Dilong Attenuates Pathological Cardiac Hypertrophy Through Akt/mTOR and NF-κB Signaling in Pressure Overload-induced Rats

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Research Article

Keywords: cardiac hypertrophy, Dilong, pressure overload, cardiomyocyte fibrosis, inflammation
Abstract

**Background:** Dilong (earthworm), a traditional Chinese medicine obtained from the dried body of *Pheretima aspergillum* (E. Perrier), has proven cardioprotective effects in cardiovascular disorders. We aimed to investigate the potential therapeutic application of Dilong in preventing cardiomyocyte fibrosis and inflammation during cardiac hypertrophy (CH) after pressure overload (PO).

**Methods:** We used the active protein compounds of Dilong (DAPC) to treat CH in vivo and in vitro. Hypertrophic rats were generated by abdominal aortic constriction (AAC)-surgery in male Wistar rats. DAPC (low-dose, 400 mg/kg per day; high-dose, 800 mg/kg per day, intraperitoneal injection [i.p.]) or captopril (50 mg/kg per day, i.p.) were given to rats for 3 weeks post AAC-surgery. Primary neonatal rat ventricular myocytes (NRVMs) isolated from 1-to 2-day-old SD rats were CH induced by phenylephrine (PE, 50 μM) with or without DAPC (low-dose, 4 mg/mL; high-dose, 8 mg/mL) or captopril (10 μM). Myocardial hypertrophy was identified by the heart weight index. Left ventricle remodeling and fibrosis of hypertrophic rats were evaluated by hematoxylin and eosin (H&E) staining, Mason staining, and Wheat germ agglutinin (WGA) staining. Using quantitative real-time PCR to examine the expressions of hypertrophic markers and inflammatory cytokines at mRNA levels. Additionally, protein expression of the Akt/mTOR and NF-κB signaling pathways were evaluated by western blot assay.

**Results:** DAPC markedly decreased heart weight index and improved pathological morphology of the myocardium in AAC-induced rats. Of note, DAPC inhibits hypertrophic biomarkers ANP and BNP in vivo and in vitro. Meanwhile, the levels of IL-1β, IL-6, and TNF-α were decreased. Interestingly, DAPC inhibited the activation of Akt/mTOR and NF-κB proteins, which were in line with the in vitro findings.

**Conclusion:** DAPC treatment in rats with PO-induced CH prevented cardiac fibrosis and had anti-inflammatory effects via regulation of the Akt/mTOR and NF-κB pathways.

Introduction

Pressure overload (PO) cardiac hypertrophy (CH) is an important independent cardiovascular risk factor for heart failure (HF) [1]. Due to the limited compensatory ability of the heart, progressive hypertrophic stimulations commonly cause cardiac decompensation, ultimately leading to cardiac arrhythmias and HF [2–4]. Thus, CH may be a major therapeutic target for the prevention of cardiovascular disorders [5, 6]. Treatment for CH typically focuses on preventing the underlying cause or reducing the ventricular overload.

In the progression of CH to HF, plasma inflammatory cytokines are markedly elevated, including tumor necrosis factor-α (TNF-α) [7] and interleukin-1β (IL-1β) [8]. These inflammatory cytokines furthermore promote CH through a variety of signaling pathways. It is accepted that Akt/mTOR and NF-κB signaling are common downstream mediators during the development of CH [9]. Of note, mTOR signaling plays a major role in myocardial cell hypertrophy in response to physiological or pathological stimuli [10].
Therefore, reducing inflammatory state through targeting Akt/mTOR and NF-κB signaling may be therapeutic to block CH progression to HF.

Recently, it is accepted that renin-angiotensin system inhibitors, sympathetic inhibitors and diuretics are commonly used to treat CH and HF [11]. Although these chemical-synthetic western drugs have the effect on reducing symptom but cannot enhance the overall cardiac function. Moreover, limited data is available on the long-term therapeutic effect of using these chemical drugs for the treatment of CH and HF on the increased risk of mortality and sudden cardiac death. Therefore, natural bioactive drugs, with their advantages of safety, effectiveness and fewer side effects, may be the alternative drugs for the treatment of CH and HF.

