Baicalin Attenuates Cardiac Dysfunction and Myocardial Remodeling in a Chronic Pressure-Overload Mice Model

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Key Words

Hypertrophy • Pressure overload • Baicalin

Abstract

Background/Aims: Baicalin has been shown to be effective for various animal models of cardiovascular diseases, such as pulmonary hypertension, atherosclerosis and myocardial ischaemic injury. However, whether baicalin plays a role in cardiac hypertrophy remains unknown. Here we investigated the protective effects of baicalin on cardiac hypertrophy induced by pressure overload and explored the potential mechanisms involved. Methods: C57BL/6J-mice were treated with baicalin or vehicle following transverse aortic constriction or Sham surgery for up to 8 weeks, and at different time points, cardiac function and heart size measurement and histological and biochemical examination were performed. Results: Mice under pressure overload exhibited cardiac dysfunction, high mortality, myocardial hypertrophy, increased apoptosis and fibrosis markers, and suppressed cardiac expression of PPAR\textgreek{a} and PPAR\textgreek{b}/\textgreek{\delta}. However, oral administration of baicalin improved cardiac dysfunction, decreased mortality, and attenuated histological and biochemical changes described above. These protective effects of baicalin were associated with reduced heart and cardiomyocyte size, lower fetal genes expression, attenuated cardiac fibrosis, lower expression of profibrotic markers, and decreased apoptosis signals in heart tissue. Moreover, we found that baicalin induced PPAR\textgreek{a} and PPAR\textgreek{b}/\textgreek{\delta} expression in vivo and in vitro. Subsequent experiments demonstrated that long-term baicalin treatment presented no obvious cardiac lipotoxicity. Conclusions: The present results demonstrated that baicalin attenuates pressure overload induced cardiac dysfunction and ventricular remodeling, which would be due to suppressed cardiac hypertrophy, fibrosis, apoptosis and metabolic abnormality.

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Introduction

During the past half-century, great advances in the prevention, diagnosis, and treatment of cardiovascular diseases have been made with the notable exception of heart failure, which is a major global health problem with an estimated prevalence of 38 million patients worldwide [1]. An important cause of heart failure is chronic pressure overload due to aortic stenosis or hypertension, which leads to cardiac hypertrophy that may progress to heart failure. Uncompensated cardiac hypertrophy is characterized by enhanced expression of fetal genes, excessive increase in myocardial mass, abnormal accumulation of extracellular matrix, and severe abnormality of energy metabolism [2-4]. Growing evidence has confirmed that uncompensated cardiac hypertrophy is correlated with an increase in glucose utilization and a decrease in fatty acid utilization (FAU). This metabolic substrate switch seems to involve changes in mRNA level of genes implicated in the transport and metabolism of glucose and fatty acids, which are primarily regulated by a class of nuclear receptors called peroxisome proliferator-activated receptors (PPARs) [3].

PPARα is a key regulator of myocardial fatty acid uptake and oxidation in the heart [5]. During pathological growth of heart failure, down-regulation of FAU genes correlates with decreased PPARα level [6]. It has been shown that activation of PPARα with medium-chain triglycerides is able to prevent the reduction of FAU and reverse the development of cardiac hypertrophy [6, 7]. However, mice with cardiac-restricted overexpression of PPARα (MHC-PPARα) has been shown to develop cardiomyopathy with cardiac dysfunction, and enhanced myocardial lipid accumulation followed by high fat diet (HFD) [5, 8]. Similar to PPARα, PPARβ/δ is also highly expressed in cardiac myocytes and necessary for maintaining myocardial lipid homeostasis [9]. Cardiac PPARβ/δ level was repressed by pressure overload in a hypoxia dependent pathway [10]. Mice with cardiac-specific deletion of the PPARβ/δ has been shown to develop cardiomyopathy with cardiac dysfunction and myocardial lipid accumulation [10, 11]. Conversely, mice with cardiac-restricted overexpression of PPARβ/δ (MHC-PPARβ/δ) did not accumulate myocardial lipid, and exhibited increased myocardial glucose utilization and normal cardiac function [8]. Additionally, reactivation of cardiac PPARβ/δ is sufficient to reverse cardiac hypertrophy in vitro and in vivo [10, 12].

