Citri Reticulatae Pericarpium Alleviates Postmyocardial Infarction Heart Failure by Upregulating Ppar γ Expression

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Abstract

Purpose

Heart failure after myocardial infarction (MI) is the leading cause of death worldwide. Citri Reticulatae Pericarpium (CRP) is a traditional Chinese herbal medicine that has been used in the clinic for centuries. In this study, we aimed to investigate the roles of CRP in cardiac remodeling and heart failure after MI, as well as the molecular mechanisms involved.

Methods

Male C57BL/6 mice aged 8 weeks were subjected to coronary artery ligation to mimic the clinical situation *in vivo*. Echocardiography was used to assess the systolic function of the mouse heart. Masson trichrome staining and Wheat germ agglutinin (WGA) staining were utilized to determine the fibrotic area and cross-sectional area of the mouse heart, respectively. Cardiomyocytes and fibroblasts were isolated from neonatal rats aged 0–3 days *in vitro* using enzyme digestion. TUNEL staining and EdU staining were performed to evaluate apoptosis and proliferation, respectively. Gene expression changes were analyzed by qRT-PCR, and protein expression changes were assessed by Western blotting.

Results

Our findings revealed that CRP attenuated cardiac hypertrophy, fibrosis and apoptosis and alleviated heart failure after MI *in vivo*. Furthermore, CRP mitigated cardiomyocyte apoptosis and fibroblast proliferation and differentiation into myofibroblasts. In addition, the PPAR γ inhibitor T0070907 completely abolished the abovementioned beneficial effects of CRP, and the PPAR γ activator rosiglitazone failed to further ameliorate cardiac apoptosis and fibrosis *in vitro*.

Conclusion

CRP alleviates cardiac hypertrophy, fibrosis, and apoptosis and can ameliorate heart failure after MI via activation of PPAR γ .

1. Introduction

Myocardial infarction (MI) induced by coronary artery occlusion is the leading cause of death globally[1]. A series of pathological processes occur after cardiac ischemia. For example, due to a lack of blood supply, cardiomyocytes undergo necrosis and apoptosis, while myofibroblasts are activated to preserve ventricular shape. In addition, the noninfarcted myocardium undergoes eccentric hypertrophy, ultimately leading to heart dilation[2]. Post-MI adverse cardiac remodeling, especially cardiac fibrosis, can develop into advanced heart failure [3]. Currently, there are a number of pharmacological treatments and preventive strategies for improving the prognosis of patients with post-MI heart failure, such as angiotensin II receptor antagonists, beta-blockers, acetyl choline esterase inhibition and Entresto [4-6]. However, the incidence of heart failure after MI is increasing year to year, ranging from 14% to 36%

according to different studies[7], which places a heavy burden on society and individuals. Therefore, identifying novel therapeutics for post-MI heart failure is of great significance.

PPARs are nuclear hormone receptor superfamily transcription factors activated by ligands. PPARs, or peroxisome proliferator-activated receptors, were discovered in 1990[8], and mainly regulate fatty acid oxidation in fat and other tissues and glucose metabolism[9]. There are three PPAR isoforms: PPAR α , PPAR β/δ , and PPAR γ [10]. Accumulating evidence indicates that PPAR γ plays key roles in controlling cardiac metabolism[11-13]. Previous studies have revealed that activation of PPAR γ can significantly ameliorate cardiac hypertrophy and cardiac fibrosis in mice with pressure overload-induced[14], angiotensin II (Ang II)-induced[15] and isoproterenol (ISO)-induced[16] pathological myocardial remodeling. In addition, upregulating PPAR γ expression can increase cardiac output in rats with myocardial ischemia-reperfusion (I/R) injury[17]. Moreover, the PPAR γ agonist pioglitazone can probably reduce the expression of inflammatory cytokines, including TNF α , TNF β and MCP-1, and attenuate cardiac remodeling and heart failure in mice with MI[18]. A recently published review systematically identified PPAR γ as a novel therapeutic target for cardiac fibrosis[19]. However, other studies have found that PPAR γ activation can result in fluid retention, weight gain, osteoporosis and even cause heart failure. Thus, identifying potential PPAR γ regulators that do not cause side effects may provide options for the clinical treatment of post-MI heart failure.

