised 1996).

Animal experimental protocols. Twenty-eight male Wistar rats, weighing 180-220 g, were allowed free access to food and water, and were maintained on a 12-h light/dark cycle at room temperature (21-23°C). When the 28 male Wistar rats weighed 300±10 g, they were randomly allocated into 4 groups: the sham operation-vehicle (SH-V, n=7), abdominal aortic constriction-vehicle (AAC-V, n=7), abdominal aortic constriction-RSV 10 mg/kg/day (AAC-LO, n=7) and the abdominal aortic constriction-RSV 20 mg/kg/day (AAC-HI, n=7) group. RSV was dissolved in stroke-physiological saline solution daily; the freshly prepared RSV solution was not preserved for >30 min prior to gavage. Five days prior to aortic constriction, the rats of the AAC-LO and AAC-HI groups were administrated various doses of RSV, while the rats of the SH-V and AAC-V groups were administrated an equal volume of vehicle by a lavage needle, until 4 weeks after surgery. Drug or vehicle were administrated once daily during the entire experimental period. The rats underwent aortic constriction or sham operation 5 days after initiation of gavage and were sacrificed 4 weeks following surgery. Although higher doses of RSV (30 and 50 mg/kg/day) were also administrated, male Wistar rats did not suffer any side-effects, such as intestinal obstruction of the drug, during the the experiment.

Abdominal aortic constriction model. Abdominal aortic constriction-induced pressure overload has been previously described (14). The rats were anesthetized with 10% chloral hydrate [0.3 ml/100 g, intraperitoneal (i.p.) injection]. The adequacy of anesthesia was measured by assessing cardiac and respiratory rates as well as pattern, lash reflex and muscle relaxation. Following asepsis, abdominal aorta was exposed through a ventral median line incision. Subsequently, ligatures were placed around both the abdominal aortas (0.5 cm above renal artery bifurcation) and an obtuse 22-gauge needle (outside diameter, 0.7 mm) with a 4-0 silk suture. The needle was promptly removed following constriction. The rats of the SH-V group underwent abdominal aortic constriction without ligation.

Echocardiographic evaluation. All the rats were anesthetized with 10% chloral hydrate (0.3 ml/100 g, i.p.) and echocardiography was performed to evaluate left ventricular function 4 weeks following the operation (15). Left parasternal short-axis views of the left ventricle were obtained using Sonos 5500 Ultrasound Machine with a S12 Pediatric Sector Probe at 10 MHz (Hewlett-Packard Development Company, L.P., Palo Alto, CA, USA), at the level of the papillary muscles. Dimensions of end diastolic interventricular septum (IVSd), diastolic left ventricular posterior wall (LVPWd) and left ventricular ejection fraction (LVEF) were noninvasively measured. Three consecutive cycles of measurements were performed and the results were averaged (16). Echocardiography and the measurement procedure were performed in a double-blind manner.

Body weight and cardiac characteristics. All of the rats were precisely weighed prior to echocardiography and the weight recorded was considered as the terminal body weight. The rats were perfused with stroke-physiological saline solution through the left ventricle immediately after echocardiography was performed and were then sacrificed by decapitation. The hearts of decollated rats were carefully excised and washed in double distilled water. The left ventricle including the septum was carefully clipped from the atrium and the right ventricle, and then weighed. The ratio of the left ventricular weight to body weight (LVW/BW) was described as a relative ratio.

Hematoxylin and eosin (H&E) staining. For the investigation of the myocyte cross-sectional area (CSA) of the different groups of rats, the heart tissues were stained with H&E. The cross-sections of the left ventricle of the rats in all 4 groups were submerged in 4% paraformaldehyde solution for fixation and then embedded in wax. They were transversely cut into 4-µm slices and the cross-sections were selected adequately. The slices were dehydrated through a series of graded alcohols (100, 95 and 75%), 15 min for each process. The slices were then stained with Mayer’s hematoxylin for 10-15 min and washed under running tap water for 5-10 min. Each slice was submerged in warm water until it appeared bright purple and then counterstained in eosin solution for 2-3 min. After gently washing with water, each slice was submerged in 85% alcohol, 100% alcohol I and II for 15 min each. Finally, all of the slices were submerged in Xylene I-Xylene II, for 15 min each. Photomicrographs were obtained using an Olympus
FSX100 microscope (magnification, x30; Tokyo, Japan) under the same conditions. The surplus tissues were stored at -80°C for subsequent molecular biology research.