Baicalin (Fig. 1) is a natural flavone compound abundant in the rhizome of the perennial herb Scutellaria baicalensis, known as huangqin in Chinese traditional medicine [13]. By using ApoE−/− mice, our group previously demonstrated that baicalin attenuates atherosclerosis through lipids regulation and dendritic cells inhibition [14, 15]. Another evidence revealed protective effects of baicalin on ER stress-induced cardiomyocytes apoptosis, acute myocardial infarction injury, and hypoxic pulmonary hypertension [16-18]. However, no study has addressed the effect of baicalin on pressure overload-induced cardiac hypertrophy. Therefore, in this study, we aimed to investigate the potential role of baicalin on pressure overload induced heart failure.

Materials and Methods

Ethics statement

All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the NIH and approved by The Institutional Animal Care and Use Committee at Tongji Medical College, Huazhong University of Science and Technology (IACUC Number: 477).

Animals and experimental groups

Male C57BL/6 mice aged 8-9 weeks were purchased from Beijing HFK Bioscience (Beijing, China). All animals were kept on a chow diet in a 12-hour light/12-hour dark environment at 25°C in the Tongji Medical School Experimental Animal Center. Mice were randomly assigned to four groups: Sham with vehicle (Sham+Veh), Sham with baicalin (Sham+BAI), TAC with vehicle (TAC+Veh), TAC with baicalin (TAC+BAI). Mice were treated orally with baicalin (gavage, 100 mg/kg/day) or vehicle for 4 weeks following TAC or
Sham operation. In some experiments, mice were treated orally with baicalin or vehicle for 8 weeks following TAC operation. For diet studies, mice were allowed ad libitum access to HFD for 4 weeks, which provides 43% of the calories from fat (Beijing HFK Bioscience, Beijing, China), accompanied by daily administration (gavage, 100 mg/kg/day) of baicalin or vehicle. At the end of each experiment, mice were sacrificed by cervical vertebra dislocation following anesthesia with pentobarbital sodium (50 mg/kg) intraperitoneally. Then, hearts were harvested, weighed, excised and snap-frozen in liquid nitrogen for protein and RNA extraction or fixed for histological analysis. In the present study, baicalin was purchased from Sigma (St. Louis, Missouri, USA) and dissolved in normal saline and administrated by gavage. The concentration of baicalin (100 mg/kg/day) were chosen according to our previous studies[14, 15].

**Transverse aortic constriction (TAC)**

We established pressure overload-induced cardiac hypertrophy via TAC as previously described [19]. Briefly, mice were anesthetized with 3% pentobarbital sodium (50 mg/kg). A thoracotomy was performed to visualize the aortic arch. TAC was created using a 6-0 suture banded between the carotid arteries over a 27-gauge needle. The needle was then gently removed, creating a 60-80% constriction with an aortic outer diameter of approximately 0.3 mm. Sham control animals underwent corresponding surgery without aortic restriction. Following chest closing and skin suturing, mice were allowed to recover and continued with the experimental process.

**Echocardiography**

We used a Vevo 2100 high-resolution microimaging system equipped with a 30-MHz transducer (VisualSonics, Toronto, Canada) for echocardiography. Mice were anesthetized with 1.5% isoflurane and two-dimensional echocardiographic views of the parasternal long axes and the mid-ventricular short axis were obtained. We measured the left ventricular end-diastolic diameter (LVEDD), and left ventricular end-systolic diameter (LVESD), interventricular septal thickness in diastole (IVSd) and in systole (IVSs) in M-mode. Left ventricular fractional shortening (FS) and ejection fraction (EF) were calculated as previously described [20]. All of these parameters were obtained from at least three beats and averaged subsequently.

