

Salvianolic Acid C Activates the AMPK Pathway to Inhibit Ferroptosis and Alleviate Cardiomyocyte Injury after Acute Myocardial Infarction

Yanping Zeng¹✉ · Lin Chen² · Zhenhua Qian¹ · Weixing Ma¹ · Cheng Ma³ · Yan Wang¹ · Guanfeng Zhu¹

Abstract

Myocardial infarction (MI) is one of the leading causes of death worldwide and results in substantial harm to human health and family well-being. Salvianolic acid C (SAC), a bioactive compound isolated from *Salvia miltiorrhiza* Bge., exhibits anti-oxidant, anti-inflammatory, and anti-apoptotic capacities. However, the protective effects of SAC against MI-induced cardiomyocyte injury and the underlying molecular mechanisms, particularly its role in ferroptosis and AMPK signaling, remain unclear. Infarct size in rat hearts was assessed through triphenyltetrazolium chloride (TTC) staining. Myocardial tissue damage was examined through hematoxylin-eosin (HE) staining. The mRNA and protein levels of inflammatory cytokines, including Interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α , were measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and enzyme-linked immunosorbent assay (ELISA), respectively. Apoptosis in myocardial tissues was evaluated through the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay. Protein expression levels were determined by Western blot. The results demonstrated that SAC significantly reduced infarct size in MI rats. Inflammation and cardiomyocyte apoptosis were markedly increased in the MI group, but these impacts were neutralized after SAC treatment. In addition, SAC effectively suppressed ferroptosis in MI rats. Mechanistically, SAC activates the AMPK pathway. Finally, SAC was shown to alleviate oxygen-glucose deprivation (OGD)-induced cardiomyocyte injury by activating the AMPK pathway. This study is the first to demonstrate that SAC activates the AMPK pathway to inhibit ferroptosis and alleviate cardiomyocyte injury after acute MI. These findings suggest that SAC may present a promising therapeutic candidate for the treatment of MI.

Keywords

Myocardial infarction · Salvianolic acid C · Ferroptosis · AMPK pathway · Inflammation · Cell apoptosis

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Abbreviations

AMPK, Adenosine 5'-Monophosphate (AMP)-Activated Protein Kinase; ARRIVE, Animal Research: Reporting of In Vivo Experiments; DAPI, 4',6-Diamidino-2-Phenylindole; DMEM, Dulbecco's Modified Eagle Medium; ELISA, Enzyme-Linked Immunosorbent Assay; GPX4, Glutathione Peroxidase 4; mRNA, Messenger Ribonucleic Acid; mTOR, Mammalian Target of Rapamycin; NF- κ B, Nuclear Factor Kappa B; p-AMPK, Phosphorylated AMPK; PCR, Polymerase Chain Reaction; p-mTOR, Phosphorylated mTOR; Rap1GAP, Rap1 GTPase-Activating Protein; RIPA, Radioimmunoprecipitation Assay; RT, Reverse Transcription; SDS-PAGE, Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis; SLC7A11, Solute Carrier Family 7 Member 11; SYBR, SYBR Green.

1. Introduction

With the improvement of the living standards, cardiovascular diseases, especially myocardial infarction (MI), have become the leading causes of death worldwide (Salari et al. 2023). In China, the incidence of MI has exceeded 1% and continues to increase annually (Zhang et al. 2025). After MI, cardiomyocytes undergo irreversible death and are gradually replaced by fibroblasts, leading to ventricular remodeling, ventricular wall thinning, and scar formation, which ultimately result in heart failure or sudden death (Galli et al. 2024). Therefore, identifying novel and effective therapeutic strategies remains essential for improving MI outcomes.

Shexiang tongxin dropping pills (STDP) is a traditional Chinese medicine formulation widely used for the treatment of cardiovascular diseases in China and Southeast Asia (Tan et al. 2023; Cui and Pu 2024; Zhu et al. 2024). Previous studies have demonstrated that STDP exerts protective effects against acute MI in rat models (Yan et al. 2024). *Salvia miltiorrhiza* Bge. is a key component of STDP, and multiple bioactive constituents derived from this herb exhibit cardioprotective effects (Shan et al. 2024a). For instance, tanshinone IIA suppresses cardiomyocyte pyroptosis after acute MI (Chai et al. 2023). In addition, salvianolic acid B

¹Intensive Care Unit, Shaoxing Central Hospital (The Central Hospital Affiliated with Shaoxing University), Shaoxing, Zhejiang, 312030, China

²Department of Nursing, Shaoxing Central Hospital (The Central Hospital Affiliated with Shaoxing University), Shaoxing, Zhejiang, 312030, China

³Department of Respiratory, Shaoxing Central Hospital (The Central Hospital Affiliated with Shaoxing University), Shaoxing, Zhejiang, 312030, China

✉ zengyanping0621@163.com

has been shown to alleviate ferroptosis in MI rats (Shen et al. 2022b).