Dilong, a compound isolated from *Lumbricus rubellus* in the form of raw animal “herb”, is a traditional Chinese medicine that has long-standing medical history, particularly empirically in treating vascular disorders in Asia [12, 13]. Previous studies showed that Dilong could enhance blood circulation and remove blood stasis [14, 15]. In addition, Dilong contains one potent fibrinolytic enzyme named lumbrokinase, isolated from dried earthworm, which has been investigated for its thrombosis prevention in rats with myocardial infarction [16]. Furthermore, Dilong prevented apoptosis and fibrosis in H9c2 cells [17], and reduced LPS-induced myocardial apoptosis [15]. More recently, Dilong extracted from *Pheretima aspergillum* could alleviate KCl-induced cardiac remodeling [18]. However, the relevance of Dilong in ventricular load CH is still less known. Based on its cardioprotection properties, we hypothesize that Dilong could also have anti-hypertrophic effects. Therefore, we aimed to investigate the potential protective role of Dilong against cardiac fibrosis and inflammation. Furthermore, we aimed to elucidate the related signaling mechanism involved in PO-induced CH.

**Materials And Methods**

**Chemicals and agents**

Dulbecco’s modified Eagle’s medium (DMEM), M199 modified Eagle’s medium, and fetal bovine serum (FBS) were all obtained from Gibco Lab (Grand Island, NY, USA). Phenylephri (PE) were obtained from Sigma Chemical Co. (St. Louis, MO). BCA Protein Assay Reagent was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Primary antibodies against α-actinin (#6487S; CST, Danvers, MA, USA), GAPDH (TA-08, ZSGB-BIO, Beijing, China), p-NF-κB p65 (#8242S; CST), IκB (#9242S; CST), p-Akt (#4060S; CST), Akt-pan (#4691S; CST), p-mTOR (#2972S; CST), and goat anti-rabbit HRP-conjugated secondary antibody (sc-2004; SANTA CRUZ, Santa Cruz, CA, USA) were obtained commercially. The primers of GAPDH, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), TNF-α, IL-1β, and IL-6 were all synthesized by Sangon-Bio (Shanghai, China).

**Dilong extraction**

The dried powder of Dilong, extracted from *Pheretima aspergillum*, was obtained from Weifang Hospital of Traditional Chinese Medicine (Weifang, China). Dilong powder (2 g) was dissolved in 10 mL distilled water at 60 °C for 1 h, centrifugated at 12,000 g for 20 min at 4 °C using a Biofuge Stratos Heraeus...
(Thermo Fisher Scientific), and the supernatant purified by gel filtration just prior to the experiments. The extract was named: Dilong active protein compounds (DAPC).

**Animals**

All animals were kept at the Center for Experimental Animals in Weifang Medical University and housed on a 12 h light/dark cycle in a 24±2 °C environment with ad libitum access to food and water. All animal experiments were approved by the Ethics Committee of Weifang Medical University (2019SDL090) according to the “Guide for the care and use of Laboratory Animals” adopted by the National Institutes of Health's (NIH) Guidance.

**Abdominal aortic constriction (AAC) induced rats and DAPC-treatment protocol**

It is well-established that abdominal aortic constriction (AAC) surgery can be used to produce a pathological hypertrophic rat model as AAC is similar to the human pattern of CH and HF [19]. In this study, male SD rats aged 6–8 weeks, weighing 180–200 g were obtained from the Shandong Pengyue Experimental Animal Center (Jinan, China). Rats were randomly assigned into a control group (Sham) (n=6) and AAC-induced rats (groups 1–4) (n=24). The Sham controls (n=6) received normal saline (NS) by intraperitoneal injection (i.p.). The first AAC group (n=6) received NS by i.p. as a negative control group. The second AAC group (n=6) received a low-dose DAPC (LD, 400 mg/kg/d, i.p.). The third AAC group (n=6) was treated with a high-dose DAPC (HD, 800 mg/kg/d, i.p.) as required according to the experimental protocol. The fourth AAC group (n=6) was treated with captopril (50 mg/kg/d, i.p.) as a positive control group.

