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Baicalein Protects Against Cardiac Hypertrophy Through Blocking MEK-ERK1/2 Signaling

Jing Zong,[1,2] Da-ping Zhang,[1,2] Heng Zhou,[1,2] Zhou-yan Bian,[1,2] Wei Deng,[1,2] Jia Dai,[1,2] Yuan Yuan,[1,2] Hua-wen Gan,[1,2] Hai-peng Guo,[3] and Qi-zhu Tang[1,2]*

[1]*Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan 430060, China*

[2]*Cardiovascular Research Institute of Wuhan University, Wuhan 430060, China*

[3]*The Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Ministry of Health, Qilu Hospital of Shandong University, Jinan 250012, China*

ABSTRACT

Baicalein, a flavonoid present in the root of *Scutellaria baicalensis*, is well known for its antibacterial, antiviral, anti-inflammatory, antithrombotic, and antioxidant effects. Here we show that baicalein also attenuates cardiac hypertrophy. Aortic banding (AB) was performed to induce cardiac hypertrophy secondary to pressure overload in mice. Mouse chow containing 0.05% baicalein (dose: 100 mg/kg/day baicalein) was begun 1 week prior to surgery and continued for 8 weeks after surgery. Our data demonstrated that baicalein prevented cardiac hypertrophy and fibrosis induced by AB, as assessed by echocardiographic and hemodynamic parameters and by pathological and molecular analysis. The inhibitory action of baicalein on cardiac hypertrophy was mediated by effects on mitogen-activated protein kinase kinase (MEK)-extracellular signal-regulated kinases (ERK1/2) signaling and GATA-4 activation. In vitro studies performed in rat cardiac H9c2 cells confirmed that baicalein attenuated cardiomyocyte hypertrophy induced by angiotensin II, which was associated with inhibiting MEK-ERK1/2 signaling. In conclusion, our results suggest that baicalein has protective potential for targeting cardiac hypertrophy and fibrosis through suppression of MEK-ERK1/2 signaling. Baicalein warrants further research as a potential antihypertrophic agent that might be clinically useful to treat cardiac hypertrophy and heart failure. J. Cell. Biochem. 114: 1058–1065, 2013.

KEY WORDS: BAICALEIN; CARDIAC HYPERTROPHY; FIBROSIS; MEK; ERK1/2

Cardiac hypertrophy gradually develops in response to the long-term systolic over load produced by aortic banding (AB). Although this initially appears to be an adaptive response, cardiac hypertrophy can result in dilated cardiomyopathy, heart failure, or sudden death [Rosen et al., 2009; Jefferies and Towbin, 2010]. Previous reports have confirmed that left ventricular hypertrophy can significantly increase the incidence of sudden death, ventricular arrhythmia, myocardial ischemia, heart failure and mortality, and cardiac hypertrophy has become an independent risk factor for cardiovascular disease [Wachtell et al., 2007]. However, there is no effective clinical strategy for the prevention of cardiac hypertrophy. Thus, it is of importance to find new targets for the prevention of myocardial hypertrophy.

Baicalein (5,6,7-trihydroxyflavone) is one of the major flavonoids from the root of *Scutellaria baicalensis* Georgi (Huangqin), a traditional Chinese medicine used for hundreds of years. Baicalein has been widely employed as a popular antibacterial, antiviral, and anti-inflammatory agent. It can inhibit eotaxin production through the prevention of eotaxin mRNA expression. Baicalein has also been shown to be a lipoxygenase inhibitor, and it induces apoptosis in several cancer cells such as breast carcinoma cells, colon carcinoma cells, and leukemia cells [Tong et al., 2002; Kovarikova et al., 2004; Chow and Shen, 2006]. Recent investigations have shown that baicalein possesses anti-inflammatory [Hsieh et al., 2007] and antioxidant effects [Shieh et al., 2000]. Baicalein exhibits free radical-scavenging activity and attenuates oxidative stress in

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*Correspondence to: Qi-zhu Tang, MD, Department of Cardiology, Renmin Hospital of Wuhan University, Cardiovascular Research Institute of Wuhan University, JieFang Road 238, Wuhan 430060, China. E-mail: qztang@whu.edu.cn

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cardiomyocytes [Shao et al., 1999, 2002]. In several settings, baicalein has been shown to be neuroprotective [Chen et al., 2004]. However, the effects of baicalein on cardiac hypertrophy and the related signaling mechanisms have not yet been reported. In the present study, we show that baicalein protects against cardiac hypertrophy by blocking MEK-ERK1/2 signaling. Our studies suggest that baicalein might have the therapeutic utility in the treatment of cardiac hypertrophy.

