ORIGINAL ARTICLE

Vitexin protects against cardiac hypertrophy via inhibiting calcineurin and CaMKII signaling pathways

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Abstract Vitexin is a flavone glycoside isolated from the leaf of Crataegus pinnatifida Bunge, the utility of which has been demonstrated in several cardiovascular diseases. However, its role in cardiac hypertrophy remains unclear. In the present study, we aimed to determine whether vitexin prevents cardiac hypertrophy induced by isoproterenol (ISO) in cultured neonatal rat ventricular myocytes in vitro and pressure overload-induced cardiac hypertrophy in mice in vivo. The results revealed that vitexin (10, 30, and 100 μM) dose-dependently attenuated cardiac hypertrophy induced by ISO in vitro. Furthermore, vitexin (3, 10, and 30 mg kg⁻¹) prevented cardiac hypertrophy induced by transverse aortic constriction as assessed by heart weight/body weight, left ventricular weight/body weight and lung weight/body weight ratios, cardiomyocyte cross-sectional area, echocardiographic parameters, and gene expression of hypertrophic markers. Further investigation demonstrated that vitexin inhibited the increment of the resting intracellular free calcium induced by ISO. Vitexin also inhibited the expression of calcium downstream effectors calcineurin-NFATc3 and phosphorylated calmodulin kinase II (CaMKII) both in vitro and in vivo. Taken together, our results indicate that vitexin has the potential to protect against cardiac hypertrophy through Ca²⁺-mediated calcineurin-NFATc3 and CaMKII signaling pathways.

Keywords Vitexin · Cardiac hypertrophy · Calcineurin · NFAT · CaMKII

Introduction

Cardiac hypertrophy is defined as an augmentation in heart mass that occurs in response to diverse pathophysiological stimuli such as ischemic heart disease, infectious agents, valvular insufficiency, etc. (Heineke and Molkentin 2006; Oka and Komuro 2008; Selvettella and Lembo 2005). Cardiac hypertrophy is initially an adaptive response to preserve cardiac function. However, sustained hemodynamic overload will result in ventricular dilatation, contractile dysfunction, and heart failure (Gunther et al. 2010; Rohini et al. 2010). Therefore, slowing the progression of heart failure or maintaining the normal modality and function of myocardium is necessary.

Many studies have demonstrated that persistent hypertrophic stimuli increase the sustained intracellular Ca²⁺ concentration ([Ca²⁺]i) (Feron et al. 1996). Furthermore, the increases in [Ca²⁺]i activate both the Ca²⁺/calmodulin (CaM)-dependent phosphatase, calcineurin, and the Ca²⁺/CaM-dependent phosphorylated calmodulin kinase II (CaMKII). It has been reported that calcineurin and CaMKII act as an important part in modulating the development of cardiac hypertrophy (Asakawa and Komuro 2001; Saito et al. 2003). Calcineurin is a serine/threonine phosphatase, which is identified as a nodal signaling protein for the induction of cardiac hypertrophy (Montero et al. 2004; Obata et al. 2005). Once activated, calcineurin facilitates binding to its primary downstream
Effectors, nuclear factor of activated T cells (NFAT), and dephosphorylates NFAT transcription factors, which are rapidly translocated to the nucleus, inducing a robust hypertrophic response in the heart (Wilkins and Molkentin 2004; Kim et al. 2004). It has been reported that cardiac-specific activation of calcineurin is sufficient to induce a significant hypertrophic response in transgenic mice (Molkentin et al. 1998; Bernardo et al. 2010). Furthermore, it is also certified that inhibiting Ca$$^{2+}$$–calcineurin pathway is profitable for reversing the cardiac hypertrophy process (Bueno et al. 2002; Zou et al. 2001). CaMKII is a serine/threonine kinase, and there is growing evidence for a pathophysiological role of CaMKII in cardiac hypertrophy and heart failure. Upon stimulation, CaMKII phosphorylates class II histone deacetylase 4, which in turn dissociates from the myocyte enhancer factor 2 (MEF2), and is translocated from the nucleus to the cytoplasm (Anderson et al. 2011). This process activates MEF2, which is sufficient to promote pathological hypertrophy. In most models of pathological hypertrophy studies, suppression of CaMKII signaling has yielded either a reduction in the hypertrophic response and/or a delay in the progression from hypertrophy to heart failure (Anderson 2005).