The AAC surgery procedure was performed as follows: First, all rats were anesthetized with 10% chloral hydrate, after disinfection with iodine gauze on the abdomen. Then, a small incision of 3~4 cm was made along the xiphoid sternum to expose the abdominal aorta. Here, 21G-blunt needle was put on the side of the isolated aorta to generate aortic constriction to mimic pressure overload to the heart. Then, after ligation of the abdominal aorta with the blunt needle by using 1# silk thread, the blunt needle was removed. Finally, the viscera were carefully returned to their original position, and closed the abdomen. The Sham surgery was operated the same as the AAC surgery only without ligation. After AAC or sham operation, the rats were given antibiotics for 1 week.

Rats were euthanized by 10% chloral hydrate at four weeks post-AAC surgery. The heart tissues were quickly excised, cleaned with PBS at 4 °C, and blotted with filter paper. Lastly, the left ventricles were fragmented: 1) for further analysis of the mRNA and protein levels, and 2) other segments were fixed in 4% triformol for histopathology analysis.

**Heart weight index and histopathological analysis**

We used the heart weight index to examine the heart dysfunction of hypertrophic rats, such as the ratios of heart weight (HW)/body weight (BW), left ventricle weight (LVW)/BW, and LVW/HW. After careful excising, left ventricles were washed and the myocardium was fixed in 4% paraformaldehyde (pH 7.4) for
24 h for further histopathological analysis. Paraffin embedding of paraformaldehyde-fixed heart tissues was sectioned at 5-µm thickness. H&E staining, Masson staining and WGA staining analyses were performed according to standard procedure.

**Isolation of neonatal rat ventricular myocytes (NRVMs) culture and treatment**

NRVMs were obtained from within 48 h SD rats through enzymatic dissociation with 0.1% trypsin and 0.03% collagenase as described previously [20]. NRVMs at a density of 3×10⁶/mL were cultured for 24 h with PE (50 µM) treatment with or without DAPC (LD, 4 mg/mL; HD, 8 mg/mL), or captopril (10 µM), before being analyzed using western blotting and quantitative real-time PCR (qRT-PCR).

**Quantitative real-time PCR (qRT-PCR)**

Total heart tissue RNA was extracted, and reverse transcribed to cDNA by a reverse transcription kit (Toyobo, Osaka, Japan). qRT-PCR was performed using gene primers and the real-time PCR Master Mix of SYBR Green (Toyobo), and run with the Cycler 480 System (Roche, Basel, Switzerland). The results were analyzed using the $2^{-\Delta\Delta CT}$ method. The primers used in the present study are listed in Supplemental Table1.

**Western blotting**

Left ventricular tissue homogenates were used for immunoblotting. The protein concentration was determined by the BCA protein assay. Proteins were equal-loaded onto each SDS-PAGE gel and then transferred to a PVDF membrane. The membrane was incubated with primary antibodies against Akt-pan (1:1,000), p-Akt (1:1,000), mTOR (1:1,500), p-mTOR (1:1,000), p-NF-κB p65 (1:1,000), and IκB (1:1,500). The protein bands were quantitated and normalized using a mouse monoclonal anti-GAPDH antibody (ZSGB-BIO). The HRP-conjugated goat anti-rabbit and anti-mouse secondary antibodies were both used at the ratio of 1:2,000. The membranes were visualized and analyzed using the Chemiluminescence Fluor Chem FC3 system (Protein Simple, Toronto, Canada).

**ELISA measurement**

The TNF-α protein levels in the rat hearts were measured using an ELISA kit (Westang Biotechnology, Shanghai, China) according to the manufacturer’s instructions.

**Statistical analysis**

All data are shown as means ± standard deviation (SD). Comparisons among three or more groups were evaluated using one-way ANOVA with the Tukey-Kramer post-hoc test. Statistically significant differences were set at $P<0.05$.