Traditional Chinese medicine (TCM) plays important roles in the clinical treatment of cardiovascular diseases[20]. Citri Reticulatae Pericarpium (CRP) is a famous TCM that is used for the treatment of multiple diseases, such as cardiovascular diseases, digestive diseases, respiratory diseases, and even tumors[21, 22]. CRP is the dried fruit peel of *Citrus reticulata* Blanco. The southeastern region of China is the main production area of CRP, such as Fujian, Guangdong and Zhejiang provinces. Phytochemical studies have identified more than one hundred chemical components of CRP, and its abundant bioactive compounds, including flavonoids, phenolic acids and limonoids, can exert positive effects on health [22]. CRP has been described as a qi regulator for centuries and was registered in the first edition of the Chinese Pharmacopoeia in 1953. CRP has attracted increasing attention from researchers because it has multiple pharmacologic effects and rich resources with low toxicity and costs [23]. Modern pharmacological investigations have revealed that CRP can fight against inflammation, oxidative stress, atherosclerosis, thrombus, liver injury and tumors[22, 24-27]. Our prior studies showed that CRP can alleviate cardiac hypertrophy and fibrosis induced by Ang II and ISO via activating PPAR γ [15, 16]. However, whether CRP has a protective effect on post-MI heart failure is unclear.

In the current research, we demonstrated that CRP can protect against post-MI heart failure by activating PPAR γ , suggesting the potential of CRP in the clinical treatment of heart failure after MI.

2. Material And Methods

2.1. Animal models

Adult male C57BL/6 mice were purchased from the Experimental Animal Center of Nanjing Medical University (Nanjing, China). The mice were randomly divided into groups. MI was established by ligation of the left anterior descending coronary artery (LAD) utilizing a 7/0 silk thread. The sham group mice underwent the same process except LAD ligation. CRP was obtained from Shijiazhuang Yiling Pharmaceutical Co., Ltd. (Shijiazhuang, Hebei, China). The animals were intragastrically administered CRP (0.5 g/kg/day) for 3 weeks after MI. A PPAR γ inhibitor (T0070907) was used to investigate the mechanism by which CRP protects against post-MI heart failure. CRP was administered after MI, and T0070907 was intraperitoneally injected (1 mg/kg/day) concurrently for 21 days.

2.2. Echocardiography

At the experimental endpoint, left ventricular fractional shortening (LVFS) and left ventricular ejection fraction (LVEF) were determined by echocardiography to assess the systolic function of the mouse heart. Isoflurane (1.5–2%) was applied to anesthetize the mice, and transthoracic echocardiography was carried out by a Vevo 2100 instrument (Visual Sonics Inc, Toronto, Ontario, Canada) with a 30 MHz central frequency scan head.

2.3. Masson trichrome staining

Heart tissues were fixed with 4% paraformaldehyde for 48 h and then embedded in paraffin. To assess the extent of fibrosis, heart sections were stained with Masson trichrome following a standard procedure. Images were captured by computer-assisted video densitometry and analyzed with ImageJ software (National Institutes of Health). The percentage of fibrosis was calculated as the fibrotic area/total myocardial area $\times$ 100%.

2.4. Wheat germ agglutinin (WGA) staining

Heart sections were subjected to WGA (1:200, Sigma, St. Louis, MO, USA) staining to evaluate the size of cardiomyocytes. Images were captured with a fluorescence microscope (Carl Zeiss, Thuringia, Germany). More than 10 fields of view from each section were analyzed. The cardiomyocyte size was assessed utilizing ImageJ software (National Institutes of Health).