Histopathological characteristics. Myocyte CSAs in epicardial, midwall and endocardial regions were randomly selected once for each location in the rats of all 4 groups. In each selected location, a visual field of ≥30-50 cardiomyocytes was observed. Thus, ≥100 cardiomyocytes were observed in all the visual fields in each sample of heart tissue. The myocyte CSA (magnification, x30) was investigated using the Image-Pro Plus software (Media Cybernetics, Carlsbad, CA, USA).

RT-PCR of cellular RNA. Total cellular RNA was extracted and purified from freezing left ventricular tissues of rats using TRIzol reagent (TransGen Biotech, Beijing, China). Primers for atrial natriuretic factor (ANF), β-myosin heavy chain (β-MHC) and peroxisome proliferator-activated receptor α (PPARα) were designed and synthesized by Sangon Biotechnology (Shanghai, China; Table I).

Oligo(dT)-primed RNA (2 µg) was reverse transcribed to cDNA using a ReverTra Ace® qPCR RT kit (Toyobo Co., Ltd., Life Sciences Department, Osaka, Japan) in a volume system of 20 µl. As a template for PCR, cDNA was amplified with a Taq PCR Master mix (CWBio, Beijing, China) using a Gene-Pro™ PCR Thermal Cycler. The thermocycling conditions were as follows: PCR products were predegenerated at 94°C for 2 min, followed by 30 cycles of 30 sec at 94°C, 30 sec at different annealing temperatures (60°C for ANF, β-MHC and GAPDH, 62°C for PPARα), then 30 sec at 72°C, and finally a 2-min cycle at 72°C. Amplified PCR products were electrophoresed on 2% agarose gels stained with ethidium bromide; the agarose gels were then visualized with a UV illuminator (Tiangen Biotech, Inc., Beijing, China). To quantify the relative density of each DNA band, all of the gel images were analyzed using Image software (NIH Image, Madison, WI, USA). GAPDH was used as an internal control. The band intensities were measured and normalized to the intensity of the respective GAPDH signal. Three independent experiments were performed in each group.

Western blot analysis. Total Akt, total ERK1/2 and their phosphorylated proteins (activation forms) were obtained using RIPA Lysis Buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, sodium orthovanadate, sodium fluoride, EDTA, leupeptin) containing 1 mM phenylmethanesulfonyl fluoride (PMSF). GATA4 and its phosphorylated protein (activation form) were extracted from the nuclei of cardiomyocytes using the Nuclear and Cytoplasmic Protein Extraction kit (Beyotime Institute of Biotechnology, Haimen, China). Protein concentrations were determined by the BCA protein assay (Beyotime Institute of Biotechnology). Equivalent amounts of protein samples (20 µg) from homogenates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at a 12% acrylamide resolving gel. Separated target proteins were transferred onto polyvinylidene difluoride membranes (Millipore). The membranes were blocked with 5% bovine serum albumin (BSA) in TBST buffer (20 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween-20) for 2 h at room temperature. The membranes were then probed with primary antibodies against ERK1/2, phospho-ERK1/2 (Thr202/Tyr204), Akt, phospho-Akt (Ser473) (dilution, 1:1,000), GATA4, phospho-GATA4 (Ser105) (dilution, 1 µg/ml) and GAPDH (dilution, 1:1,000) followed by incubation with the proper secondary horseradish peroxidase (HRP)-conjugated antibodies (dilution, 1:5,000). Subsequently, the membranes were washed with TBST thrice at room temperature, each for 5 min. The reactive proteins were visualized using enhanced chemiluminescence (ECL) reagent. The intensity of the bands was analysed using ImageJ software (NIH Image) and the relative expression levels of proteins were determined using proportionality of target proteins and GAPDH.

Statistical analysis. The results are presented as the mean ± SEM. Comparisons between groups were conducted using one-way analysis of variance (ANOVA) followed by Bonferroni’s post hoc test. P<0.05 and P<0.01 were considered to indicate statistically significant and highly significant differences, respectively, for all the analyses. All of the statistical analyses were conducted with SPSS 17.0 statistical software package (SPSS, Inc., Chicago, IL, USA).