**Results**
**Effects of DAPC on the pathology of PO-induced CH**

The PO-induced CH rat model was established by AAC surgery according to a previous study [19]. To investigate the impact of DAPC on the changes of CH, we used DAPC to treat AAC-induced rats. The experimental protocol is shown in Fig. 1A. Furthermore, extensive evaluations were performed 4 weeks post AAC surgery. As shown in Fig. 1B-F, cardiac hypertrophic responses were incisive after AAC-induced, whereas the responses of DAPC treatment were obviously blunted in comparison with the negative control group. HW/BW and LVW/HW of rats after DAPC treatment was as well as notably decreased.

To observe the histological effects of DAPC on AAC hearts, we used H&E, WGA, and Masson staining to evaluate the pathological changes. As shown in Fig. 2A, B, left ventricle pachynsis and fibrosis were observed in the AAC-surgery group with vehicle-treatment compared to the Sham group. Furthermore, diffused myocytes hypertrophy significantly increased and myocardial cell nucleus enlarged in AAC-induced rats, whereas DAPC treatment could almost completely reverse and attenuate the myocardial cell pachynsis. Consistently, WGA staining to compare the surface area of the cardiomyocytes in each group also supported the above results (Fig. 2C, D). Interestingly, the average collagen volume showed a markedly decrease of DAPC-treated rats, and the effect of DAPC-HD treatment more pronounced than that of the DAPC-LD treatment (Fig. 2E, F). Thus, these results suggested DAPC treatment had a protective effect in relieving AAC-induced cardiac fibrosis.

**DAPC inhibits hypertrophic biomarkers and inflammatory responses in vivo and in vitro.**

To validate the effect of DAPC on CH, we examined the changes of the hypertrophic markers ANP and BNP at the mRNA level. The ANP and BNP levels were detected by qRT-PCR. As expected, AAC surgery triggered an increase of the hypertrophic markers ANP and BNP at the mRNA level as seen from PCR results (Fig. 3A, B), which were inhibited in rats with DAPC treatment compared to the Sham group, while DAPC-HD treatment showed a more pronounced effect than the DAPC-LD treatment. In addition, the AAC-surgery groups had elevated cytoskeletal protein α-actinin levels compared to the Sham group, while DAPC-treatment inhibited the increase in α-actinin levels (Fig. 3C, D). DAPC-HD treatment also had a more pronounced effect than either DAPC-LD or captopril treatments.

It is well-established that CH is associated with an increased inflammatory response [21]. Therefore, we analyzed the pro-inflammatory mediators IL-6 and TNF-α at mRNA level by quantitative RT-PCR. Furthermore, we observed the expression of TNF-α at protein level by using ELISA kit. As shown in Fig. 3E, F, AAC-induced rats with vehicle treatment showed higher levels of the inflammatory cytokines TNF-α and IL-6 than Sham group. But DAPC treatment decreased the mRNA levels of TNF-α and IL-6 in rats after AAC surgery. Meanwhile, the TNF-α protein level in the serum of DAPC- treated rats was lower than the vehicle-treated rats (Fig. 3G). Taken together, DAPC treatment inhibited inflammation in PO-induced rats.

To further evaluate the protective effects of Dilong extracts in CH, we used PE (as a hypertrophic inducer) to stimulate NRVMs to produce cardiomyocyte cellular hypertrophy. Consequently, ANP and BNP levels were both increased in PE groups without DAPC treatment, while DAPC-HD treatment had protective
effects against CH compared to the vehicle-control or captopril-treatment groups (Fig. 4A, B). As shown by the α-actinin staining in Fig. 4C, D, the cell surface area significantly increased in the PE-stimulated groups with or without DAPC treatment, while DAPC-HD treatment blunted the changes compared to the vehicle-control group. Consistently, we also found that DAPC treatment decreased the proinflammatory factors TNF-α and IL-1β at the mRNA level in PE-stimulated NRVMs. Interestingly, similar findings also occurred in the captopril treatment group (Fig. 4E, F).