2.5. Neonatal cardiomyocyte (CM) and cardiac fibroblast (CF) isolation, culture and treatment

Neonatal Sprague–Dawley rats were purchased from the Experimental Animal Center of Nanjing Medical University (Nanjing, China). Heart tissues were gradually minced and digested in a mixed enzyme solution comprising 60% trypsin and 40% collagenase II. After approximately 10 digestions (usually 30 rats in total), the suspended cells were centrifuged and resuspended in high-glucose Dulbecco's modified Eagle's medium (DMEM, Gibco, Pasadena, CA, USA) containing 10% fetal bovine serum (FBS, Gibco, Pasadena, CA, USA) and 1% penicillin-streptomycin (PS, Gibco, Pasadena, CA, USA) and then plated in a cell incubator (37 °C, 5% CO₂ and 95% O₂). Nearly 1 h later, the CFs had attached to the culture plates and were ready for subsequent culture and passage. To obtain purer CMs, Percoll gradient centrifugation was

used to further purify the unattached cells in the medium. Purified CMs were cultured in appropriate culture plates in DMEM containing 10% horse serum (HS, Gibco, Pasadena, CA, USA), 5% FBS and 1% PS.

To mimic the lack of blood and oxygen in mice with MI, CMs were maintained in glucose-free Dulbecco's modified Eagle's medium (Gibco, Pasadena, CA, USA) in an anaerobic chamber containing 95% N₂ and 5% CO₂ at 37 °C for 8 h to mimic oxygen-glucose deprivation (OGD). To explore the roles of CRP in OGD-induced cardiomyocyte injury, CMs were treated with CRP two days in advance at a dose of 0.5 µg/ml. A PPAR γ agonist (rosiglitazone, 1 µM) or T0070907 (1 µM) combined with CRP was added to the CM culture medium for 48 h before OGD to investigate whether CRP can protect CMs from OGD-induced injury by upregulating PPAR γ expression.

CFs were used in our study at passage 2. CFs were treated with recombinant human TGF- β (Peprotech, Rocky Hill, NJ, USA) for 24 h to study the roles of CRP in CF proliferation and differentiation into myofibroblasts. CFs were treated with CRP (0.5 µg/ml) for 48 h. To evaluate the mechanisms by which CRP alleviates TGF- β -induced CF activation, rosiglitazone or T0070907 was mixed with CRP and added to the cells 24 h before administration of TGF- β .

2.6. Western blotting

Tissues or cells were harvested at the experimental endpoint and lysed with RIPA buffer (Beyotime, Nantong, China) with 1 mM PMSF (ST505, Beyotime, Nantong, China). A bicinchoninic acid protein assay kit (Thermo Fisher, Waltham, MA, USA) was used to measure the protein concentration of each sample. Equal amounts of proteins were separated by SDS-PAGE and transferred onto PVDF membranes. Then, the PVDF membranes were blocked using 5% milk for 2 h and subsequently incubated with primary antibodies overnight at 4 °C. The following primary antibodies were utilized: β -tubulin (1:1,000, Bioworld Technology, Minnesota, USA), GAPDH (1:1,000, Kangchen, Shanghai, China), α -SMA (1:1,000, Proteintech Group, Wuhan, China), Collagen type I (1:1,000, Proteintech Group, Wuhan, China), Bax (1:1000, Cell Signaling Technology, Boston, Massachusetts, USA), Bcl-2 (1:1,000, Cell Signaling Technology, Boston, Massachusetts, USA), and PPAR γ (1:1,000, Proteintech Group, Wuhan, China). β -Tubulin or GAPDH was used as a loading control. On the second day, the membranes were incubated in the appropriate secondary antibodies for 2 h at room temperature, and then the signals were visualized with an ECL Chemiluminescence Kit (Thermo Fisher). Analysis of each band was carried out with ImageJ software (National Institutes of Health).