MATERIALS AND METHODS

MATERIALS, ANIMAL, AND ANIMAL MODELS

The primary antibodies included antibodies specific for p-MEK1/2 (Cell Signaling Technology, 9154), T-MEK1/2 (Cell Signaling Technology, 9122), p-ERK1/2 (Cell Signaling Technology, 4370), T-ERK1/2 (Cell Signaling Technology, 4695), p-P38 (Cell Signaling Technology, 4511), T-P38 (Cell Signaling Technology, 9212), p-JNK (Cell Signaling Technology, 4668), T-JNK (Cell Signaling Technology, 9258), p-GATA4 (Santa Cruz Biotechnology, Sc32823), T-GATA4 (Santa Cruz Biotechnology, Sc9053), and GAPDH (Bioworld Technology, MB001). Baicalein (98% purity as determined by HPLC analysis) was ordered from Shanghai Medical Technology Development Co., Ltd, Harmony. Baicalein (98%) was purchased from Aldrich (CAS 491-67-8) and dissolved in dimethyl sulfoxide (DMSO) (Sigma, Lot RNBC0311) for the in vitro bioassay. All protocols were approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University. The surgery and subsequent analyses were performed in a blinded fashion for all groups. Adult male C57/BL6 mice (8–10 weeks old) were used. AB was performed as described previously [Bian et al., 2010]. Mice (15–20 per group) received normal feed or feed containing 0.05% baicalein (dose: 100 mg/kg/day baicalein). After one week we subjected the mice to either chronic pressure overload generated by AB or sham surgery as the control group. After the mice had been killed, the hearts and lungs were dissected out and weighed to compare heart weight/body weight (HW/BW, mg/g), lung weight/body weight (LW/BW, mg/g), and heart weight/tibia length (HW/TL, mg/ml) ratios in baicalein-treated and vehicle-treated mice.

ECHOCARDIOGRAPHY AND HEMODYNAMICS

Echocardiography was performed by Mylab30CV ESAOTE S.P.A. with a 10 MHz linear array ultrasound transducer. The dimensions of left ventricle (LV) were assessed in both parasternal long-axis and short-axis views at a frame rate of 50 Hz. End-systole or end-diastole was defined as the phase in which the smallest or largest area of LV, respectively.

For hemodynamic measurements, mice were anesthetized with 1.5% isoflurane, microtip catheter transducer (SPR-839, Millar Instruments, Houston, TX) was inserted into the right carotid artery and advanced into the LV. The pressure signals and heart rate were recorded continuously with a Millar Pressure-Volume System (MPVS-400, Millar Instruments), and the data were processed by PVAN data analysis software.

HISTOLOGICAL ANALYSIS

Hearts were excised, washed with saline solution, and placed in 10% formalin. Hearts were cut transversely close to the apex to visualize the left and right ventricles. Several sections of heart (4–5 μm thick) were prepared and stained with H&E for histopathology or PSR for collagen deposition and then visualized by light microscopy. For myocyte cross-sectional area, sections were stained for membranes with FITC-conjugated WGA (Invitrogen) and for nuclei with DAPI. A single myocyte was measured with an image quantitative digital analysis system (Image Pro-Plus, version 6.0). The outline of 100 myocytes was traced in each group.

QUANTITATIVE REAL-TIME RT-PCR

Real-time PCR was used to detect RNA expression levels of hypertrophic and fibrotic markers. Total RNA was extracted from frozen, pulverized mouse cardiac tissue using TRIZol (Roche 15596-026). Their yields and purities were spectrophotometrically estimated using the A_{260}/A_{280} and A_{230}/A_{260} ratios via a SmartSpec Plus Spectrophotometer (Bio-Rad). The RNA (2 μg of each sample) was reverse-transcribed into cDNA using oligo(DT) primers and the Transcriptor First Strand cDNA Synthesis Kit (Roche, 04896866001). The PCR amplifications were quantified by a LightCycler 480 SYBR Green 1 Master Mix (Roche, 04707516001). The results were normalized against glyceraldehydes-3-phosphate dehydrogenase (GAPDH) gene expression. The oligonucleotide primers are shown in Table I.

WESTERN BLOTTING

Cardiac tissues and cultured H9c2 cells were lysed in RIPA lysis buffer. Fifty micrograms of cell lysate was used for SDS/PAGE, and proteins were then transferred to a polyvinylidene difluoride membranes (Millipore). Specific protein expression levels were normalized to the GAPDH protein for total cell lysate and cytosolic proteins on the same polyvinylidene difluoride membranes. Quantification of Western blots was performed by Odyssey infrared imaging system (Li-Cor Biosciences). The secondary antibodies goat anti-Rabbit IRdye@ 800 CW (LI-COR, 926-32211) IgG and goat anti-Mouse IRdye@ 800 CW (LI-COR, 926-32210) were used at 1:10,000

TABLE I. Primer Sequences for RT-PCR Assays

mRNA	Forward	Reverse
ANP	ACCTGCTAGACCACCTGGAG	CCTTGGCTGTTATCTTCGGTACCGG
BNP	GAGGTCACTCCTATCCTCTGG	GCCATTTCCTCCGACTTTTCTC
β-MHC	CCGAGTCCCAGGTCAACAA	CTTCACGGGCACCCTTGGA
α-MHC	GTCCAAGTTCCGCAAGGT	AGGGTCTGCTGGAGAGGTTA
TGF-β1	AACAACGCCATCTATGAG	TATTCCGTCTCCTTGGTT
TGF-β2	TCGACATGGATCAGTTTATGCG	CCCTGGTACTGTTGTAGATGGA
CCN2	TGACCCCTGCGACCCACA	TACACCGACCCACCGAAGACACAG
Col1agenIα	AGGCTTCAGTGGTTTGGATG	CACCAACAGCACCATCGTTA
Col1agen	CCCAACCCAGAGATCCCATT	GAAGCACAGGAGCAGGTGTAGA
GAPDH	ACTCCACTCACGGCAAATTC	TCTCCATGGTGGTGAAGACA
ANP[a]	AAAGCAAACTGAGGG-CTCTGCTCG	TTCGGTACCGGAAGCTGTTGCA
BNP[a]	CAGCAGCTTCTGCATCGTGGAT	TTCCTTAATCTGTCGCCGCTGG
β-MHC[a]	TCTGGACAGCTCCCCATTCT	CAAGGCTAACCTGGAGAAGATG
GAPDH[a]	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT

Sequences are listed 5′–3′.
[a]The PCR used the primers in vitro.

in Odyssey blocking for 1 h. The blots were scanned with the infrared Li-Cor scanner, allowing for simultaneous detection of two targets (antiphospho and anti-total protein) in the same experiment.