**DAPC blunts CH by inhibiting the activation of the Akt/mTOR and NF-κB signaling pathways in vivo and in vitro.**

As is known to all, Akt/mTOR and NF-κB signaling pathways are activated during CH [22]. Using western blotting, we further explored the effect of DAPC on the expression of Akt, mTOR, and NF-κB proteins in CH. We found that AAC-induced rats exhibited elevated levels of p-Akt and p-mTOR (Fig. 5A–E). DAPC-HD treatment significantly ablated the expression of phosphorylated Akt and mTOR in PO-induced rats in comparison with the Sham or captopril groups. DAPC treatment could also inhibit the degradation of IκB and the phosphorylation of NF-κB compared to these groups (Fig. 5F–I). These results suggest that DAPC could inhibit the activation of the Akt/mTOR and NF-κB signaling pathway in the AAC-induced rats.

Lastly, to further study the changes in the pathways mentioned above, we also used PE-stimulation to induce CH in NRVMs. Consistent with the PO-induced rats, DAPC treatment inhibited the phosphorylation of Akt, mTOR, and p65 (Fig. 6A–E), and increased the expression of IκB compared to the untreated controls (Fig. 6F–I). Therefore, DAPC effectively inhibited the activation of the Akt/mTOR and NF-κB signaling pathways in vivo and in vitro.

**Discussion**

Dilong, a traditional Chinese medicine, is widely used to treat vascular disorders [12, 13]. It is accepted that Dilong has an anti-hypertensive effect to remove blood stasis—abnormal blood flow [14, 15]. Additionally, Dilong can alleviate fibrosis and apoptosis in cardiomyocytes [17]. In this study, we extracted the active protein components from Dilong by gel filtration. We then proceeded to show that Dilong has a cardioprotective effect in AAC-induced rats. Dilong might function by reducing PO-induced inflammation. More importantly, our results further indicated that Dilong may have promising therapeutic potential for CH via inhibition of the Akt/mTOR and NF-κB signaling both in vivo and in vitro. A schematic representation of our findings is shown in Fig. 7.

In this study, rats were subjected to the AAC surgery to mimic PO-induced CH as described previously [19]. AAC surgery induced an increase in the heart weight index (HW/BW and LVW/BW) (Fig. 1). Consistent with previous studies [23, 24], ANP and BNP levels were both upregulated during CH, while Dilong administration directly inhibited the pathological changes of CH (Fig. 3). Simultaneously, similar findings were observed using an in vitro CH model established by stimulating NRVMs with the hypertrophic inducer PE (Fig. 4). This is the first study to identify the therapeutic potential of Dilong treatment in CH models.
As inflammation participates in CH, fibrosis, and heart dysfunction, the progression of CH to HF may be slowed by anti-inflammatory treatment [25, 26]. Moreover, in the development and progression of HF, proinflammatory factors are activated, such as TNF-α, IL-6, and IL-1β [27]. Our study showed the anti-inflammatory effects of Dilong in vivo and in vitro. Interestingly, the results demonstrated that DAPC treatment markedly reversed the effect of PO-induced CH on up-regulated level of the inflammatory cytokines TNF-α, IL-1β, and IL-6 (Figs. 3–4). In addition, DAPC administration also inhibited the NF-κB transcription suggesting it has a key anti-inflammatory mechanism in treating PO-induced CH (Figs. 5–6).