2.7. Quantitative real-time polymerase chain reaction (qRT-PCR)

A TRIzol RNA extraction kit (Invitrogen, USA) and iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) were used to lyse tissues or cells and to reverse transcribe RNA into cDNA, respectively, according to the manufacturer's instructions. SYBR Green qPCR Master Mix (Bio-Rad, Hercules, CA, USA) was utilized to perform qRT-PCR on an ABI 7900HT fast Real-Time PCR System (Applied Biosystems). 18S RNA was used as an internal control. The following primer sequences (forward and reverse) were used in this study:

Anp (rat): F: 5'-GAGCAAATCCCGTATACAGTGC-3',

R: 5'-ATCTTCTACCGGCATCTTCTCC-3'.

Bnp (rat): F: 5'-GCTGCTGGAGCTGATAAGAGAA-3',

R: 5'-GTTCTTTTGTAGGGCCTTGGTC-3'.

α -SMA (rat): F: 5'-GTCCCAGACATCAGGGAGTAA-3',

R: 5'-TCGGATACTTCAGCGTCAGGA-3'.

Col1a1 (rat): F: 5'-GAGCGGAGAGTACTGGATCGA-3',

R: 5'-CTGACCTGTCTCCATGTTGCA-3'.

Col3a1 (rat): F: 5'-TGCCATTGCTGGAGTTGGA-3',

R: 5'-GAAGACATGATCTCCTCAGTGTTGA-3'.

18s (rat): F: 5'- TCAAGAACGAAAGTCGGAGG -3',

R: 5'- GGACATCTAAGGGCATCAC -3'.

Anp (mouse): F: 5'-AGGCAGTCGATTCTGCTT-3',

R: 5'-CGTGATAGATGAAGGCAGGAAG-3'.

Bnp (mouse): F: 5'-TAGCCAGTCTCCAGAGCAATTC-3',

R: 5'-TTGGTCCTTCAAGAGCTGTCTC-3'.

α -SMA (mouse): F: 5'-GTCCCAGACATCAGGGAGTAA-3',

R: 5'-TCGGATACTTCAGCGTCAGGA-3'.

Col1a1 (mouse): F: 5'-GCTCCTCTTAGGGGCCACT-3',

R: 5'-CCACGTCTCACCATTGGGG-3'.

Col3a1 (mouse): F: 5'-CTGTAACATGGAACTGGGGAAA-3',

R: 5'-CCATGACTGAACTGA AAACCACC-3'.

18s (mouse): F: 5'- TCAAGAACGAAAGTCGGAGG -3',

R: 5'- GGACATCTAAGGGCATCAC -3'.

2.8. α -Actinin and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) costaining

Paraformaldehyde (4%) and Triton X-100 (0.5%) were used to fix and permeabilize CMs, respectively, for 20 min. Then, 10% goat serum in PBS was applied to block nonspecific protein sites for 1 h. Both of these processes were performed at room temperature. CMs were incubated with an α -actinin primary antibody (1:200, Sigma–Aldrich, St, MO, USA) overnight and subsequently with secondary antibody (1:200, Jackson ImmunoResearch, Lannister, PA, USA). Then, TUNEL staining was performed using a TUNEL Apoptosis Detection Kit according to the manufacturer's instructions (Yeasen, Shanghai, China). DAPI (Sigma–Aldrich, St, MO, USA) was applied to stain the nuclei, and a fluorescence microscope (Carl Zeiss, Thuringia, Germany) was used to capture images. The number of TUNEL-positive CMs was counted using ImageJ software (National Institutes of Health).

2.9. Alpha smooth muscle actin (α -SMA) and EdU costaining

CFs were fixed, permeabilized and blocked as described for CMs. After blocking, the CFs were incubated with an α -SMA primary antibody (1:500, Sigma–Aldrich, St, MO, USA) overnight and then with secondary antibody (1:200, Jackson ImmunoResearch, Lannister, PA, USA). To assess the proliferation of CFs, EdU staining was performed using the Click-iT Plus EdU Alexa Fluor 555 Imaging Kit (Invitrogen) according to the manufacturer's instructions. DAPI was utilized to stain the nuclei. A fluorescence microscope (Carl Zeiss, Thuringia, Germany) was employed to visualize the signals, and the number of EdU-positive CFs was calculated.