Vitexin, an apigenin flavone glucoside, isolated from the leaf of Crataegus pinnatifida Bunge, exhibited potent hypotensive, anti-inflammatory, and anti-spasmodic properties and myocardial ischemia/reperfusion protective effects (Martino et al. 2008; Yang et al. 2012). Previous studies confirmed that vitexin preconditioning could inhibit Ca$$^{2+}$$ overload in cardiomyocytes induced by anoxia and reoxygenation (Dong et al. 2011). However, the effect of vitexin on cardiac hypertrophy and the underlying mechanism remain unknown. Therefore, this study aimed to determine whether vitexin attenuates cardiac hypertrophy in vitro and in vivo by impairing Ca$$^{2+}$$-mediated signaling pathway.

Materials and methods

Animals

Adult male Kunming mice (8–10 weeks old) were used in the current study. The mice were kept at standard room conditions (temperature 21±1 °C, humidity 55–60 %) and had free access to food and water. All animal procedures were approved according to the guidelines of the Ethics Committee of Harbin Medical University, People’s Republic of China.

Transverse aortic constriction

The pressure overload hypertrophy models were performed by transverse aortic constriction (TAC) according to previous studies (Liao et al. 2002). Adult male mice were anesthetized with pentobarbital sodium (60 mg kg$$^{-1}$$) administrated intraperitoneally. After having been successfully anesthetized, the mouse was orally intubated with a 20-gauge tube and ventilated (UGO BASILE, Biological Research Apparatus, Italy). The transverse aorta was isolated gently and a 7–0 silk suture was snared with a 27-gauge blunt needle and pulled back around the aorta. Then, the blunt needle and the aorta were tied snugly around by the suture. After ligation, the needle was quickly and carefully removed to yield a constriction of 0.4 mm in diameter. The chest and skin were closed and the pneumothorax was evacuated. Finally, the mice were allowed to recover from anesthesia. In order to evaluate the effects of vitexin on cardiac hypertrophy, vitexin (3, 10, and 30 mg kg$$^{-1}$$ day$$^{-1}$$) was injected intraperitoneally after surgery. Vitexin (purity >95 %) was purchased from Sichuan Weikeqi Biology Company. At 4 weeks later, the hearts and lungs of the euthanized mice were dissected, and the left and right ventricles were separated and weighed to calculate the heart weight/body weight (HW/BW, mg/g), left ventricular weight/body weight (LVW/BW, mg/g), lung weight/body weight (LW/BW, mg/g) ratios.

Histological analysis

Hearts were excised, washed in saline solution, and placed in 4 % paraformaldehyde. Then, samples were paraffin-embedded and cut into 4–5-mm sections. Tissue sections were prepared and stained with hematoxylin and eosin (H&E) for quantification of myocyte size and then visualized by light microscopy. A single myocyte was measured with an image quantitative digital analysis system (Image Pro-Plus 4.5). The outline of 100–120 myocytes from 30–45 randomly selected fields per heart was measured and averaged.

Culture of neonatal rat ventricular myocytes

Primary cultures of cardiac myocytes were isolated and cultured as described previously. Briefly, ventricles were dissected from 1–2-day neonatal Wistar rats. Then, the ventricular pieces were incubated with 0.25 % trypsin and the cell suspensions were collected. The cell suspensions were centrifuged at 2,000 rpm for 3 min. The collected cells were cultured in DMEM supplemented with 10 % FBS and 1 % penicillin/streptomycin at 37 °C in humid air with 5 % CO$$^{2}$$.