CELL CULTURE AND SURFACE AREA

Rat cardiac H9c2 cells (Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were cultured in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, C11995) supplemented with 10% fetal bovine serum (FBS; HyClone, SV30087.02), 100 U/ml penicillin/100 mg/ml streptomycin (Gibco, 15140) and 5% CO_2 at 37°C. The cells were fed every 1–2 days and subcultured once they reached 70–80% confluence. Cells were plated at an appropriate density according to each experimental design. H9c2 cells were seeded in 6-well plates at a density of 0.25×10^6 cells per well. Following 24 h adherence, the culture medium was changed to serum-free DMEM for 12 h before the experiment, and then, cells were incubated with baicalein (5, 25, 50, and 100 μM) in DMEM supplemented with serum-free DMEM at 37°C with/without Ang (Sigma, A9525) (1 μM) for 24 or 48 h.

To identify the rat cardiac H9c2 cells and assess cardiomyocyte hypertrophy, we characterized cells by immunofluorescence for cardiac α-actinin. The cells were washed with PBS, fixed with RCL2 (ALPHELYS, RCL2-CS24L), permeabilized in 0.1% Triton X-100 in PBS, and stained with anti-α-actinin (Millipore, 05-384) at a dilution of 1:100 in 1% goat serum. The secondary antibody was Alexa FluorH 488 goat anti-mouse IgG (Invitrogen, A11001). The cells on coverslips were mounted onto glass slides with SlowFade Gold antifade reagent with DAPI (Invitrogen, S36939).

STATISTICAL ANALYSIS

Data were expressed as means ± SEM. Differences among groups were determined by two-way ANOVA followed by a post hoc Tukey test. Comparisons between two groups were performed by unpaired Student's *t*-test. $P < 0.05$ was considered to be significantly different.

RESULTS

BAICALEIN ATTENUATED CARDIAC HYPERTROPHY INDUCED BY PRESSURE-OVERLOAD

To determine whether baicalein antagonized the hypertrophic response to pressure overload, mice were subjected to either AB surgery or sham surgery. Anatomical and hemodynamic parameters in mice before AB surgery were no significant difference (Table II). The echocardiographic data and pressure–volume (PV) loop analyses were supported by morphologic analysis. Baicalein treatment prevented the development of adverse cardiac remodeling and ventricular dysfunction, as evidenced by decreased IVSd (left ventricular septum, diastolic), IVSs (left ventricular septum, systolic), LVEDD (left ventricular end-diastolic diameter), LVESD (left ventricular end-systolic diameter) and increased percent FS (fractional shortening) and EF (Fractional shortening), maximal LV dP/dt (dp/dt max) and minimum LV dP/dt (dp/dt min, absolute value). After 8 weeks of AB, end-systolic pressure was increased. But there were no significant changes between the AB-operated mice (Fig. 1A,B). We also monitored the cardiac function by echocardiography at 2, 4, and 12 weeks after AB surgery (Table III). The ratios of HW/BW, LW/BW, and HW/TL were decreased in baicalein treated mice after AB surgery (Fig. 1C). Gross hearts, H&E, WGA–FITC staining and myocyte cross-sectional areas showed consistent results (Fig. 1D,E). The hypertrophic markers ANP, BNP, and β-MHC were markedly blunted, while α-MHC was increased in baicalein treated mice in response to AB (Fig. 1F). These results suggest that baicalein negatively regulates the extent of cardiac hypertrophy in response to pressure overload.

BAICALEIN ATTENUATED CELL HYPERTROPHY IN VITRO

To further confirm the effect of baicalein on cardiac hypertrophy, we used an in vitro model with Ang II (1 μM) in cultured H9c2 cells. After stimulation with Ang II (1 μM), H9c2 cells showed enlarged cell surface area compared to those induced by baicalein (5, 25, 50, and 100 μM) with Ang II for 48 h (Fig. 2A). Real-time PCR demonstrated that baicalein (5, 25, 50, and 100 μM) markedly decreased the induction of ANP, BNP, and β-MHC mRNA expression by Ang II (1 μM), especially in baicalein (50 μM) group (Fig. 2B). In addition, real-time PCR demonstrated that cells treated with baicalein (50 μM) for 6, 12, and 24 h markedly decreased the induction of ANP, BNP, and β-MHC mRNA expression by Ang II (1 μM) (Fig. 2C). These findings indicated that baicalein attenuated cell hypertrophy in vitro.