2.10. Statistical analysis

All data were expressed as the mean $\pm$ SD. One-way ANOVA followed by Bonferroni's post hoc test was applied for multiple-group comparisons. A P value < 0.05 was considered statistically significant. All analyses were performed using GraphPad Prism 8 software.

3. Results

3.1. CRP attenuates cardiac injury after MI *in vivo*

Since our previous studies demonstrated that CRP protects against Ang II-induced and ISO-induced cardiac dysfunction [15, 16], we further investigated the role of CRP in post-MI heart failure in the present study. C57BL/6 mice aged eight weeks were subjected to LAD and then given CRP for three weeks. Echocardiography suggested that CRP significantly improved cardiac systolic function after MI, including by increasing the LVEF and LVFS (Figure 1A). In addition, WGA staining and qRT–PCR showed that CRP reduced cardiac hypertrophy (Figure 1B) and decreased the activation of fetal genes (Anp and Bnp) in the hearts of mice with MI (Figure 1C). Cardiac fibrosis is a pivotal hallmark of cardiac remodeling and heart failure after MI. Our data revealed that compared with vehicle, CRP obviously attenuated collagen deposition in the heart post-MI (Figure 2A–C). Cardiac apoptosis is another feature of cardiac remodeling and heart failure after MI. We found that the expression of the antiapoptotic protein Bcl2 was increased

and that the expression of the proapoptotic protein Bax was decreased by CRP treatment (Figure 2D). Taken together, our results demonstrated that CRP can alleviate cardiac hypertrophy and cardiac fibrosis as well as cardiac apoptosis after MI injury and thus plays important roles in post-MI heart failure.

3.2. CRP ameliorates OGD-induced cardiomyocyte apoptosis and TGF β -induced cardiac fibroblast activation *in vitro*

To further investigate the protective effects and underlying mechanisms of CRP *in vitro*, neonatal rat cardiomyocytes (CMs) and cardiac fibroblasts (CFs) were obtained by enzyme digestion. As shown in Figure 3A and 3B, CRP decreased the number of TUNEL-positive nuclei and reduced the Bax/Bcl2 ratio in OGD-treated CMs, providing evidence for the antiapoptotic function of CRP. In addition, prior to administering TGF β to activate CFs, CRP was mixed in the culture medium for 24 h. Immunofluorescence analysis indicated that TGF β enhanced CF proliferation and differentiation into myofibroblasts and that CRP administration reversed these effects (Figure 4A). We further examined the mRNA levels of Col1a1, Col3a1 and α -SMA by qRT-PCR, and the results illustrated that CRP may reduce the increase in the expression of fibrotic genes in TGF β -stimulated CFs (Figure 4B). Collectively, these results showed that CRP can alleviate OGD-induced cardiomyocyte apoptosis and TGF β -induced cardiac fibroblast activation *in vitro*.

3.3. PPAR γ is activated by CRP treatment both *in vivo* and *in vitro*

Metabolic disturbance is a crucial feature of cardiac remodeling and heart failure after MI, and PPAR γ is a well-known regulator of metabolism[28-31]. Our Western blotting results showed that PPAR γ expression was decreased in the hearts of mice with MI and in OGD-induced CMs and TGF β -stimulated CFs. Interestingly, under the pathological conditions mentioned above, CRP dramatically upregulated the expression of PPAR γ (Figure 5A-C). These data suggested that PPAR γ activation contribute to the beneficial effects of CRP in protecting against post-MI heart failure.

3.4. CRP alleviates OGD-induced cardiomyocyte apoptosis and TGF β -induced cardiac fibroblast activation by upregulating PPAR γ expression

To study whether PPAR γ is a pivotal downstream effector of CRP, rosiglitazone and T0070907 were applied. We found that T0070907 inhibited the beneficial effects of CRP on OGD-induced cardiomyocyte apoptosis, while rosiglitazone failed to provide additional protection in the presence of CRP (Figure 6A-B). Similarly, the protective function of CRP in TGF β -induced cardiac fibroblast activation was impaired by T0070907, and rosiglitazone did not further decrease CF proliferation and differentiation into myofibroblasts caused by TGF β in combination with CRP (Figure 7A-B). Generally, these findings suggested that CRP attenuates OGD-induced cardiomyocyte apoptosis and TGF β -induced cardiac fibroblast activation by maintaining the activation of PPAR γ .