The mechanism by which AAC surgery induces CH is complex. It is well-established that Akt is considered to be a hypertrophic inducer of CH [28]. Akt knock-down in mice could alleviate the physiological CH [28]. Moreover, mTOR as a negative regulator of inflammation has been confirmed [29]. Here, PE-induced NRVMs and PO-induced rats were used to extract the effect of Dilong on CH in hypertrophic cardiomyocytes and in vivo. Phosphorylation of Akt and mTOR were upregulated in AAC-induced rats and PE-induced NRVMs, while Dilong treatment inhibited Akt/mTOR signaling pathway (Figs. 5-6). In addition, NF-κB, a transcription factor, regulates the expression of multiple genes particularly those in early responses to the adaptive body’s function and inflammatory response, such as IL-1, IL-2, and TNF-α [30–32]. Moreover, the phosphorylation and expression of IkB and p65 were assessed to investigate the underlying mechanisms of Dilong against CH. In this study, we found that Dilong extract inhibited IkB and NF-κB levels in AAC-induced rats (Fig. 5F–I) and PE-stimulated NRVMs (Fig. 6F–I). Therefore, we suppose that Dilong may reduce the activation of inflammatory factors and the expression level of hypertrophic genes by inhibiting CH triggering signal transduction. Taken together, our results showed Dilong has anti-hypertrophic effects, which proved to be protective against PO-induced CH in rats. Moreover, the protective effect of Dilong against CH may be related to inhibition of the Akt/mTOR and NF-κB signaling pathway.

**Conclusion**

Our findings demonstrated for the first time that Dilong administration could ameliorate PO-induced CH in rats. The anti-hypertrophy mechanism of Dilong is related to reduced expression of pro-inflammatory cytokines and transcription factors. Our results also confirmed the hypothesis that Dilong treatment significantly reversed abnormal Akt/mTOR and NF-κB signaling. Therefore, the beneficial effects of Dilong are related to suppressing Akt/mTOR and NF-κB signaling pathways to trigger anti-inflammatory action. Dilong holds promise as an anti-hypertrophic and anti-inflammatory treatment for CH.

**Abbreviations**

AAC, abdominal aortic constriction; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CH, cardiac hypertrophy; DAPC, Dilong active protein compounds; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; H&E, Hematoxylin and eosin staining; HF, heart failure; HW/BW, heart weight/body weight; i.p., intraperitoneal injection; IL-1β, interleukin-1β; IL-6, interleukin-6; LVW/BW, left
ventricle weight/body weight; NRVMs, neonatal rat ventricular myocytes; NS, normal saline; PE, phenylephrine; PO, pressure overload; qRT-PCR, quantitative real-time PCR; SD, standard deviation; WGA, Wheat germ agglutinin staining; TNF-α, tumor necrosis factor-α.

**Declarations**

**Acknowledgments**

We thank the Language Editing Service of Sanger for assistance with the language editing.

**Author's contributions**

LZ designed and performed the experiments; TL, SZ, and RP performed the experiments and analyzed the data; LZ wrote the manuscript. JG, YC, and TS performed the isolation of NRVMs; XZ, JZ and WX helped with the purification from Dilong of pheretima aspergillum; LL, RF and ZZ helped with the establishment of cardiac hypertrophy model. All authors reviewed the manuscript. The authors declare that they have no conflict of interest.

**Funding**

This work was sponsored by grants from Traditional Chinese Medicine Science and Technology Development Project of Shandong Province (No. 2015-333 & 2017-213), the National Science Foundation of China (No. 8170021245), Natural Science Foundation of Shandong Province (No. ZR2021LZY033), Natural Science Foundation of Shandong Province (No. ZR2019BH036), Science and Technology Development Project of Weifang (No. 2018YX058) and Weifang Medical University Ph. D Project (No. 2017BSQD21). Additionally, LZ was sponsored by Shandong Provincial Government Overseas Study Project and Weifang Medical University Overseas Study Project in 2019.

**Availability of data and materials**

The datasets used and analyzed during current study are available from the corresponding author on reasonable request.

**Ethics statement**

The animal study was approved by the Animal Research Ethics Committee of Weifang Medical University.

**Consent for publication**

All authors have approved the publication of this submission.

**Competing interests**

The authors declare that there are no conflicts of interests regarding the publication of this paper.
References


13. Popovic M, Hrcenjak TM, Babic T, et al. Effect of earthworm (G-90) extract on formation and lysis of clots originated from venous blood of dogs with cardiopathies and with malignant tumors. Pathol...