**Histology and TUNEL assay**

Fixed heart tissues were embedded in paraffin and cut into 5-µm thickness sections, followed by staining with hematoxylin and eosin (H&E) or Masson’s trichrome. Frozen sections were prepared from frozen heart tissue embedded with optimum cutting temperature (OCT) media and stained with Oil Red O. For Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) assay, myocardial sections were treated as instructed in the protocol of In Situ Cell Death Detection kit (Roche Diagnostics GmbH, Mannheim, Germany). Digital images were obtained with a 40× objective lens by microscopy (Olympus, Tokyo, Japan). H&E-stained transverse sections were chosen to measure single cardiomyocytes cross-sectional area. We selected and counted more than 100 myocytes to assess the mean cross-sectional area using Image-Pro Plus (Media Cybernetics, Houston, USA). For the measurement of fibrosis, we randomly selected 30 fields from Masson trichrome-stained sections and analyzed the degree of fibrosis using Image-Pro Plus. We counted the ratio of TUNEL-positive nuclei and total nuclei per 40× objective field to determine the percentage of apoptotic cells. More than 5 fields per animal were examined in a blinded fashion by a technician who was not informed about the experiment groups.

**Biochemical analysis**

Blood samples were collected into tubes and centrifuged for serum separation. Serum non-esterified fatty acids (NEFA) levels were measured using a commercially available reagent according to manufacturer’s guideline. Measurement of heart lipid content was performed as previously described [21]. Briefly, 50 mg of heart tissue was weighed and homogenized with 1 ml of buffer containing 18 mM Tris, pH 7.5, 300 mM mannitol, 50 mM EGTA, and 0.1 mM phenylmethylsulfonyl fluoride. 400 µl of above homogenate was mixed...
with 4 ml of chloroform/methanol (2:1) and incubated at room temperature with continuous shaking overnight. Then 800 μl of H2O was added, vortexed, and centrifuged at 3,000g for 5 min, and then the lower lipid phase was collected and dried under nitrogen gas. The lipid pellets were dissolved with a mixture of 60 μl of tert-butyl alcohol and 40 μl of Triton X-114/methanol (2:1) mixture. The concentrations of triglyceride were then measured using the Stanbio assay kits.

**Cell culture and treatment**

H9C2 cell line were purchased from the Shanghai Cell Institute Country Cell Bank. Cells were cultured in low-glucose Dulbecco Modified Eagle Medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, USA), 100 mg/ml streptomycin and 100 U/ml penicillin (Hyclone, USA), under a 5.0% CO2 atmosphere, at 37°C. After 48 hours, the culture medium was replaced with DMEM containing baicalin or dimethylsulfoxide (DMSO, Sigma, USA), and baicalin plus PE. Following 48 hours of incubation, the cells were washed with cold Phosphate Buffer Solution (PBS) and fixed for immunofluorescent assay, or collected for Real time polymerase chain reaction (RT-PCR) or Western Blot assay. In the above experiment, baicalin and PE was dissolved in DMSO. The concentrations of baicalin (50 μM) or PE (100 μM) were chosen according to previous studies [17, 22].

**Immunofluorescent assay**

Cell contour was analyzed by immunofluorescence staining of α-actinin protein in cultured H9C2 cells. Briefly, cultured cells were washed three times in PBS before being fixed in 4% paraformaldehyde for 15 minutes, then washed three times in PBS before being ruptured with 0.1% Triton-100. Slides were stained for cells shapes with α-actinin antibody and for nuclei with DAPI. Digital images were obtained with a 40× lens by microscopy. Cell surface was measured by Image-Pro Plus. More than 60 random cells from three independent experiments were selected for measurement and analyses of cell surface area.

**Gene expression analyses**

Total RNA was extracted from heart tissue or cultured cells with Trizol (Takara Biotechnology, Japan) and reverse-transcribed into cDNA, according to the manufacturer’s protocol. Polymerase chain reactions were performed in an ABI PRISM 7900 Sequence Detector system (Applied Biosystems, Foster City, USA) using SYBR green as the fluorescence dye. The mRNA expression level of the target genes was normalized to the control β-actin using the comparative threshold cycle (2−ΔΔCt) method. The sequences of primers for RT-PCR were listed in Table 1.