BAICALEIN INHIBITS MEK–ERK1/2 SIGNALING PATHWAY IN RESPONSE TO HYPERTROPHIC STIMULI

To explore the molecular mechanisms through which baicalein decreased the hypertrophic response, we examined the state of activation of MAPK in baicalein treated and vehicle treated hearts in response to pressure overload. We found that the phosphorylated levels of MEK1/2, ERK1/2, p38, JNK1/2, and GATA-4 were significantly increased in vehicle treated hearts subjected to AB. However, the increased level of MEK1/2, ERK1/2, and GATA-4 was blocked in baicalein treated hearts, whereas p38 and JNK1/2 was similarly activated in the two groups (Fig. 3A,B). In vitro data confirmed that the activation of MEK, ERK1/2, and GATA-4 in H9c2 cells treatment with baicalein (50 μM) was lower compared to those in response to Ang II (1 μM) (Fig. 3C,D). The results showed that baicalein significantly inhibits cardiac hypertrophy through direct inhibition of MEK-ERK1/2 signaling Pathway.

TABLE II. Anatomical and Hemodynamic Parameters in Mice Before AB Surgery

Parameter	Vehicle (n = 8)	Baicalein (n = 8)
HW/BW (mg/g)	4.58 ± 0.11	4.28 ± 0.09
LW/BW (mg/g)	5.09 ± 0.11	4.98 ± 0.14
HW/TL (mg/mm)	6.53 ± 0.23	6.26 ± 0.22
LVEDD (mm)	3.56 ± 0.05	3.58 ± 0.06
LVESD (mm)	2.01 ± 0.02	2.01 ± 0.03
EF (%)	80.88 ± 0.79	81.25 ± 0.62
FS (%)	44.13 ± 0.55	43.63 ± 0.56

HW/BW, heart weight/body weight; LW/BW, lung weight/body weight; HW/TL, heart weight/tibia length; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; EF, ejection fraction; FS, fractional shortening.

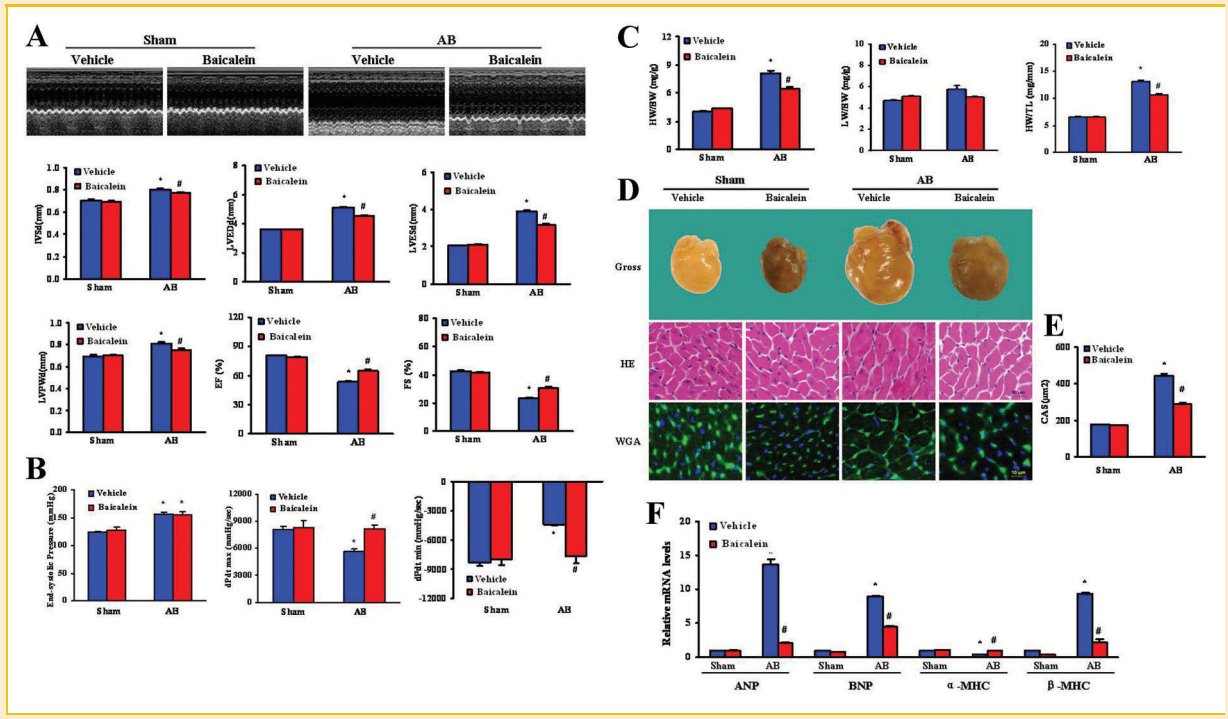

Fig. 1. Baicalein attenuated cardiac hypertrophy induced by pressure-overload. A: Echocardiography and (B) pressure volume results from the four groups of mice at 8 weeks post-AB or sham surgery. C: Statistical results of HW/BW ratio, LW/BW ratio, HW/TL ratio and myocyte cross-sectional areas (n = 100 cells per section) at 8 weeks post-AB surgery (n = 14). D: Histology. Top, gross hearts; middle, representative H&E staining; bottom, WGA–FITC staining at 8 weeks post-AB surgery. E: Expression of transcripts for ANP, BNP, β-MHC and α-MHC induced by AB were determined by RT-PCR analysis (n = 4). $^{*}P < 0.05$ for difference from corresponding sham group. $^{\#}P < 0.05$ versus AB/vehicle group after AB.