Table

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<th>Gene</th>
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Figures
**Figure 1**

Effects of DAPC on the pathology of cardiac hypertrophy under PO. **A** The experimental protocol. **B-F** Effects of DAPC on **B** body weight (BW), **C** Heart weight (HW), **D** Left ventricular weight (LVW), **E** HW/BW, **F** LVW/HW. The Data are showed as means ± SD. *P < 0.05, **P < 0.01, ***P < 0.001, versus the Sham group 4 weeks post AAC surgery; ##P < 0.01, ###P < 0.001, versus the AAC group 4 weeks post AAC surgery with supplementation of DAPC.
Figure 2

Effect of DAPC on PO-induced cardiac hypertrophy in rats. **A** Representative images of the heart tissues in different groups examined by H&E staining. Scale bar 50 µm. **B** The related calculation of cross-sectional area. **C** Representative images of the heart tissues in different groups examined by WGA staining. **D** Comparison of the surface area of the cardiomyocytes. **E** Representative images of the left ventricular tissues in different groups examined by Masson's staining. **F** Quantification of the relative fibrosis area in DAPC- and vehicle-treated rats 4 weeks post-AAC surgery (200 cells counted per heart). Data are showed as means ± SD. **P < 0.01, versus the saline control group; ##P < 0.01, versus the AAC group.

Figure 3

DAPC inhibits hypertrophic biomarkers and inflammatory responses in vivo. **A-B** The mRNA levels of **A** ANP and **B** BNP. **C** Immunoblotting analyses of α-actinin. **D** Quantification of the relative protein expression of α-actinin to GAPDH. **E-F** qRT-PCR analysis of mRNA levels of **E** TNF-α and **F** IL-6 in hearts. The data are normalized to the GAPDH content. **G** The protein expression of TNF-α by ELISA. Data are showed as means ± SD. **P < 0.01, versus the saline control group; #P < 0.05, ##P < 0.01 versus the AAC group.
DAPC inhibits hypertrophic biomarkers and inflammatory responses in vitro. A-B The mRNA levels of A ANP and B qPCR by qRT-PCR. C Representative images of NRVMs in different groups examined by α-actinin staining. D The cell surface area of NRVMs in different groups. E-F The mRNA levels of E TNF-α and F IL-1β by qRT-PCR. The data are normalized to the GAPDH content. Data are showed as means ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 versus the PBS control group; #P < 0.05, ##P < 0.01 versus PE (50µM) stimulated group after 24h in NRVMs.
**Figure 5**

DAPC inhibited cardiac inflammation through Akt/mTOR and NF-κB signaling *in vivo*. A Western blotting assay was performed to measure the expressions of p-Akt, Akt, p-mTOR, and mTOR in heart tissues. B-E Relative expressions of p-Akt, Akt, p-mTOR, and mTOR were quantified. F-G Immunoblotting analyses of IκB, p-NF-κB, NF-κB. H-I Quantification of relative protein expression of p-NF-κB, NF-κB, and IκB. The data are normalized to the GAPDH content. Data are showed as means ± SD. *P < 0.05, **P < 0.01, versus the saline control group; #P < 0.05, ##P < 0.01, versus the AAC group.
Figure 6

DAPC inhibited cardiac inflammation through Akt/mTOR/NF-κB signaling in vitro. A Immunoblotting analyses of p-Akt Akt, p-mTOR, mTOR. B-E Quantification of protein expression of p-Akt, Akt, p-mTOR, and mTOR in NRVMs. F Immunoblotting analyses of IκB, p-NF-κB, NF-κB. G-I Quantification of protein expression of p-NF-κB, NF-κB, and IκB. The data are normalized to the GAPDH content. Data are showed as means ± SD. *P < 0.05, **P < 0.01, ***P < 0.001, versus the PBS control group; #P < 0.05, ##P < 0.01, ###P < 0.001, versus the PE (50µM) stimulated group after 24h in NRVMs.
Figure 7

A schematic representation of our findings.