**Western Blot**

Total protein from heart tissues or cultured cells was extracted using radioimmunoprecipitation lysis buffer (Beyotime, Shangh, China). After denaturation and SDS-PAGE electrophoresis, separated proteins were transferred to nitrocellulose membranes. The primary antibodies used were as follows: anti-ANP (1:1000, Sigma, St. Louis, Missouri, USA); anti-PPARα (1:1000, Santa Cruz Biotechnology, USA); anti-PPARβ/δ (1:1000, Santa Cruz Biotechnology, USA); anti-Bcl2 (1:1000, Proteintech, Wuhan, China); anti-Bax (1:1000, Proteintech, Wuhan, China); and anti-GAPDH (1:1000; Abcam, Cambridge, USA). Membranes were blocked in Tris Buffered Saline with Tween (TBST) containing 5% skim milk and then incubated with primary antibodies at 4°C overnight. After 3 times of washing with TBST, the membranes were further incubated with HRP-conjugated secondary antibodies at room temperature for 2 hours. Bands were visualized with chemiluminescence assays (ECL detection reagents, Pierce, USA) and quantified by densitometry using BioRad’s Quantity One software (version 4.4).

**Statistical analysis**

Data are presented as the mean ± SEM. Differences between groups were compared by one-way ANOVA. We used the Kaplan-Meier method for survival analysis; we tested between-group differences in survival using the log-rank (Mantel-Cox) test. All statistical analyses were performed using Prism 5 (GraphPad Software Inc., San Diego, USA). P < 0.05 was considered statistically significant.
Results

Baicalin improves cardiac function and reduces mortality following TAC

To investigate the effect of baicalin on pressure overload-induced cardiac dysfunction, adult mice were randomly assigned to 4 groups: treatment with either baicalin or vehicle for
4 weeks following either TAC or Sham operation. Then, left ventricular ejection fraction (EF), left ventricular fractional shortening (FS), interventricular septal thickness in diastole (IVSd) and in systole (IVSs), left ventricular end-diastolic diameter (LVEDD), and left ventricular end-systolic diameter (LVESD) were measured by echocardiography. Following 4 weeks of pressure overload, TAC mice displayed depressed cardiac function, increased left ventricular wall thickness, and increased left ventricular diameter (Table 2). However, we found that baicalin treatment resulted in attenuation of ventricular dysfunction, as evidenced by improvements in EF and FS (Table 2). Moreover, baicalin attenuated the increases of IVSd, IVSs, LVEDD, and LVESD induced by pressure overload (Table 2). No significant changes were observed in sham mice treated with baicalin or vehicle. Furthermore, we specially followed contractile functions and left ventricular diameters of TAC mice treated with baicalin or vehicle for up to 8 weeks. As shown in Fig. 2, baicalin treatment ameliorated adverse contractile dysfunction following TAC surgery, as measured by improvements in EF and FS (Fig. 2A and 2B). Additionally, baicalin attenuated left ventricular diameters dilation following TAC surgery, as assessed by decreases in LVESD and LVEDD (Fig. 2C, 2D and 2E). In a long-term animal experiment, we treated mice daily with baicalin or vehicle following TAC surgery. At the end of the 14 weeks, mortality in the baicalin-treated group was lower than that in the TAC group (Fig. 2F). Therefore, we concluded that baicalin treatment improved cardiac dysfunction and decreased mortality following TAC.

**Baicalin prevents pressure overload-induced cardiac hypertrophy**

In order to further evaluate the effects of baicalin on cardiac hypertrophy, we compared heart morphology and weight following TAC. As shown in Fig. 3, mice under pressure overload displayed marked elevation of heart weight/body weight (HW/BW) ratio (Fig. 3A), heart weight/tibia length (HW/TL) ratio (Fig. 3B), and cardiomyocyte cross-sectional area (Fig. 3D and 3E). However, baicalin treatment resulted in attenuation of cardiac hypertrophy.
as measured by HW/BW ratio (Fig. 3A), HW/TL ratio (Fig. 3B), and cardiomyocyte cross-sectional area (Fig. 3D and 3E). We also measured lung weight/body weight (LW/BW) ratio to further define the effect of baicalin on left ventricular dysfunction following TAC. As shown in Fig. 3C, the LW/BW ratio was elevated in the TAC+Veh group, and attenuated in the TAC+BAI groups (Fig. 3C). Furthermore, mRNA levels of hypertrophic hallmarks atrial natriuretic peptide (ANP) and myosin, heavy polypeptide 7 (β-MHC) were shown to be induced by TAC and attenuated by baicalin treatment (Fig. 3F and 3H). However, we found no significant attenuation of natriuretic peptide type B (BNP) mRNA levels in TAC+BAI group compared with TAC+Veh group (Fig. 3G). Collectively, treatment of baicalin attenuates pressure overload-induced cardiac hypertrophy.

