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Caffeic acid phenethyl ester attenuates pathological cardiac hypertrophy by regulation of MEK/ERK signaling pathway *in vivo* and *vitro*

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ABSTRACT

Aim: To explore the effects of caffeic acid phenethyl ester (CAPE) on cardiac hypertrophy induced by pressure overload.
Main methods: Male wild-type C57 mice, aged 8–10 weeks, were used for aortic banding (AB) to induce cardiac hypertrophy. CAPE or (resveratrol) RS was administered from the 3rd day after AB surgery for 6 weeks. Echocardiography and hemodynamic analysis were performed to estimate cardiac function. Mice hearts were collected for H&E and PSR staining. Western blot analysis and quantitative PCR were performed for to investigate molecular mechanism. We further confirmed our findings in H9c2 cardiac fibroblasts treated with PE or CAPE.
Key findings: CAPE protected against cardiac hypertrophy induced by pressure overload, as evidenced by inhibition of cardiac hypertrophy and improvement in mouse cardiac function. The effect of CAPE on cardiac hypertrophy was mediated *via* inhibition of the MEK/ERK and TGFβ-Smad signaling pathways. We also demonstrated that CAPE protected H9c2 cells from PE-induced hypertrophy *in vitro via* a similar molecular mechanism as seen in the mouse heart. Finally, CAPE seemed to be as effective as RS for treatment of pressure overload induced mouse cardiac hypertrophy.
Significance: Our results suggest that CAPE may play an important role in the regulation of cardiac hypertrophy induced by pressure overload *via* negative regulation of the MEK/ERK and TGFβ/Smad signaling pathways. These results indicate that CAPE could potentially be used for treatment of cardiac hypertrophy.

1. Introduction

Cardiac hypertrophy is a common pathway of pathological stimulus such as excessive pressure overload, sympathetic activation, and aberrant expression of myocardial contractile proteins caused by gene mutations [1]. It is a complex process involving the activation or inhibition of multiple signaling pathways. The maladjustment of these signaling pathways eventually promotes the progression of pathological cardiac hypertrophy evidenced by increased left ventricle volume, accumulation of proteins, and re-expression of fetal genes resulting in cardiac dysfunction and heart failure. Available reports have pointed out that the MAPK signaling pathway plays important roles in the regulation of cardiac hypertrophy [2]. ERK1/2, JNK1/2, and p38 can be activated by oxidative stress, pressure overload, and neurohumoral factors [3]. MAPK has been considered a therapeutic target for intervention in cardiac hypertrophy.

Caffeic acid phenethyl ester (CAPE), a natural flavonoid-derivative, is an active phenolic part of bee propolis which has been used in folk medicine in Asia since ancient times. CAPE has been shown to have extensive biological effects, by modulating processes such as immune responses, cell proliferation, and apoptosis [4], and there have been several reports on attempts to investigate the underlying mechanisms. Recent publications have illustrated that its anti-inflammatory effect was more apparent compared to other components of propolis, because it strongly modulates the arachidonic acid cascade [5]. Its anti-inflammatory function is also associated with the reduction of c-jun-N-terminal kinase and nuclear factor kappa-B (NF-κB), and the down-regulation of cyclooxygenase (COX)-2 expression. CAPE has also been suggested to be involved in the regulation of the MAPK signaling pathway [6,7]. In human breast cancer MCF-7 cells, CAPE activates the phosphorylation of p38 and JNK leading to apoptosis through activation of Fas [6], while in mouse intestinal epithelial cells, CAPE appears to be p38-independent for the regulation of inflammation mediated by TNF-α. In HepG2 cells, CAPE regulates oxidative stress associated with the post-translational

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phosphorylation of ERK [8]. These reports demonstrate that CAPE is closely linked to the regulation of the MAPK signaling pathway, but the roles of CAPE appear to be context-dependent.

Epidemiological study has shown that regular honey intake is associated with a reduced risk of cardiovascular diseases [9]. It was also reported that CAPE shows cardioprotective effects in short-term myocardial ischemia in rats by reducing activities of xanthine oxidase (XO) and adenosine deaminase (ADA), and direct antioxidant effects [10]. The suggested mechanism was a reduction of cardiomyocyte apoptosis *via* CAPE-mediated inhibition of p38 MAPK activation and caspase-3 activity, along with reduction of the proinflammatory cytokines (IL-1b and TNF-a) in cardiac tissues [11]. Therefore, CAPE-mediated protection of cardiac myocytes from I/R injury is possibly through suppression of both inflammatory signaling and cell death. However, the molecular mechanisms underlying cardiac hypertrophy are different from those of I/R injury. The purpose of the current study was to investigate the effects of CAPE on cardiac hypertrophy induced by pressure overload, and the underlying mechanisms.

2. Materials and methods

2.1. Chemicals

Caffeic acid phenethyl ester (CAPE) (>98% purity) was purchased from Shanghai Winherb Medical Science Co., Ltd. (Shanghai, China).