After 2 h for fibroblast adherence, neonatal cardiomyocytes were plated into a six-well plate or culture flask. At 48 h later, the culture medium was changed with fresh DMEM medium with BRDU (0.1 mM). After 12 h of serum starvation, 10 μM isoproterenol (Sigma, St. Louis, MO, USA) alone or together with vitexin at doses of 10, 30, and 100 μM was added to the medium, and the cultures were incubated for another 48 h.
Quantification of cell surface area

Following drug administration under the indicated conditions, cultured myocytes were fixed with 4 % paraformaldehyde for 30 min, permeabilized with 0.4 % Triton X-100 for 1 h, and blocked with goat serum for 2 h. Then, cells were incubated with a mouse monoclonal antibody to α-actinin (Sigma, St. Louis, MO, USA). After having been washed with PBS, cells were treated with FITC-conjugated goat anti-mouse IgG. Then, the nucleus was stained with DAPI. Immunofluorescence was visualized using a fluorescence microscope (Nikon 80i). A total of 80 random cells from three experiments were used for the measurement of cell surface area.

Measurement of intracellular resting free calcium

As shown in a previous study (Pan et al. 2010), cultured neonatal rat ventricular myocytes were loaded with Fluo-3/AM (5 mM) and F127 (0.02 %) at 37 °C for 40 min and then washed with Tyrode solution (in mM: 140 NaCl, 4 KCl, 2 CaCl₂, 1 MgCl₂, 10 glucose, 10 HEPES, pH 7.4) for three times. Relative alteration in [Ca²⁺]i was analyzed by FV300 Vision software was used to calculate the fluorescent intensities. Relative alteration in [Ca²⁺]i was analyzed by normalizing the fluorescent intensity to control group.

Quantitative real-time PCR

The total RNA samples were extracted using Trizol from sectorial heart tissues or neonatal myocytes. After reverse transcription, quantitative PCR was performed using real-time detection technology and analyzed on ABI 7500 fast Real Time PCR system (Applied Biosystems, USA). The real-time PCR primer:

atrial natriuretic peptide (ANP) sense: 5′-CTTCCGATA GATCTGCCCTCTTGAA-3′ and antisense: 5′-GGTACC GGAAGCTGTTGCAGCCTA-3′; brain natriuretic peptide (BNP) sense: 5′-TTGGGCAGAAGATAGACCGGAT-3′ and antisense: 5′-GGTCTTTCTAAAACACCTCA-3′; β-myosin heavy chain (β-MHC) sense: 5′-AACCTGTCCA AGTTCGCAAAGGTG-3′, and antisense 5′-GAGCTGGGTA GCACAAGAGCTACT-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) sense: 5′-TCTACATGTTCAGAT GACTC-3′ and antisense: 5′-ACTCCACGACATCTCA GCACC-3′; GAPDH was used as an internal control.

Western blot analysis

The protein samples were extracted from left ventricular tissues and cultured neonatal cardiac myocytes. A total of 50 μg protein samples were fractionated by SDS-PAGE (8–12 % polyacrylamide gels) and transferred to nitrocellulose membranes. The membranes were blocked with 5 % evaporated milk at room temperature for 2 h. The membranes were incubated overnight at 4 °C with the primary antibodies ANP, BNP (Santa Cruz Biotechnology Inc., USA), β-MHC (Abcam, Cambridge, MA, USA), NFATc3 (Santa Cruz Biotechnology Inc., USA), calcineurin (BD PharMingen, San Diego, CA, USA), CaMKII (Santa Cruz Biotechnology Inc., USA), and phospho-CaMKII (Promega, USA). Then, the membranes were washed and incubated with secondary antibodies (Invitrogen, Carlsbad, CA, USA) diluted in PBS for 50 min at room temperature. The immunoreactivity was quantified using Odyssey software and normalized to GAPDH (Kangchen, Shanghai, China) as an internal control.

Echocardiography

Cardiac function of the mice was evaluated with echocardiography. Mice were anesthetized with pentobarbital sodium (60 mg kg⁻¹) and the chest was shaved. Echocardiography was performed using a 10.0-MHz 121 phase-array transducer (GE Vivid 7, GE, USA). At least three independent M-mode measurements per animal were performed by an examiner blinded to the group of the animal. End-systole and end-diastole were defined as the phases in which the smallest and largest areas of the left ventricular were obtained, respectively.

Statistic analysis

Data were expressed as means±SEM. Comparisons among groups were tested by one-way ANOVA. Differences between two groups were determined by Student’s t-test. p < 0.05 was considered to be statistically significant.