**Baicalin attenuates cardiac fibrosis after TAC surgery**

An important hallmark of pathological hypertrophy and heart failure is cardiac fibrosis [23], which is characterized by an increase in collagens and other extracellular matrix components in the interstitial and perivascular regions of the myocardium [4]. Therefore, we assessed cardiac fibrosis in the left ventricular myocardium following Masson trichrome staining. We found that both interstitial fibrosis and perivascular fibrosis were induced in TAC hearts and attenuated in baicalin-treated hearts (Fig. 4A). We further measured the collagen fractional area in the left ventricular myocardium, and obtained similar results (Fig. 4B). It is well known that the most abundant collagen types in the heart are type I and III, which account together for over 90% of the total collagen [23]. Previous studies have demonstrated that connective tissue growth factor (CTGF) is a major growth factor that promotes fibrosis in the hypertrophic heart [23, 24]. Therefore, we detected mRNA levels of collagen I, collagen III and CTGF in these hearts under different stimuli. RT-PCR results demonstrated that pressure overload triggered elevation of collagen I, collagen III and CTGF mRNA levels in vehicle-treated hearts (Fig. 4C, 4D and 4E). In line with the Masson trichrome staining results, baicalin administration attenuated TAC-induced elevation of collagen I,
collagen III and CTGF mRNA levels (Fig. 4C, 4D and 4E). Collectively, these data indicated that oral administration of baicalin attenuates pressure overload-induced cardiac fibrosis.

**Baicalin reduces myocardial apoptosis following TAC surgery**

Previous studies have demonstrated that cardiomyocyte apoptosis is a characteristic cellular event and a causal mechanism in the progression of hypertrophy and heart failure [25, 26]. Therefore, TUNEL staining was performed in order to investigate the effect of
Baicalin stimulates cardiac FAU genes expression without altering those involved in glucose utilization

Cardiac hypertrophy is associated with a decrease in FAU and an increase in glucose utilization [3]. To analyze the effect of baicalin treatment on energy metabolic genes expression, we performed RT-PCR to detect genes implicated in the transport and metabolism of glucose and fatty acids. We found that 4 weeks of pressure overload increased mRNA levels of glucose transporter member 1 (GLUT1) and hexokinase 1 (HK1) (Fig. 6A). However, treatment of baicalin did not influence their expression (Fig. 6A). Additionally,
neither cardiac pressure overload nor baicalin treatment altered the expression of GLUT4 (Fig. 6A), which could translocate from intracellular stores to the cell surface through an insulin-dependent pathway [27]. Moreover, 4 weeks of cardiac pressure overload induced pronounced decrease in key genes involved in FAU (Fig. 6B and 6C). These genes included cluster of differentiation 36 (CD36), diacylglycerol O-acyltransferase 2 (DGAT2), carnitine palmitoyltransferase 1α (CPT1α), long-chain acyl-coenzyme A dehydrogenase (LCAD), and medium-chain acyl-coenzyme A dehydrogenase (MCAD). Surprisingly, treatment of baicalin elevated mRNA levels of CD36, DGAT2, CPT1α, LCAD, and MCAD in both sham hearts and TAC hearts (Fig. 5B and 5C).

These findings suggest: (1) suppressed FAU genes expression and increased glucose utilization genes expression in hearts under 4 weeks of pressure overload; (2) increased FAU genes expression and unchanged glucose metabolism genes expression in hearts treated with baicalin in both sham and TAC hearts.