BAICALEIN INHIBITED THE FIBROSIS INDUCED BY PRESSURE-OVERLOAD

To determine the extent of fibrosis in the heart, paraffin-embedded slides were stained with picrosirius red (PSR) and Masson trichrome staining. Perivascular and interstitial fibrosis was detected in both vehicle treated and baicalein treated mice subjected to AB, but the extent of cardiac fibrosis was remarkably reduced in baicalein treated mice (Fig. 4A,B). Fibrotic areas from histological sections were quantified using an image-analyzing system (n = 6) (Fig. 4C). Subsequent analysis of mRNA expression levels of known mediators of fibrosis including TGF-β1, Col1agen Iα, Col1agen III, and CCN2, also demonstrated a blunted response in baicalein treated mice (Fig. 4D).

TABLE III. Body Weight and Hemodynamic Parameters in Mice After AB Surgery

	Sham	Sham	AB	AB
Parameter	Vehicle	Baicalein	Vehicle	Baicalein
2 weeks	n = 6	n = 7	n = 9	n = 9
BW (g)	26.64 ± 0.52	25.28 ± 0.26	26.53 ± 0.54	27.09 ± 0.29
LVEDD (mm)	3.41 ± 0.03	3.53 ± 0.03	4.36 ± 0.10*	4.10 ± 0.03*
LVESD (mm)	1.85 ± 0.03	1.91 ± 0.04	3.06 ± 0.12*	2.59 ± 0.04#
FS(%)	46.17 ± 0.63	45.57 ± 1.14	29.93 ± 1.32*	36.22 ± 0.49#
4 weeks	n = 8	n = 7	n = 8	n = 9
BW (g)	27.61 ± 0.19	27.54 ± 0.36	27.63 ± 0.47	27.11 ± 0.26
LVEDD (mm)	3.70 ± 0.03	3.73 ± 0.07	4.73 ± 0.01*	4.42 ± 0.07#
LVESD (mm)	2.08 ± 0.05	2.10 ± 0.09	3.52 ± 0.04*	3.02 ± 0.07#
FS(%)	43.88 ± 0.95	43.71 ± 1.48	26.34 ± 0.22*	31.89 ± 0.77#
12 weeks	n = 7	n = 7	n = 6	n = 6
BW (g)	30.22 ± 0.73	29.74 ± 0.63	29.61 ± 0.31	29.40 ± 0.19
LVEDD (mm)	3.92 ± 0.04	3.64 ± 0.02	5.50 ± 0.18*	4.70 ± 0.15#
LVESD (mm)	2.20 ± 0.04	2.06 ± 0.03	4.40 ± 0.20*	3.45 ± 0.15#
FS (%)	43.71 ± 0.89	42.71 ± 0.47	20.67 ± 1.17*	26.50 ± 1.02#

HW, body weight; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; EF, ejection fraction; FS, fractional shortening.
$^{*}P < 0.05$ for difference from corresponding sham group.
$^{\#}P < 0.05$ versus AB/vehicle group after AB.

DISCUSSION

In the present study, we examined the role of baicalein in cardiac hypertrophy and fibrosis induced by pressure overload in vivo and by Ang II in vitro. The results demonstrated that baicalein significantly blunted hypertrophy, chamber dilation and fibrosis by disruption of MEK-ERK1/2 signaling following chronic pressure overload. To our knowledge, this is the first study to demonstrate an important role of baicalein in the regulation of cardiac hypertrophy and fibrosis.

The mechanism by which baicalein mediates its antihypertrophic effect remains unclear. Cardiac hypertrophy is a pathophysiological process involving a variety of complex factors. A great number of signaling pathways and several transcription factors are implicated in the pathogenesis of cardiac hypertrophy. Recent studies demonstrated that MAPK signaling pathways play a considerable