3.5. CRP improves post-MI heart failure by activating PPAR γ

Based on the data above, we explored whether PPAR γ activation is involved in the protective effects of CRP in post-MI heart failure *in vivo*. Mice with MI were intraperitoneally injected with T0070907 intragastrically administered CRP. According to the echocardiography results, the LVEF and LVFS were increased by CRP treatment, while both were significantly decreased by T0070907 injection (Figure 8A). The cardioprotective effect of CRP in cardiac hypertrophy after MI was also blocked by a PPAR γ inhibitor (Figure 8B), as was the expression of fetal genes (ANP and BNP) (Figure 8C). Regarding cardiac fibrosis, the CRP-mediated reduction in the fibrotic area and downregulation of fibrotic molecule expression were reversed by T0070907 (Figure 9A-C). As shown in Figure 9D, T0070907 inhibited the ability of CRP to alleviate cardiac apoptosis after MI. Consequently, these results indicated that CRP exerts positive effects on post-MI heart failure by upregulating PPAR γ expression.

4. Discussion

Reversing cardiac remodeling after MI is a major challenge worldwide[32]. The efforts of scientists worldwide have led to a decrease in the death rate in the acute phase after MI. However, chronic injury after MI, pathological cardiac remodeling and heart failure result in high morbidity and mortality in MI patients[33]. Currently, there are limited pharmacological therapies for heart failure after MI. Novel strategies are urgently needed.

CRP, a traditional Chinese herbal medicine used in the clinic for centuries, has anti-inflammatory, antioxidant, anticancer and other beneficial properties according to modern pharmacological studies[22, 24, 34]. In addition, CRP may be effective for diseases affecting multiple systems, such as the digestive system, the respiratory system and particularly the cardiovascular system[22, 27, 35]. Accumulating evidence suggests that CRP has cardioprotective effects[15, 16, 36]. Our previous investigations have demonstrated that CRP can ameliorate cardiac hypertrophy and fibrosis induced by Ang II and ISO by upregulating PPAR γ expression. In this study, we found that CRP attenuated cardiac injury and improved cardiac function after MI by alleviating cardiac hypertrophy, fibrosis and apoptosis. *In vitro*, CRP not only decreased OGD-induced CM apoptosis but also inhibited TGF β -stimulated CF proliferation and differentiation. Our findings suggested that CRP plays important roles in cardiac remodeling after MI and may be a new potential therapeutic target for post-MI heart failure.

Cardiac remodeling and heart failure after MI involve metabolic dysfunction, in which PPAR γ is a pivotal regulator[37-39]. PPAR γ is a member of the PPAR nuclear receptor family and is expressed abundantly in multiple types of cells. Our results showed that PPAR γ expression was downregulated in the hearts of mice with MI, OGD-induced CMs and TGF β -stimulated CFs. Interestingly, PPAR γ expression was upregulated by CRP treatment. In addition, T0070907, a known PPAR γ inhibitor, completely blunted the inhibitory effect of CRP on cardiac dysfunction after MI, OGD-induced CM apoptosis and TGF β -stimulated CF activation. However, rosiglitazone did not exert further protective effects *in vitro* in the presence of CRP, which indicated that rosiglitazone and CRP may work through the same pathway that is completely activated by either medicine alone. Therefore, CRP alleviates post-MI cardiac injury and heart failure via activation of PPAR γ . Increasing evidence shows that activation of PPAR γ can attenuate

cardiac hypertrophy[40], alleviate cardiac fibrosis[41] and decrease myocardial apoptosis[42], which is consistent with our findings. However, PPAR γ activators have been found to have side effects, including fluid retention and edema[43], as PPAR γ was broadly used as a hypoglycemic drug decades ago. No signs of edema were observed in the mice treated with CRP, but whether CRP causes the other reported side effects of PPAR γ activators needs to be elucidated in further studies.