Salvianolic acid C (SAC) is a major polyphenolic compound isolated from *S. miltiorrhiza* Bge., and is structurally composed of two salvianolic acid units and one caffeic acid unit (Xing et al. 2025). SAC has been reported to possess antioxidant, anti-inflammatory, and antiapoptotic properties and to participate in the regulation of multiple pathological processes. For example, SAC modulates the TLR4/NF- κ B pathway to reduce neuroinflammation, thereby improving cerebral ischemic injury (Guo et al. 2024). Moreover, SAC alleviates renal tubulointerstitial fibrosis (Wu et al. 2023a) and reduces inflammation and apoptosis to cisplatin-induced acute kidney injury (Chien et al. 2021). SAC has also been shown to promote cerebral angiogenesis and mitigate cerebral ischemia-reperfusion injury (Shen et al. 2022a). Importantly, SAC has been verified to attenuate apoptosis, inflammation, and oxidative stress (Duan et al. 2019; Wu et al. 2019). However, the protective effects of SAC in MI and the underlying molecular mechanisms have not yet been fully elucidated.

In this study, we demonstrated that SAC activated the AMPK pathway to inhibit ferroptosis and alleviate cardiomyocyte injury after acute MI. These findings provide novel mechanistic insights into the cardioprotective role of SAC and suggest its potential value in the clinical treatment of MI.

2. Materials and Methods

2.1. MI rat model

This work was approved by the Institutional Animal Care and Use Committee (IACUC) of Zhejiang Provincial Laboratory Animal Center, China (Approval No. ZJCLA-IACUC-20011035), with the application submitted through Shaoxing Central Hospital, China. The animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Sprague-Dawley rats (specific pathogen-free grade, $n = 24$) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). After anesthesia with pentobarbital sodium, MI was induced by ligation of the left anterior descending (LAD) coronary artery. Successful establishment of the MI model was confirmed by visible whitening of the ligation area. In the Sham group, rats underwent thoracotomy without LAD ligation. SAC (IS05509; 10 mg/kg or 20 mg/kg), purchased from Solarbio Biotechnology Co., Ltd. (Beijing, China), was administered intragastrically to rats once daily for 30 days.

2.2. Triphenyltetrazolium chloride staining

Myocardial tissues were sliced into -mm-thick sections. Sections were incubated in 1% triphenyltetrazolium chloride

(TTC) solution for 0.5 h, followed by 10% formaldehyde for 6 h. The infarct area (white) and non-infarct area (red) were photographed, and the infarct size was calculated as a percentage of the total myocardial area.

2.3. Hematoxylin-eosin staining

The myocardial tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Paraffin-embedded tissues were sectioned at a thickness of 4 μ m, followed by hematoxylin-eosin (HE) staining according to standard procedures. Histological images were captured using a light microscope (BX53, Olympus, Japan).

2.4. Reverse transcription quantitative polymerase chain reaction

Total RNA was extracted from myocardial tissues using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). Complementary DNA was synthesized using the PrimeScript™ RT Reagent Kit (Takara, Dalian, China). Quantitative PCR was performed using the SYBR Green PCR kit (Toyobo, Japan). Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal control.

The primer sequences were as follows:

Tumor necrosis factor (TNF)- α :

F: 5'-AGCCGATGGGTTGTACCT-3'

R: 5'-TGAGTTGGTCCCCCTTCT-3'

Interleukin (IL)-1 β :

F: 5'-CCAGCTTCAAATCT CACAGCAG-3'

R: 5'-CTTCTTTGGGTATTGCTTGGGATC-3'

IL-6:

F: 5'-TCCAGTTGCCTTCTTGGGAC-3'

R: 5'-GTACTCCAGAAGACCAGAGG-3'

GAPDH:

forward, 5'-CTGGGCTACACTGAGCACC-3'

reverse, 5'-AAGTGGTCGTTGAGGGCAATG-3'

2.5. Enzyme-linked immunosorbent assay

Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to measure cytokine levels according to the manufacturers' instructions, including IL-1 β (ab255730, Abcam, Shanghai, China), IL-6 (ab234570, Abcam), and TNF- α (ab236712, Abcam).

2.6. Reactive oxygen species examination (DHE staining)

The myocardial tissue sections (4 μ m) were incubated with dihydroethidium (DHE; 10 μ mol, Sigma-Aldrich, USA) for

30 min in the dark. After being washed twice with phosphate-buffered saline (PBS), fluorescence images were acquired using an automatic fluorescence microscope (BX63, Olympus Optical Ltd. Tokyo, Japan).

2.7. Measurement of superoxide dismutase, malondialdehyde, and ferrous iron (Fe²⁺)

The levels of superoxide dismutase (SOD), malondialdehyde (MDA), and Fe²⁺ were quantified using commercial assay kits, including SOD (ab65354, Abcam, Shanghai, China), MDA (ab118970), and the Fe assay kit (ab83366), following the manufacturer's protocols.