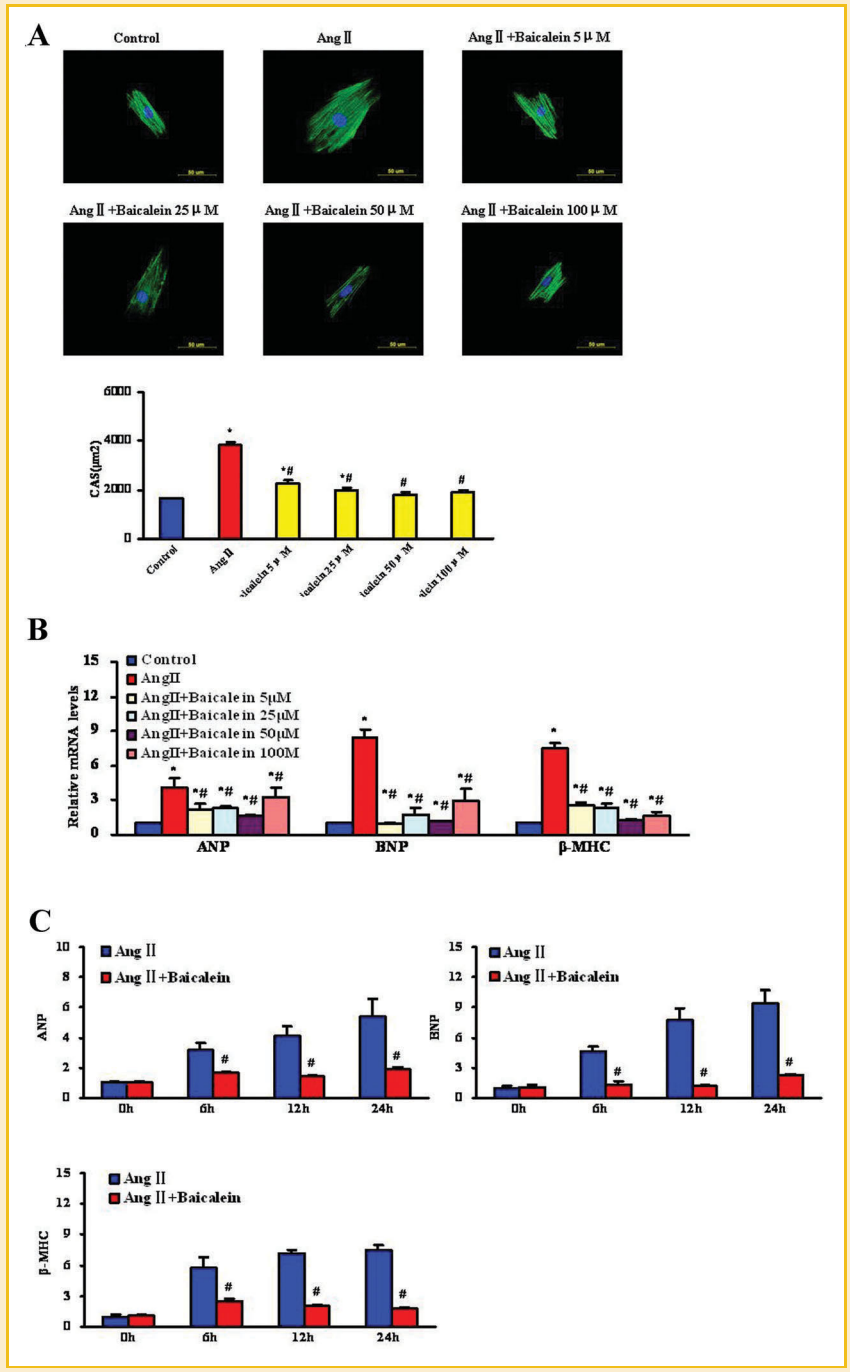

Fig. 2. Baicalein attenuated cell hypertrophy in vitro. A: Effect of baicalein on the shrink of H9c2 cells induced by Ang II (1 μM) for 48 h. B: Real-time PCR analysis of the mRNA levels of ANP, BNP, and β-MHC induced by baicalein (5, 25, 50, and 100 μM) with Ang II (1 μM) for 24 h. C: Real-time PCR analysis of the mRNA levels of ANP, BNP, and β-MHC induced by baicalein (50 μM) with Ang II (1 μM) at the time points indicated. $^{*}P < 0.05$ versus vehicle group at the 0 time point. $^{\#}P < 0.05$ versus vehicle group at the same time point.

role in the progress/pathogenesis of cardiac hypertrophy [Bueno et al., 2001; Lorenz et al., 2009a]. Blockade of MAPK signaling pathways prevented the progression of cardiac hypertrophy [Bueno et al., 2001; Lorenz et al., 2009b]. Several members of MAPK family have been isolated which include ERKs, JNKs, and p38 MAPK. ERK1 and ERK2 play a critical role in cardiac hypertrophy, and are activated by mitogen-activated protein kinase kinase (MEK or MAPKK)-dependent phosphorylation [Lorenz et al., 2009a]. Since ERK1/2 is activated by a large variety of prohypertrophic stimuli, they may appear to be an ideal target for small molecules aimed at