Results

Concentration-dependent reversibility of cardiac hypertrophy by vitexin

To assess the role of vitexin in cellular hypertrophy, we first analyzed the surface area of cultured myocytes in different groups. ISO 10 μM for 48 h significantly increased cell surface area by ≈twofold compared with control group. Vitexin 30 and 100 μM obviously inhibited ISO-induced hypertrophic growth (Fig. 1a, c). To determine the physiological relevance of our findings in vitro, we next examined whether vitexin could prevent the hypertrophic response to pressure overload. H&E stain revealed that 4 weeks of application of vitexin...
decreased cell cross-sectional area significantly compared to the TAC group (Fig. 1b, d). The HW/BW ratio, LVW/BW ratio, and LW/BW ratio increased significantly from 4.57±0.30 to 7.39±0.81 mg/g (p<0.01), 3.38±0.24 to 5.70±0.64 mg/g (p<0.01), and 7.98±0.16 to 8.98±0.62 mg/g (p<0.01), respectively. However, vitexin (10 and 30 mg kg⁻¹) treatment resulted in a significant attenuation of hypertrophy as measured by HW/BW ratio, LVW/BW ratio, and LW/BW ratio (Fig. S2). Echocardiographic parameters also indicated that vitexin prevented adverse cardiac remodeling and improved cardiac performance (Table 1).

Effects of vitexin on hypertrophic markers at both protein and mRNA levels

It is well known that the fetal genes ANP, BNP, and β-MHC are important hypertrophic markers. Therefore, we first examined the effects of vitexin on ANP, BNP, and β-MHC gene expression in cardiomyocytes. Real-time quantitative RT-PCR analysis revealed that ISO markedly up-regulated ANP, BNP, and β-MHC in cardiac cells, which was reversed by vitexin at the dose of 100 mM (Fig. 2a). To further evaluate the preventive effect of vitexin on ISO-induced cardiac hypertrophy, western blot was performed to detect the protein levels of ANP, BNP, and β-MHC. Vitexin treatment obviously reversed ISO-induced up-regulation of ANP, BNP, and β-MHC proteins in cardiac cells (Fig. 3a). Meanwhile, the mRNA level of ANP, BNP, and β-MHC was reduced by vitexin at the dose of 30 mg kg⁻¹ as compared with TAC group (Fig. 2b). Western blot further confirmed the protective effect of vitexin in pressure overload-induced hypertrophy (Fig. 3b).

Vitexin attenuated the resting intracellular free calcium in cardiomyocytes

Ca²⁺ is the most important second messenger in cardiomyocytes. There is growing evidence that the intracellular Ca²⁺ plays a pivotal role in cardiac hypertrophy and progression to heart failure. Thus, we investigated the possible involvement of Ca²⁺ in the preventive effects of vitexin on cardiac hypertrophy. Consistent with previous studies, our data also confirmed that 10 μM ISO incubation for 48 h obviously increased the resting intracellular free calcium in cultured myocytes. Vitexin
Effects of vitexin on calcineurin-NFATc3 and CaMKII protein expression

One potential focal signal pathway of cardiac hypertyrophy that responds to altered calcium is calcineurin–NFAT. Thus, we investigated the possible involvement of calcineurin–NFAT in the cardioprotective activity of vitexin against cardiac hypertrophy. In accordance to previous reports, ISO obviously increased the expression of calcineurin and NFATc3 proteins as compared with NC cells. Vitexin (100 μM) significantly attenuated the up-regulation of calcineurin and NFATc3 proteins in ISO-treated cardiac cells (Fig. 5a). CaMKII is another downstream mediator and transducer of calcium for promoting cardiac hypertrophy. To determine whether vitexin also blocks the CaMKII signaling pathway in response to hypertrophic stimuli, we next examined the expression of CaMKII. As expected, we found that there were no changes in total CaMKII and ISO increased the expression of p-CaMKII, which was obviously reversed by simultaneous treatment with 100 μM vitexin (Fig. 5a). Consistent with our in vitro data, the expression of calcineurin, NFATc3, and p-CaMKII was significantly increased in TAC mice, and such changes were inhibited by treatment with vitexin 30 mg kg⁻¹ day⁻¹ (Fig. 5b). In conclusion, our results suggest that vitexin mediates the inhibitory effects on cardiac hypertrophy by