2.2. Animal models

The animal protocol was approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University (protocol number: 00013274 Wuhan, China) and was in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Forty male mice (C57 background) aged 8–10 weeks (weight, 23.5–27.5 g) were used in this study. The C57 male mice were purchased from the Institute of Laboratory Animal Science, CAMS & PUMC (Beijing, China). All animals were allowed to acclimatize to the laboratory environment for 1 week. Aortic banding (AB) [12] was done to induce cardiac hypertrophy following reported methods [13]. Briefly, mice were anaesthetized and a horizontal skin incision was made at the 2–3 intercostal space. The descending aorta was isolated and a 24-gauge needle was placed next to the aorta, and then a 7–0 silk suture was tied around the needle and the aorta. Finally, the needle was quickly removed after ligation. The mice in sham groups underwent the same procedure without ligation. All animals were randomly assigned to four groups, 10 mice in each group: in the blank control group (CON), mice were treated with normal saline (NS) containing 0.5% carboxymethylcellulose; in the caffeic acid phenethyl ester treatment group (CAPE), mice were treated with CAPE suspension at 100 mg/kg/day by oral gavage once a day; in the aortic banding group (AB), mice were treated with normal saline (NS) containing 0.5% carboxymethylcellulose; in the AB + CAPE group, mice were treated with CAPE suspension at 100 mg/kg/day by oral gavage once a day. All of these treatments began 3 days after AB surgery, for a period of 6 weeks. The dose of CAPE administered was based on the protocol described in a previous study [14]. CAPE suspension at a concentration of 10 mg/ml was prepared in 0.5% carboxymethylcellulose normal saline (NS) for the animal experiments. Suspensions were freshly prepared and administered at 100 mg/kg/day by oral gavage once a day. Six weeks after surgery, the mice were euthanized with 1.5% isoflurane for echocardiography and hemodynamics testing, and then the mice were sacrificed using cervical dislocation. Hearts, lungs, and tibiae of the sacrificed mice were dissected and weighed or measured to compare the heart weight (HW)/body weight (BW) ratio (in mg/g), the HW/tibial length (TL) ratio (in mg/mm) and the lung weight (LW)/BW (in mg/g) ratio in the different groups. All surgeries and analyses were performed in a blinded manner. To further validate the effects of CAPE in preventing the mouse heart from cardiac hypertrophy, we selected a well-known compound, Resveratrol (RS) [15], for comparing the treatment effects between CAPE and RS. Briefly, the RS (100 mg/kg·d) and CAPE was administration for mouse with or without AB surgery in a period of 4 weeks. And then, echocardiography was performed to evaluate the mouse function. The mouse hearts, LW and BW were used to assay the cardiac hypertrophy among different group. This experiment was designed into 5 groups:CAPE treatment group (CAPE), RS treatment group (RS), AB surgery group (AB), AB + CAPE group and AB + RS group.

2.3. Echocardiography and hemodynamics

Echocardiography measurements were performed using a MyLab 30CV ultrasound (Biosound Esaote Inc.) with a 10-MHz linear array ultrasound transducer. The left ventricle (LV) dimensions were evaluated in the parasternal short-axis view. End-systole and end-diastole were identified as the phases in which the smallest and largest areas of the LV were obtained, respectively. The ejection fraction (EF) and fractional shortening (FS) were calculated based on the detected parameters.

For hemodynamics determination, a micro-tip catheter transducer (SPR-839, Millar Instruments, Houston, Texas, USA) was inserted into the right carotid artery and advanced into the left ventricle. The pressure signals and heart rate were recorded continuously using an ARIA pressure-volume conductance system coupled to a Powerlab/4SP A/D converter. The signals were continuously recorded by a Millar Pressure Volume system, and the maximal rate of pressure development (dP/dtmax) and minimal rate of pressure decay (dP/dtmin) were processed using PVAN data analysis software [16].

2.4. Histological analysis

Hearts of mice were excised, arrested in diastole with 10% KCl to ensure that they were stopped in diastole, and placed in 10% formalin. After rehydration, heart tissue sections (5 mm) were prepared and stained with hematoxylin-eosin (H&E) for histopathology, or Picrosirius red (PSR) for interstitial fibrosis, detected by light microscopy. A single myocyte was observed using an image quantitative digital analysis system (Image-Pro Plus 6.0). The outline of 150 myocytes in the left ventricle was traced for evaluation of cardiomyocyte hypertrophy in each group.

2.5. Western blot

LV tissues were lysed in RIPA lysis buffer. The lysates were placed on ice for 15 min, followed by centrifugation at 12000*g* for 30 min at 4 °C. The isolated proteins were quantified using a BCA Protein Assay Kit. Fifty micrograms of the extracted protein was used for SDS-PAGE gel electrophoresis. Subsequently, the protein blots were transferred to nitrocellulose membrane and were blocked with 5% (w/v) non-fat milk for 1 h at room temperature. Then, the protein blots were incubated with the specific primary antibodies overnight. The blots were then incubated with the secondary antibody for 1 h at room temperature in the dark. Finally, immunoblots were scanned using an Odyssey Imaging System. The expression levels of specific phosphorylated proteins were normalized to total protein or GAPDH on the same membrane.

The following primary antibodies were used in this study: GAPDH (#2118), p-MRK (#9154S), T-MRK1/2 (#9122S), p-ERK1/2$^{thr202/Tyr2041/2}$ (#4370p), T-ERK1/2(#4695), p-p38 (#4511), T-p38 (#9212), p-JNK$^{T183/Y185}$ (#4668p), T-JNK (#9258), TGF-β (SC-9053), p-Smad1/5$^{Ser463/465}$ (#9516), T-Smad1/5 (SC-6210), p-Smad3 $^{Ser423/425}$ (#8769), and T-Smad3 (SC-101154).