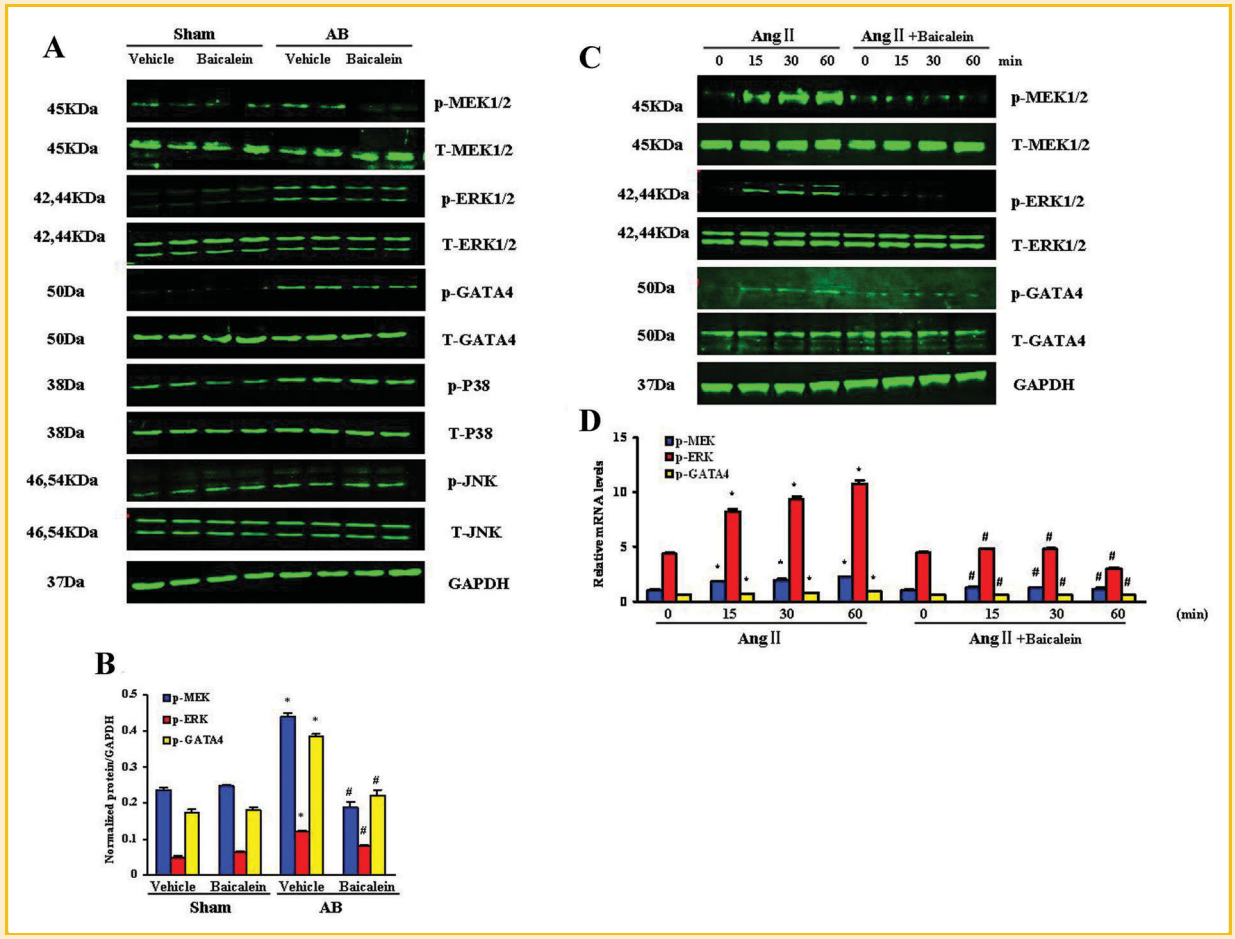

Fig. 3. Baicalein inhibits MEK–ERK1/2 signaling Pathway in response to hypertrophic stimuli. A,B: The levels of total and phosphorylated MEK1/2, ERK1/2, GATA-4 expression in the heart tissues of mice in the indicated groups ($n = 4$). A: Representative blots. B: Quantitative results. $^{*}P < 0.05$ for difference from the corresponding sham group. $^{\#}P < 0.05$ versus AB/vehicle group after AB. C,D: The levels of total and phosphorylated MEK1/2, ERK1/2, GATA-4 expression in H9c2 cells treated with Baicalein (50 μM) with Ang II (1 μM) at the time points indicated. C: Representative blots. D: Quantitative results. $^{*}P < 0.05$ versus Ang II group at the 0 time point. $^{\#}P < 0.05$ versus Ang II group at the same time point.

reducing cardiac hypertrophy. However, a number of similarly designed culture-based studies have disputed this conclusion with those data suggesting that MKK1-ERK1/2 does not regulate cardiac hypertrophy [Thorburn et al., 1994; Ramirez et al., 1997]. To examine the molecular mechanisms involved in baicalein protection against cardiac hypertrophy, we examined the status of MAPKs signaling in our hypertrophic models. An important finding of this study is that MEK and ERK1/2 activation were inhibited in baicalein treated hearts and H9c2 cells (50 μM) compared with vehicle treated hearts in response to chronic pressure overload and H9c2 cells stimulated with Ang II (1 μM). ERK1/2 signaling has been associated with phosphorylation and activation of the cardiac-enriched transcription factor GATA4 [Morimoto et al., 2000; Liang et al., 2001]. Previous studies have found GATA4 may function as a transcriptional effecter acting downstream from the ERK1/2 signaling pathway activated by hypertrophic stimulation [Akazawa and Komuro, 2003]. Therefore, the effects of baicalein on GATA-4 expression were further examined in vivo and vitro. The findings indicate that the inhibitory effects of baicalein on cardiac hypertrophy are mediated through MEK-ERK1/2 signaling.

Cardiac fibrosis is an important pathologic finding in various cardiovascular diseases and refers to an excessive deposition of extracellular matrix components in the heart, which leads to cardiac dysfunction and can result in heart failure [Daniels et al., 2009]. TGF-β1 is a critical factor which stimulates both myofibroblast formation and collagen production [Berk et al., 2007]. In the pressure-overloaded rat heart, using specific neutralizing antibodies to block the function of TGF-β1 prevented the induction of collagen mRNA, myocardial fibrosis, and diastolic dysfunction [Kuwahara et al., 2002]. Overexpression of TGF-β1 in transgenic mice results in cardiac hypertrophy that is characterized by both interstitial fibrosis and hypertrophic growth of cardiac myocytes [Rosenkranz et al., 2002]. The crucial role of CCN2 as a regulator of tissue fibrosis has been described in the heart [Koitabashi et al., 2007]. Together with ANF, BNP, and α-actin, CCN2 is considered one of the marker genes of hypertrophy and heart failure [Koitabashi et al., 2007; Kong et al., 2011]. The antifibrotic effects of baicalein were illustrated by the