**Fig. 7.** Treatment with baicalin inhibits cardiac hypertrophy in vitro. (A) Representative fields of H9C2 cells stained with α-actinin. Original magnification, 40× objective (scale bar = 50 μm). (B) Quantification of cell cross-sectional area from experiments shown in (A) by measuring more than 60 random cells. (C) mRNA levels of ANP, BNP, and β-MHC were analyzed by RT-PCR. (D) Protein levels of PPARα, PPARβ/δ, ANP and GAPDH in the indicated groups were shown. (E and F) Quantification of GAPDH-corrected protein levels of ANP, PPARα and PPARβ/δ from (D). These results were reproducible in 3 dependent experiments. *P < 0.05 vs. DMSO; #P < 0.05 vs. PE.

### Baicalin improves cardiac energy metabolic rebalance through induction of PPARα and PPARβ/δ

PPARs regulate the expression of genes involved in glucose utilization, fatty acid metabolism and mitochondrial oxidative phosphorylation in the heart [8, 28]. Therefore, we
performed RT-PCR to detect the myocardial expressions of PPARα and PPARβ/δ. We found decreased mRNA levels of PPARα and PPARβ/δ in hearts under pressure overload (Fig. 6D). However, treatment of baicalin induced restoration in mRNA levels of these genes (Fig. 6D). Surprisingly, baicalin administration stimulated mRNA levels of PPARα in Sham hearts (Fig. 6D). We further detected protein levels of PPARα and PPARβ/δ by performing Western blot assay. Consistently, cardiac pressure over-load decreased protein levels of PPARα and PPARβ/δ (Fig. 6E and 6F). Baicalin administration increased protein levels of PPARα both in Sham and TAC hearts and restored PPARβ/δ protein level in hearts under hemodynamic stress (Fig. 6E and 6F). Collectively, these findings suggest decreased expression of PPARα and PPARβ/δ in TAC hearts and restoration of these genes in baicalin-treated TAC hearts.

**Baicalin inhibits cardiomyocyte hypertrophy in vitro**

To detect the effect of baicalin on cardiomyocyte hypertrophy in vitro, H9C2 cells were cultured and treated with PE or DMSO in the presence or absence of baicalin. Cardiac hypertrophy can be monitored by increased myocyte cross-sectional area, and induction of fetal gene expression [29]. Cardiac myocytes were incubated with 50 μM baicalin for 24 hours and subsequently treated with PE for 48 hours. There were no observable adverse effects by the treatment of DMSO or baicalin alone (data not shown). We found that 48 hours of PE induction resulted in marked increase in cardiac myocyte size, which was attenuated by treatment with baicalin (Fig. 7A and 7B). Meanwhile, PE-induced high expression of the hypertrophic hallmarks ANP, BNP and β-MHC were attenuated by treatment of baicalin (Fig. 7C). Further detection of ANP protein levels showed similar result (Fig. 7D and 7E). Furthermore, we detected increased protein levels of PPARα and PPARβ/δ in cultured cardiomyocyte treated by baicalin (Fig. 7D and 7F). However, 48 hours of PE induction was not sufficient to influence protein levels of PPARα and PPARβ/δ (Fig. 7D and 7F). Taken together, these data demonstrated that (1) baicalin attenuated PE-induced cardiac hypertrophy in vitro, and (2) baicalin increased protein levels of PPARα and PPARβ/δ in cultured cardiomyocyte in the absence or presence of PE.
Long-term treatment of baicalin presents no significant lipotoxicity