There are several limitations of this study. First, CRP is a traditional herbal medicine with more than 140 ingredients, and it is unclear which of these components plays a leading role in the cardioprotective effects of CRP. It is possible that these ingredients have cooperative effects, which should be investigated in the future. In addition, it was demonstrated in our study that CRP attenuated cardiac injury after MI by activating PPAR γ ; however, the mechanism by which CRP regulates PPAR γ is still unknown.

5. Conclusion

Taken together, our results demonstrated that CRP can alleviate cardiac hypertrophy, fibrosis and apoptosis after MI and ameliorate post-MI heart failure by increasing PPAR γ expression. The data presented in this research provide experimental evidence for the potential application of CRP in treating post-MI heart failure in the future.

Abbreviations

CRP	Citri reticulatae Pericarpium
TCM	Traditional Chinese medicine
MI	Myocardial infarction
PPAR γ	Peroxisome proliferator-activated receptor γ
CMs	Cardiomyocytes
CFs	Cardiac fibroblasts
OGD	Oxygen-glucose deprivation
LVFS	Left ventricular fractional shortening
LVEF	Left ventricular ejection fraction

Declarations

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Competing interests

Not applicable.

Availability of data and materials

All data involved in this study are available from the corresponding author upon reasonable request.

Code availability

Not applicable.

Authors' contributions

X.L.L., H.F.Z. and H.C.S designed the research and instructed the whole experiments. M.L.C., H.Y.Z. and Q.Q.Z. performed the most experiments and analyzed the data. X.D.W. and Y.F.Z. participated in the animal experiments. M.L.C. wrote the manuscript and R.R.G. revised this manuscript. M.S.S., T.Z., T.Y. performed part of the experiments and analyzed the data. All authors listed read and approved this manuscript.

Ethics approval and consent to participate

All procedures with animals of this study were in accordance with the Guidelines of Laboratory Animals for biomedical research published by National Institutes of Health (NIH publication, revised in 2011). The experimental protocol was reviewed and approved by the ethical animal committees of Nanjing Medical University (license number: IACUC-1903016).

Consent for publication

This manuscript is approved by all authors for publication.

Acknowledgements

Not applicable.

Disclosure of potential conflicts of interest

Not applicable.

Research involving human participants and/or animals

All procedures with animals of this study were in accordance with the Guidelines of Laboratory Animals for biomedical research published by National Institutes of Health (NIH publication, revised in 2011). The

experimental protocol was reviewed and approved by the ethical animal committees of Nanjing Medical University (license number: IACUC-1903016).

Informed consent

Not applicable.

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Figures

Figure 1

CRP alleviates cardiac dysfunction and hypertrophy after myocardial infarction. (A) Parameters measured by echocardiography showed that CRP-treated mice with MI showed preservation of the ejection fraction (EF) and fractional shortening (FS) (n=8,7,6,7). (B) WGA staining indicated that CRP reduced the increase in the cross-sectional area in mice with MI (n=6). (C) qRT-PCR showed that activation of Anp and Bnp expression in post-MI heart failure was reversed by CRP treatment (n=6). The data are presented as the mean $\pm$ SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001. Scale bar = 20 μ m.

Figure 2

CRP decreases MI-induced cardiac fibrosis and apoptosis. (A) Masson trichrome staining (n=8,7,6,7), (B) qRT-PCR (n=6) and (C) Western blotting (n=6) revealed that CRP decreased the fibrotic area and downregulated the expression of fibrotic molecules at both the mRNA (Col1a1, Col3a1 and α-SMA) and protein levels (collagen type I and α-SMA). (D) The Bax/Bcl2 ratio was increased in the MI group but decreased upon CRP treatment (n=6). The data are presented as the mean ± SD. *, P < 0.05; ***, P < 0.001. Scale bar = 500 µm.