Results

Echocardiographic analysis. Echocardiographic analysis indicated an increased thickness of IVSd (2.31±0.12 mm vs. 3.14±0.39 mg, P<0.01) and LVPWd (2.15±0.07 mm vs. 1.68±0.09 mm, P<0.01) in vehicle-treated banded rats compared with rats in the sham-operated group 4 weeks following surgery. Treatment with RSV at a low and high dose resulted in decreased IVSd (1.95±0.08 mm vs. 2.14±0.07 mm, P<0.01) compared with rats in the untreated aortic banding group 4 weeks following surgery. Among the rats with aortic banding, LVEF evaluated by transthoracic echocardiography was found to be significantly decreased compared with the sham-operated rats (69.41±2.85% vs. 91.49±2.18%, P<0.01). Additionally, LVEF of heart tissues from rats in the RSV-treated aortic banding group was higher compared with vehicle-treated banded rats (85.07±1.87 and 89.83±1.11 vs. 69.41±2.85%, P<0.01) 4 weeks following surgery (Table II).

Animal characteristics (LVW/BW). LVW/BW was determined to evaluate the extent of myocardial hypertrophy. Constriction of abdominal aorta led to higher LVW/BW compared with the ratio of the sham-operated rats (3.14±0.39 vs. 1.93±0.17 mg/g, P<0.01) 4 weeks following surgery. A significantly decreased LVW/BW was observed in RSV-treated aortic banding rats when compared with the ratio of the vehicle-treated banded rats (2.36±0.55 vs. 3.14±0.39 mg/g, P<0.05 and 2.15±0.06 vs. 3.14±0.39 mg/g, P<0.01) 4 weeks following surgery.

Assessment of cardiomyocyte CSA. As expected, following histological analysis, a 406% increase in myocyte CSA of the AAC-V rats was observed compared with the CSA of SH-V rats 4 weeks following surgery. The CSA of RSV-treated banded rats treated with low and high RSV doses was decreased by ~39 and 66%, respectively, compared with the CSA of the AAC-V rats 4 weeks following surgery (Fig. 1).
ANF, β-MHC and PPARα mRNA expression. In addition to the morphological changes, ANF and β-MHC mRNA expression levels as hypertrophic markers were significantly increased in rats with aortic banding compared with sham-operated rats (ANF/GAPDH, 1.09±0.08 vs. 0.26±0.05; P<0.01 and β-MHC/GAPDH, 1.19±0.07 vs. 0.32±0.02; P<0.01). However, PPARα expression was decreased in aortic banded rats compared with the sham-operated rats (PPARα/GAPDH, 0.60±0.07 vs. 1.21±0.03; P<0.01). Furthermore, ANF and β-MHC expression levels were decreased according to the RSV doses used in banded rats compared with vehicle-treated rats (ANF/GAPDH, 0.73±0.04 and 0.53±0.03 vs. 1.09±0.08; β-MHC/GAPDH, 0.63±0.04 and 0.46±0.03 vs. 1.19±0.07; P<0.01), in contrast with PPARα mRNA expression, which was significantly increased (PPARα/GAPDH, 0.99±0.04 and 1.02±0.04 vs. 0.60±0.07; P<0.01). No significant differences in PPARα expression were observed between the two RSV-treated groups (P>0.05; Fig. 2).

Analysis of Akt, ERK1/2 and GATA4 protein activation. As shown in Figs. 3 and 4, quantitative total Akt and ERK1/2 protein expression (relative identities compared with GAPDH) were not significantly altered among the 4 groups (P>0.05). Phospho-Akt/Akt (p-Akt/Akt), (phospho-ERK1/2)/ERK1/2 [(p-ERK1/2)/ERK1/2] and phospho-GATA4/GATA4 (p-GATA4/GATA4) relative protein levels were significantly increased in aortic banded rats compared with the sham-vehicle rats [p-Akt/Akt, 2.40±0.06 vs. 0.74±0.05; (p-ERK1/2)/ERK1/2, 0.71±0.06 vs. 0.33±0.02; p-GATA4/GATA4, 0.30±0.03 vs. 0.06±0.02; P<0.01]. However, the relative expression of the three proteins was significantly reduced in the aortic banded rats treated with low and high doses of RSV compared with the sham-operated rats [p-Akt/Akt, 1.05±0.05 and 0.83±0.04 vs. 2.40±0.06; (p-ERK1/2)/ERK1/2, 0.45±0.06 and 0.41±0.01 vs. 0.71±0.06; p-GATA4/GATA4, 0.22±0.02 and 0.13±0.02 vs. 0.30±0.03, P<0.01].