### Table 1 Echocardiographic analysis 4 weeks after TAC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>TAC</th>
<th>TAC+Vit 3 (mg kg⁻¹ day⁻¹)</th>
<th>TAC+Vit 10 (mg kg⁻¹ day⁻¹)</th>
<th>TAC+Vit 30 (mg kg⁻¹ day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVSd, mm</td>
<td>0.68±0.03</td>
<td>0.90±0.03</td>
<td>0.85±0.05</td>
<td>0.82±0.03</td>
<td>0.75±0.02</td>
</tr>
<tr>
<td>IVSs, mm</td>
<td>1.00±0.05</td>
<td>1.13±0.05</td>
<td>1.07±0.04</td>
<td>1.00±0.05</td>
<td>0.98±0.03</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>0.72±0.03</td>
<td>0.98±0.05</td>
<td>0.90±0.03</td>
<td>0.82±0.05</td>
<td>0.80±0.04</td>
</tr>
<tr>
<td>LVPWs, mm</td>
<td>0.95±0.03</td>
<td>1.20±0.03</td>
<td>1.12±0.04</td>
<td>1.03±0.06</td>
<td>0.93±0.05</td>
</tr>
<tr>
<td>EF (%)</td>
<td>76.76±1.61</td>
<td>60.96±2.16</td>
<td>60.63±1.40</td>
<td>67.09±0.82</td>
<td>70.59±1.90</td>
</tr>
<tr>
<td>FS (%)</td>
<td>40.49±1.44</td>
<td>29.31±2.00</td>
<td>28.86±1.89</td>
<td>31.32±0.52</td>
<td>36.56±1.36</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM

IVSd interventricular septum diameter in diastole, IVSs interventricular septum diameter in systole, LVPWd left ventricular posterior wall diameter in diastole, LVPWs left ventricular posterior wall diameter in systole, EF ejection fraction, FS fractional shortening

**p<0.01 vs. Sham; #p<0.05 vs. TAC; ##p<0.01 TAC, n=6
disrupting both calcineurin–NFAT and CaMKII signaling pathways.

Discussion

The main finding of our study is that vitexin protects against cardiac hypertrophy induced by ISO in vitro and pressure overload-induced cardiac hypertrophy in vivo. The cardioprotection of vitexin is mediated by the interruption of Ca^{2+}–calcineurin and Ca^{2+}–CaMKII pathways. These findings suggest that vitexin may be a prospective therapeutic candidate against cardiac hypertrophy and progression to heart failure.

Cardiac hypertrophy develops in response to a variety of intrinsic and extrinsic stimuli on the heart as the body attempts to compensate for the reduced cardiac output. Though cardiac hypertrophy is an initial adaptive process, persistent hemodynamic overload will result in pathological cardiac hypertrophy, which is a leading predictor for the development of heart failure and sudden death (Rosca et al. 2012; Weiss et al. 2010). It has been reported that numerous traditional Chinese medicine herbs have protective effects against cardiac hypertrophy, especially the flavones isolated from many plants, such as apigenin (Eom et al. 2011) and quercetin (Wu and Gu 2007; Han et al. 2009). Vitexin, an apigenin flavone glucoside (8-C-b-D-glucopyranosylapigenin; Fig. S1), is isolated from the leaf of Crataegus pinnatifida Bunge. The main function of vitexin is activation of blood circulation to dissipate blood stasis and vital energy regulation. Clinically, it is mainly used for the treatment of congestion-induced dyspnea, cardiopalmus, giddiness, and tinnitus. Recently, vitexin has been verified to protect against myocardial ischemia/refusion injury in isolated rat heart by inhibition of the inflammation and cardiac myocyte apoptosis (Dong et al. 2011). Vitexin preconditioning attenuates cardiomyocytes anoxia and reoxygenation injury by improvement of cardiac myocyte survival and calcium overload (Dong et al. 2008). It has not been reported whether vitexin has effects on cardiac hypertrophy. Thus, our study is aimed to investigate the effects and mechanisms of vitexin on cardiac hypertrophy in vitro and in vivo.