MHC-PPARα mice has been shown to develop cardiac dysfunction over time, and were prone to enhance myocardial lipid accumulation when treated with HFD [5, 8]. However, MHC-PPARβ/δ mice did not accumulate myocardial lipid and had normal cardiac function [8]. In the current study, the presence of increased PPARα and its target genes expression and the absence of cardiac dysfunction phenotype in Sham mice treated with baicalin led us to explore whether baicalin alters myocardial lipid balance. Adult mice treated with baicalin or vehicle were fed HFD for 4 weeks to increase circulating nonesterified fatty acids (NEFA). Levels of myocardial neutral fat were visualized by oil red O staining, followed by extraction and quantification of triglyceride (TAG) contents. We found that hearts treated with baicalin did not accumulate abnormal levels of neutral lipid following 4 weeks of HFD (Fig. 8A). Consistently, quantification of TAG levels demonstrated no significant increase in myocardial TAG accumulation in baicalin-treated mice fed HFD compared with corresponding controls (Fig. 8B). While the food intake and serum NEFA levels were similar in mice treated with baicalin or vehicle in the HFD states (Table 3 and Fig. 8C), indicating that the supply of circulating NEFA was equivalent between different treatments. Moreover, we found that mice fed HFD displayed increased expression of FAU genes, and no further elevation when treated with baicalin (Fig. 8D). Taken together, these results demonstrate that Long-term treatment of baicalin presents no substantial lipotoxicity.

Discussion

Current therapeutic strategies toward heart failure emphasize inhibition of neurohumoral activation and normalization of hemodynamics [30]. Despite significant improvements in clinical diagnosis and efficiency of symptoms control, the progression of heart failure treatment remains unchanged with high rehospitalization rate. Novel therapeutic approaches are urgently needed [31]. Here, we provide an option of baicalin, which presents efficient attenuation of cardiac hypertrophy and mortality induced by pressure overload. Major findings of our study were as follows: (1) baicalin attenuated cardiac hypertrophy in response to hypertrophic stimuli both in vitro and in vivo; (2) baicalin-mediated cardioprotection was associated with suppressed cardiac hypertrophy, fibrosis, apoptosis, and metabolic abnormality; and (3) baicalin-induced high expression of PPARα presents no significant lipotoxicity.

Pressure overload is associated with numerous cardiac changes and responses including cardiac myocyte hypertrophy, fibrosis, apoptosis, and metabolic abnormality [3, 32]. These features lead to cardiac dysfunction and pathological heart dilatation which progresses to heart failure. In the present study, cardiac hypertrophy was examined by assessing cardiac function during the time course of the study by echocardiography, measuring heart weight and cardiomyocyte cross-section area, and hypertrophic genes (ANP, BNP, and β-MHC) at study end. TAC-induced cardiac hypertrophy was attenuated by baicalin with a number of favourable outcomes including attenuation in cardiac dysfunction, smaller heart weight, lower myocyte cross-section area, and lower hypertrophic genes expression.
To further explore potential mechanisms by which baicalin protect against pathological hypertrophy, we investigated the effect of baicalin on cardiac fibrosis and apoptosis. Cardiac fibrosis is a classical hallmark of pathological hypertrophy and is characterized by the expansion of collagens and other extracellular matrix components [23]. Previous research suggested that cardiac fibrosis not only causes systolic dysfunctions, but also disrupts the coordination of myocardial excitation-contraction coupling [4]. The present study demonstrated that baicalin attenuates cardiac fibrosis induced by pressure overload in vivo, as indicated by decreased extracellular matrix and lower expression of fibrogenic genes (Collagen I, Collagen III and CTGF). Additionally, apoptosis contributes to heart failure by decreasing the number of myocardial cells [33]. Even very low, but persistent, levels of apoptosis of myocardial cells gradually resulted in left ventricular dilation, systolic and diastolic dysfunction, and lethal dilated cardiomyopathy [26]. These studies show that a causal relationship exists between myocardial apoptosis and heart failure. Our results showed increased apoptosis signals in hearts under pressure overload. However, baicalin could inhibit the apoptosis by down-regulating the ratio of Bax/Bcl-2.