Discussion

It is well known that ventricular hypertrophy followed by heart failure constitute familiar outcomes of abdominal aortic constriction induced by pressure overload in rats (17,18). Previous studies on this model have shown that β-adrenergic (AR) blockers and angiotensin converting enzyme inhibitors suppress myocardial hypertrophy (19,20). To the best of our knowledge, this model was used for the first time in the present study to investigate the anti-myocardial hypertrophy effect of a novel HMG-CoA reductase inhibitor, RSV, and the potential underlying molecular mechanisms of action. The beneficial effects of statins on left ventricular hypertrophy (LVH) regression in various hypertrophy models has been previously shown. Particularly, statins have been reported

Table I. Primer sequences used for the target genes and internal standard GAPDH gene in rats.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα</td>
<td>F: 5'-GGGGTCTGATAAAGGATGT-3' R: 5'-GGGGTCTGATAAAGGATGT-3'</td>
<td>145</td>
</tr>
<tr>
<td>ANF</td>
<td>F: 5'-AGGAGAGAGAGGCGGAGAAG-3' R: 5'-AGGAGAGAGAGGCGGAGAAG-3'</td>
<td>211</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: 5'-ACAGCAAGGGTGGTGGAC-3' R: 5'-ACAGCAAGGGTGGTGGAC-3'</td>
<td>256</td>
</tr>
<tr>
<td>β-MHC</td>
<td>F: 5'-TCCAGAAGAGAGAAGCTACCTT-3' R: 5'-TCCAGAAGAGAGAAGCTACCTT-3'</td>
<td>205</td>
</tr>
</tbody>
</table>

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PPARα, peroxisome proliferator-activated receptor α; ANF, atrial natriuretic factor; β-MHC, β-myosin heavy chain; F, forward; R, reverse.

Table II. Echocardiographic analysis of IVSd, LVPWd and LVEF, and measurements of LVW/BW in the rats of all groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>IVSd (mm)</th>
<th>LVPWd (mm)</th>
<th>LVEF (%)</th>
<th>LVW/BW (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH-V</td>
<td>1.73±0.09</td>
<td>1.68±0.09</td>
<td>91.5±2.2</td>
<td>1.93±0.17</td>
</tr>
<tr>
<td>AAC-V</td>
<td>2.31±0.12a</td>
<td>2.15±0.07a</td>
<td>69.4±2.8</td>
<td>3.14±0.39</td>
</tr>
<tr>
<td>AAC-LO</td>
<td>1.95±0.08a</td>
<td>1.84±0.06a</td>
<td>85.1±1.9c</td>
<td>2.36±0.05b</td>
</tr>
<tr>
<td>AAC-HI</td>
<td>1.84±0.04c</td>
<td>1.77±0.04c</td>
<td>89.9±1.1c</td>
<td>2.15±0.06c</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± SEM. *P<0.01 vs. SH-V group; **P<0.05, †P<0.01 vs. AAC-V group. IVSd, diastolic interventricular septum; LVPWd, diastolic left ventricular posterior wall; LVEF, left ventricular ejection fraction; LVW/BW, left ventricular weight to body weight ratio; SH-V, sham-operation-vehicle control group; AAC-V, abdominal aortic constriction-vehicle control group; AAC-LO, abdominal aortic constriction-RSV 10 mg/kg group; AAC-HI, abdominal aortic constriction-RSV 20 mg/kg group.
to enhance endothelial nitric oxide synthase (eNOS) expression and to inhibit NADPH oxidase activity, which has been associated with myocardial hypertrophy endothelial function and the development of myocardial hypertrophy (9,21). Statins have also been reported to inhibit myocardial hypertrophy via the ERK1/2 activation signaling pathway in spontaneously hypertensive rats (22). However, there have been controversial results regarding the effect of RSV on cardiac hypertrophy, which have been associated with the different types of animal hypertrophy models and the different experimental protocols used. RSV has been shown to inhibit cardiac hypertrophy via suppression of Gh and cardiac oxidative stress (23,24). However, Chang et al (25) showed that RSV treatment does not reverse hypertension-induced LVH; no beneficial effects on heart failure and survival were also observed (25). The underlying mechanisms of action of RSV on abdominal aortic constriction-induced myocardial hypertrophy have yet to be fully elucidated. Therefore, the Akt, ERK1/2 and GATA4 signaling pathways were investigated in the present study.