In our study, ISO was used as stimuli for inducing myocardial hypertrophy in cultured neonatal rat ventricular myocytes, and to investigate whether vitexin has the function of inhibiting cardiac hypertrophy in vitro, the cell size was detected. Our result demonstrated that vitexin significantly reduced the increased cell size, which indicated that vitexin has an anti-hypertrophy effect in vitro. To evaluate the effect of vitexin on cardiac hypertrophy in vivo, transverse aorta

![Fig. 3](#) Effects of vitexin on cardiac hypertrophic markers at protein levels. a Vitexin 100 μM suppressed ISO-induced increases of ANP, BNP, and β-MHC at protein levels. The left panel illustrates the representative western blots and the right panel presents the summarized data. Data are expressed as mean±SEM for three individual experiments. **p<0.01 vs. NC, #p<0.05 vs. ISO. b Vitexin 30 mg kg⁻¹ compromised pressure overload-induced activation of ANP, BNP, and β-MHC at protein levels. The left panel presents the western blots and the right panel is for the summarized data. Data are expressed as mean±SEM for three individual experiments. **p<0.01 vs. sham, #p<0.05 vs. TAC, ##p<0.01 vs. TAC.

![Fig. 4](#) Effects of vitexin on ISO-induced increment of resting intracellular free calcium in neonatal rat ventricular myocytes. ISO enhanced the intracellular resting calcium, which was significantly inhibited by vitexin 100 μM. Data are expressed as mean±SEM for 60 cells from at least three individual experiments. **p<0.01 vs. NC, ##p<0.01 vs. ISO.
Banding surgery was performed. Vitexin administration attenuated pressure overload-induced increase in HW/BW, LVW/BW, and LW/BW ratios as well as cardiomyocyte cross-sectional area. To further confirm the preventive effect of vitexin on cardiac hypertrophy, we next evaluated the effects of vitexin on hypertrophy markers. Vitexin attenuated the expression of ANP, BNP, and \( \beta \)-MHC at protein and mRNA levels. Echocardiographic parameters indicated that vitexin prevented adverse cardiac remodeling and ventricular dysfunction. Taken together, our data first demonstrated that vitexin is effective in preventing cardiac hypertrophy both in vitro and in vivo.

However, the molecular mechanism by which vitexin mediates its anti-hypertrophic effects remains unclear, and the signaling pathways that interact to drive hypertrophy are very complicated. It has been reported that persistent hemodynamic overload, a main cause of cardiac hypertrophy in clinics, results in sustained intracellular Ca\(^{2+}\) increases in cardiomyocytes (Wang et al. 2012). Moreover, Ca\(^{2+}\), both a second messenger and a regulatory factor, has been reported to play a pivotal role in the progression of cardiac hypertrophy (Vicencio et al. 2011). Numerous studies have confirmed that Ca\(^{2+}\)-activated kinase, calcineurin, and CaMKII, acting an important part in modulating the development of cardiac hypertrophy. Following stimulation with hypertrophic agonists in cultured rat neonatal cardiomyocytes, calcineurin enzymatic activity, calcineurin mRNA, and protein levels were increased (Lim et al. 2000). Calcineurin activity was also up-regulated in hypertrophied hearts following aortic banding surgery in rodents and in hypertrophied and failing hearts from human patients (Haq et al. 2001). NFAT luciferase reporter activity was up-regulated in several pathological models (Wilkins et al. 2004). Additionally, transgenic overexpression of calcineurin or NFAT is sufficient to induce apparent hypertrophy and interstitial fibrosis (Bernardo et al. 2010). Transgenic mice with targeted inactivation of calcineurin A\(\beta\) (Bueno et al. 2002) or pharmacological inhibition of calcineurin activity (Zou et al. 2001) is profitable for reversing the cardiac hypertrophy process. Besides, mice with targeted disruptions of NFATc3 demonstrated a significant reduction in cardiac hypertrophy (Wilkins et al. 2002). CaMKII pathway is critical for promoting cardiac hypertrophy. The activity and expression of CaMKII are increased in hypertrophied myocardium from animal models and patients (Anderson et al. 2011a). Overexpression of CaMKII causes myocardial hypertrophy and heart failure, and CaMKII inhibition suppresses myocardial hypertrophy and progression to heart failure (Cheng et al. 2012). J Backs and H Ling reported that loss of CaMKII significantly attenuated ventricular dilation and dysfunction induced by long-term TAC (Backs et al. 2009; Ling et al. 2009) and R Zhang demonstrated that mice expressing a CaMKII-inhibitory peptide prevented hypertrophy and resultant pathological remodeling induced by AngII and/or ISO (Zhang et al. 2005). Besides, recent study has demonstrated that vitexin preconditioning could inhibit Ca\(^{2+}\) overload in cardiomyocytes induced by anoxia and reoxygenation injury (Dong et al. 2011). Therefore, we hypothesized that vitexin could protect against cardiac hypertrophy through Ca\(^{2+}\)-mediated calcineurin and CaMKII signaling pathways.