As the most avid ATP-consuming organ in the body, the heart possess quite low ATP reserve for only 10 contractions [34]. Therefore, the myocardium requires constant and robust mitochondrial ATP synthesis to maintain uninterrupted contraction and relaxation [30]. Previous studies demonstrated that abnormalities in cardiac lipid metabolism and energy production is a consistent feature of heart failure [30, 35]. Crucially, the progression of heart failure with pressure overload is associated with decreased PPARα and PPARβ/δ, and reactivation of PPARα, or PPARβ/δ, is sufficient to reverse myocardial dysfunction and hypertrophy, respectively [5, 7, 8, 10]. In the present study, we found myocardial dysfunction was ameliorated by baicalin treatment in TAC hearts, accompanied by reduced myocardial fibrosis, apoptosis signals, and profound restoration of PPARα and PPARβ/δ. These protective effects were also accompanied by unchanged expressions of glucose transport and utilization genes (GLUT1, GLUT4, and HK1) and increased FAU genes (CD36, DGAT2, CPT1α, LCAD, and MCAD). Previous studies suggested that cardiac expression of genes involved in glucose transport and utilization is repressed in MHC-PPARα mice and induced in MHC-PPARβ/δ mice [5, 8]. Additionally, PPARα and PPARβ/δ exerted opposite transcriptional control of the GLUT4 promoter [8], which may explain the observed results including unchanged GLUT4 mRNA levels and increased both PPARα and PPARβ/δ in baicalin-treated TAC hearts. Besides, PPARα and PPARβ/δ share overlapping functions in regulating myocardial lipid metabolism [36]. Therefore, we cannot clearly conclude that baicalin mediated increase of FAU genes was fulfilled by induction of PPARα, or PPARβ/δ.

Baicalin has been attracting growing interest from pharmaceutical and food industries due to its promising therapeutic uses [37]. In particular, this flavonoid has shown cardioprotective effects on variant disease models in vivo or in vitro. In cultured cardiomyocytes, ER stress-induced apoptosis were attenuated by baicalin via CHOP/eNOS/NO pathway [17]. In an acute myocardial infarction rats model, baicalin presented cardioprotective effects by modulating p38 MAPK cascades [16, 38]. Additionally, baicalin may attenuate chronic hypoxia-induced pulmonary hypertension and cor pulmonale by downregulating the p38 MAPK/MMP-9 pathway [39]. Besides, evidence showed that baicalin attenuated HFD-induced obesity and liver dysfunction, with dose-dependently inhibited hepatic CaMKKβ/AMPK/ACC pathway [40]. In the present study, we found that baicalin attenuated cardiac hypertrophy in response to hypertrophic stimuli, accompanied by restoration of PPARα and PPARβ/δ. Previously, reactivation of PPARα with different ligands exhibited opposite effects on left ventricular hypertrophy in spontaneously hypertensive rat [7]. However, the protective effects of cardiac PPARβ/δ restoration on cardiac hypertrophy were clearly demonstrated in vitro and in vivo [10, 12]. Therefore, it is sufficient to concluded that the protective effects of baicalin were mediated through, at least in part, restoring cardiac PPARβ/δ.

MHC-PPARα mice exhibited signatures of cardiomyopathy including ventricular hypertrophy, enhanced expression of fetal genes, and systolic ventricular dysfunction [5, 8]. Moreover, MHC-PPARα mice exerted at least 15-fold increase in PPARα level, and...
enhanced myocardial lipid accumulation followed by HFD, which is possibly because of TAG synthesis beyond the capacity for oxidation [5, 8]. Therefore, we performed diet studies to further assess whether long-term administration of baicalin accelerate myocardial lipid accumulation. Mice were fed HFD for 4 weeks to increase circulating NEFA, whose localization at the luminal surface of coronary blood vessels may serve to activate PPARα in heart tissue [41]. Activated PPARα promotes myocardial fatty acid uptake (CD36), TAG synthesis (DGAT2), and fatty acid oxidation (CPT1α, LCAD, and MCAD). Here we detected no significant increase in myocardial TAG accumulation in baicalin-treated mice given HFD compared with corresponding controls. Perhaps baicalin-induced PPARα is not as strong as cardiac-restricted overexpression of PPARα, and is not sufficient to induce severe myocardial neutral lipid accumulation. Therefore, oral administration of baicalin presents no significant myocardial lipotoxicity.

Taken together, these data suggest that oral administration of baicalin attenuated pressure overload induced cardiac dysfunction, high mortality and ventricular remodeling. These protective effects of baicalin treatment appear to be mediated by suppression of cardiac hypertrophy, fibrosis and apoptosis, and restoration of PPARβ/δ.

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Disclosure Statement

The authors declare that they have no interests that could influence the publication of this paper.

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