Figure 3

CRP ameliorates OGD-induced cardiomyocyte apoptosis. (A) Immunofluorescence for α-actinin was used to identify CMs (red). TUNEL staining was applied to label apoptotic nuclei (green). DAPI was utilized to stain the nuclei (blue) (n=6). (B) The Bax/Bcl2 ratio was determined by Western blotting (n=6). The data are presented as the mean ± SD. ***, P < 0.001. Scale bar = 50 μm.

Figure 4

CRP alleviates TGFβ-induced cardiac fibroblast activation. (A) Immunofluorescence for α-SMA was used to assess the extent of CF differentiation (green). EdU staining was performed to label proliferating nuclei (red). DAPI was utilized to stain nuclei (blue) (n=6). (B) qRT-PCR analysis was used to determine expression changes in fibrotic genes (n=6). The data are presented as the mean ± SD. ***, P < 0.001. Scale bar = 50 μm.

A **B** **C** **Figure 5**

PPAR γ expression is upregulated by CRP both in vivo and in vitro. (A-C) Western blotting indicated that PPAR γ expression was reduced in the hearts of mice with MI, OGD-treated CMs and TGF β -stimulated CFs but dramatically upregulated upon CRP treatment in the abovementioned pathological models (n=6). The data are presented as the mean $\pm$ SD. *, P < 0.05; ***, P < 0.001.

Figure 6

CRP decreases cardiomyocyte apoptosis by increasing PPAR γ expression. (A) TUNEL staining showed that T0070907 abolished the protective effects of CRP on OGD-induced CM apoptosis, but rosiglitazone did not induce further improvements in the presence of CRP (n=6). (B) The same conclusion was drawn by measuring Bax and Bcl2 expression using Western blotting (n=6). The data are presented as the mean $\pm$ SD. **, P < 0.01; ***, P < 0.001. Scale bar = 50 μ m.

Figure 7

CRP inhibits cardiac fibroblast activation by upregulating PPAR γ expression. (A) EdU staining and immunofluorescence for α -SMA indicated that T0070907 blocked CRP's inhibitory effects on TGF β -stimulated CF proliferation and differentiation into myofibroblasts, but rosiglitazone failed to further suppress the CF activation induced by TGF β in combination with CRP (n=6). (B) qRT-PCR showed that upon TGF β stimulation, the expression of fibrotic genes (*Col1a1*, *Col3a1* and α -SMA) that was downregulated by CRP treatment was upregulated by T0070907 but not further downregulated by rosiglitazone (n=6). The data presented as the mean $\pm$ SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001. Scale bar = 50 μ m.

Figure 8

CRP protects against post-MI cardiac dysfunction and hypertrophy by activating PPAR γ . (A) Echocardiography indicated that the MI-induced reductions in LVEF and LVFS were reversed by CRP and completely inhibited by T0070907 (n=8,7,6,7). (B) WGA staining showed that enlargement of the heart cross-sectional area in mice with MI was reduced by CRP and abolished by T0070907 (n=6). (C) qRT-PCR indicated that activation of Anp and Bnp expression in the hearts tissues of mice with MI were reversed by CRP treatment and blocked by T0070907 administration (n=6). The data are presented as the mean $\pm$ SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001. Scale bar = 20 μ m.

Figure 9

CRP attenuates cardiac fibrosis and apoptosis by upregulating PPAR γ expression. (A) Masson trichrome staining (n=8,7,6,7), (B) qRT-PCR (n=6) and (C) Western blotting (n=6) revealed that the increase in the fibrotic area, fibrotic gene expression and fibrotic protein expression in mice with MI was attenuated by CRP administration, but the protective effects were reversed by T0070907. (D) Western blotting analysis showed that the increase in the Bax/Bcl2 ratio in the MI group declined after CRP treatment, but

T0070907 inhibited the cardioprotective effect of CRP (n=6). The data are presented as the mean $\pm$ SD. *, P <0.05; **, P <0.01; ***, P <0.001. Scale bar = 500 μ m.