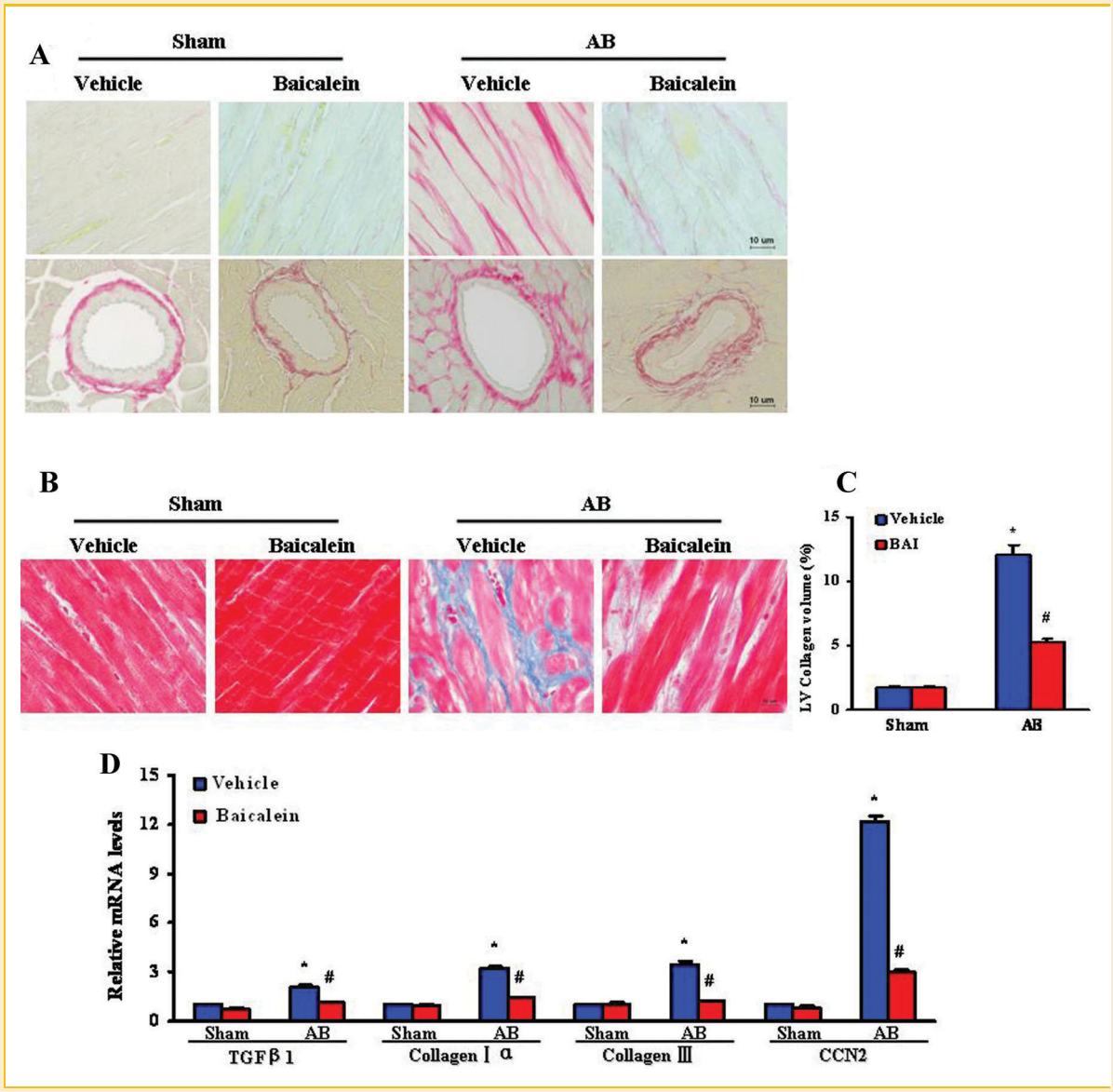

Fig. 4. Baicalein inhibited the fibrosis induced by pressure-overload. A,B: Determination of myocardial fibrosis by PSR (A) and Masson trichrome staining (B) on histological sections of the LV was performed on each group 8 weeks after AB. C: Fibrotic areas were quantified using an image-analyzing system ($n = 6$). D: Baicalein inhibited the mRNA expression of TGF-β1, Collagen Iα, Collagen III and CCN2 ($n = 6$). $^{*}P < 0.05$ for difference from corresponding sham group. $^{\#}P < 0.05$ versus AB/vehicle group after AB.

suppressed expression of LV pro-collagens I and III accompanied by the decreased expression of 12-lipoxygenase in spontaneously hypertensive rats [Daniels et al., 2009]. We found that mRNA expression levels of TGF-β1, Col1agen Iα, Col1agen III, and CCN2 were blunted in baicalein treated mice.

In conclusion, our present study indicated that baicalein protected against cardiac hypertrophy and fibrosis in response to chronic pressure overload by regulation of the MEK-ERK1/2 signaling pathway. Our observations revealed new insight into the pathogenesis of cardiac remodeling and may have considerable implications for the development of strategies for the treatment of cardiac hypertrophy and heart failure through the application of baicalein. Additional studies are necessary to explore the potential clinical use of baicalein.

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