Our results revealed that vitexin significantly suppressed the increment of resting intracellular free calcium in vivo hypertrophic models. Furthermore, vitexin suppressed the expression of calcineurin, NFATc3, and p-CaMKII, which suggests that vitexin is capable of inhibiting cardiac hypertrophy via Ca\(^{2+}\)-mediated calcineurin–NFAT and CaMKII signaling pathways.
Recently, M Scott reported that CaMKII negatively regulated calcineurin–NFAT signaling in cardiomyocytes; this interesting study illustrated the potential relationship between CaMKII and calcineurin from a new spot. In their studies, CaMKII activation preserved the hypertrophied heart by mediating calcineurin phosphorylation and NFATc3 translocation. However, our data showed that vitexin abolished cardiac hypertrophy by inhibiting these Ca\(^{2+}\)-mediated signaling pathways (MacDonnell et al. 2009), although the difference between animal models, initial stimuli, and the complexities of intracellular hypertrophied pathways themselves could contribute to those contradictory results. In my opinion, the distinct splice variants and the different space location and activating duration of CaMKII may be the stem cause of the contradiction, and it has been reported that the activation of CaMKIIδB and cytoplasmic CaMKIIδC increased the survival pathways, while activating CaMKIIδC in the nucleus is considered to be maladaptive (MacDonnell et al. 2009). These suggest that the cellular mechanism of cardiac hypertrophy is very complicated and multiple factors are associated with the mediation of CaMKII and calcineurin–NFAT. The relationship between CaMKII and NFAT in vitexin effect on cardiac hypertrophy needs further investigation.

Vitexin is also an anti-oxidant agent (Kim et al. 2005). It was well established that TAC treatment and/or ISO infusion could increase the reactive oxygen species (ROS) level, and a high level of cellular ROS was sufficient to stimulate hypertrophic response by activating Ca\(^{2+}\)-independent pathways (Gupta et al. 2007; Sun et al. 2012). Many studies have documented that many anti-oxidant agents like resveratrol and green tea extracts could reverse or attenuate hypertrophy in vitro and in vivo (Sabri et al. 2003; Cave et al. 2005). In this study, our data revealed that vitexin protected against cardiac hypertrophy by inhibiting Ca\(^{2+}\)-dependent mechanisms. The dose of vitexin used in the present study was sufficient to implicate its anti-oxidant effects. Although in this study we focus on the Ca\(^{2+}\)-dependent pathways inhibited by vitexin, we believe that vitexin could also favor the stressed heart by decreasing cardiac ROS level. Further study is still needed to investigate the anti-oxidative effects of vitexin on pathways involved in cardiac hypertrophy.

In summary, our results suggest that vitexin exerts therapeutic effects on cardiac hypertrophy both in vivo and in vitro. Additionally, our data also show that the anti-hypertrophic effect of vitexin can be attributed to, at least partly, the interruption of the Ca\(^{2+}\)-mediated pathways Ca\(^{2+}\)-calcineurin–NFATc3 and Ca\(^{2+}\)-CaMKII. These findings improve our knowledge of vitexin and provide a new perspective of developing vitexin as a therapeutic candidate for cardiac hypertrophy and heart failure.

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