Akt is a serine/threonine protein kinase and acts as an important pathway which is involved in the regulation of heart development. Short-term Akt activation, occurring in postnatal cardiac development and trained athletes, promotes physiological hypertrophy, while long-term activation of Akt occurring in unbounded hypertension, myocardial infarction and aortic stenosis, results in pathological hypertrophy and heart failure (26). In the present study, we found that the increased phosphorylated Akt protein level is associated with cardiac hypertrophy (pathological hypertrophy) in response to pressure overload (11). Mitogen-activated protein kinases (MAPKs) are 3-tiered kinase cascades that are classified into 3 distinct subfamilies, including extracellular signal receptor-regulated kinases (ERKs), c-jun NH2-terminal kinases (JNKs) and p38 MAPK. The three MAPK pathways...
have been shown to be activated in the myocardium of mice following transverse aortic constriction (TAC) operation (12). The phosphorylated transcription factor GATA4 is required for pressure overload-induced cardiac hypertrophy in vivo (13). It has been proved that the phosphorylation of GATA4 at Ser105 directly activated by ERK1/2, improves the transcriptional activity and DNA-binding affinity of GATA4 in vivo and in vitro (27,28). The activated Akt pathway managed to enforce its hypertrophic effect via GSK3β/GATA4 phosphorylation activity in cardiac myocytes in vivo and in vitro. Activity of the Akt signal pathway is not involved with activity of the ERK1/2 signal pathway. They are two different signal pathways which lead to GATA4 activation (29,30). These data suggest that the activation of the Akt, ERK1/2 and GATA4 signaling pathways is involved in pressure overload-induced cardiac hypertrophy. Thus, we further aimed to investigate the activation of Akt, ERK1/2 and GATA4 activation signaling pathways in rats disposed by abdominal aortic constriction, and to examine
whether RSV reduces pressure overload-induced cardiac hypertrophy by preventing the activation of these molecular pathways. PPARα is a member of the nuclear receptor of ligand-activated transcription factors. Relevant studies have shown that deficiency of PPARα leads to a more significant hypertrophic growth response, suggesting that PPARα attenuates pathological cardiac remodeling induced by pressure overload. Thus, PPARα has been suggested to exert beneficial effects on cardiac hypertrophy (31).

In the present study, abdominal aortic constriction-induced pressure overload resulted in left ventricular myocardial hypertrophy due to the increase in LVW/BW, echocardiography characteristics, cardiomyocyte area and mRNA expression levels of the hypertrophy markers ANF and β-MHC. In pressure overload-induced hypertrophic hearts, phosphorylated activation of Akt, ERK1/2 and GATA4 proteins was significantly increased, which was in agreement with the results of previous studies (11,32,33). Briefly, these results showed that the phosphorylation levels of Akt, ERK1/2 and GATA4 proteins are involved in myocardial hypertrophy induced by abdominal aortic constriction. Treatment with RSV at doses of 10 and 20 mg/kg/day was shown to downregulate the phosphorylation levels of Akt, ERK1/2 and GATA4 proteins, and to upregulate PPARα mRNA expression in rat myocardial cells. Consequently, these may constitute the molecular mechanisms of regression to myocardial hypertrophy. Based on this observation, the effect of RSV on promoting cardiac function is suggested to be parallel with the effect of myocardial hypertrophy regression. RSV is suggested to reverse the development of cardiac hypertrophy by affecting the phosphorylation of Akt, ERK1/2 and GATA4 molecular activation signaling pathways in cardiomyocytes.

In conclusion, based on the significant decreases in left ventricular mass and relative cardiomyocyte area in the RSV-treated banded rats, RSV was shown to have cardiac anti-hypertrophic effects and to be involved in the maintenance of hemodynamic stability. Therefore, RSV suppresses myocardial hypertrophy induced by pressure overload. The underlying molecular mechanisms of action may be associated to the regulation of activation of Akt, ERK1/2 and GATA4 pro-hypertrophic signaling pathways. However, further studies are needed for the investigation of the association between Akt, ERK1/2 and GATA4 molecular activation signaling pathways. RSV treatment also increases the mRNA expression levels of PPARα, which is beneficial to the regression of cardiac hypertrophy. To the best of our knowledge, this is the first time that RSV has been shown to prevent and reverse cardiovascular remodelling induced by abdominal aortic constriction initiated by pressure overload. The results of the present study provide additional evidence regarding the pleiotropic effects of statins. RSV is suggested to constitute a novel drug suitable for the clinical reversal of cardiac hypertrophy. However, further studies are needed for the in-depth investigation of the role of RSV in myocardial hypertrophy.

Acknowledgements
This study was supported by the General Programs of the Natural Science Foundation of Shandong Province of China (no. ZR2010HM116).

References