2.8. Terminal deoxynucleotide transferase-mediated dUTP nick end-labeling assay

Apoptosis in myocardial tissues was detected using the *In Situ* Cell Death Detection Kit (Roche, Basel, Switzerland). The myocardial tissue sections were permeabilized with 0.1% Triton X-100, followed by incubation with terminal deoxynucleotide transferase-mediated dUTP nick end-labeling (TUNEL) staining solution for 1 h in the dark. After washing, converter-peroxidase and diaminobenzidine (DAB) were subsequently applied, and nuclei were counterstained with DAPI. Fluorescence images were obtained using a fluorescence microscope (BX63, Olympus Optical Ltd. Tokyo, Japan).

2.9. Western blot

Total proteins were extracted from myocardial tissues or H9C2 cells using RIPA lysis buffer (Beyotime, Shanghai, China). Equal amounts of protein were separated by 10% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (Beyotime, Shanghai, China). After blocking, membranes were incubated overnight at 4°C with primary antibodies, including SLC7A11 (1/1000; ab307601; Abcam, Shanghai, China), GPX4 (1/1000; ab125066), p-AMPK (1/2000; ab68206), AMPK (1/1000; ab32047), p-mTOR (1/1000; ab109268), mTOR (1/5000; ab134903) and GAPDH (1/2000; ab8245) for 12 h. Afterward, membranes were incubated with the appropriate secondary antibodies (1/1000; ab7090) for 2 h, and the protein bands were visualized using a chemiluminescence detection kit (Thermo Fisher Scientific, Inc., USA).

2.10. Cell lines and treatment

Rat H9C2 cardiomyocytes were obtained from American Tissue Culture Collection (ATCC, Manassas, VA, USA), and cultured in DMEM under humidified conditions (37°C, 5% CO₂). To establish the oxygen-glucose deprivation

(OGD) model, cells were incubated in glucose-free and serum-free DMEM under hypoxic conditions (94% N₂, 5% CO₂, and 1% O₂) for 3 h. Control cells were maintained under normoxic conditions (95% air and 5% CO₂). SAC (0, 2.5, 5, 10, 20, 40, 80, and 160 μM) was adopted for treating H9C2 cells.

2.11. Cell counting kit-8 assay

H9C2 cells were seeded into 96-well plates, and 10 μL of cell counting kit-8 (CCK-8) reagent (Dojindo Laboratories, Kumamoto, Japan) was added to each well. Cell viability was measured using a microplate reader (Bio-Rad, CA, USA).

2.12. ROS detection by dichlorofluorescein staining

Intracellular reactive oxygen species (ROS) levels were assessed using a ROS kit (E004-1-1, Nanjing Jiancheng Technology Co., Ltd., Nanjing, China). H9C2 cells were incubated with dichloro-dihydro-fluorescein diacetate (DCFH-DA) for 30 min. After washing, images were captured through the fluorescence microscope (Olympus, Tokyo, Japan).

2.13. Statistical analysis

All data are presented as the mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software, La Jolla, CA, USA). The one-way analysis of variance (ANOVA) was applied for multiple group comparisons, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. SAC alleviated the infarct size in MI rats

The infarct size of the heart was significantly increased in the MI group; however, this effect was markedly attenuated following SAC treatment (10 mg/kg or 20 mg/kg) (Figure 1A, $p < 0.05$). In addition, obvious myocardial fiber rupture, cardiomyocyte swelling, and extensive inflammatory cells were observed in the MI group. These pathological changes were notably improved after SAC treatment (Figure 1B). In general, these findings indicate that SAC effectively reduces infarct size in MI rats.

3.2. SAC attenuated inflammation and cardiomyocyte apoptosis in MI rats

The mRNA expression levels of IL-1β, IL-6, and TNF-α were significantly elevated in the MI group. These increases were rescued after SAC treatment (Figure 2A, $p < 0.01$). Consistently, ELISA results showed similar trends at the protein level, with

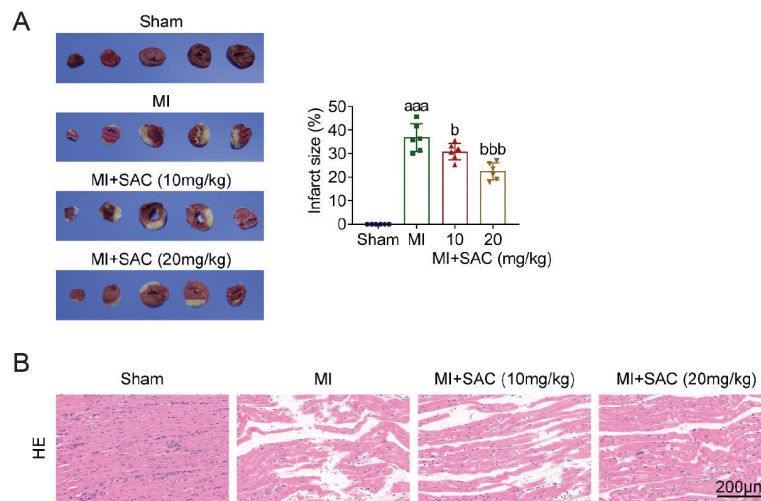


Fig 1. SAC alleviated the infarct size in MI rats. Groups were separated into the Sham, MI, MI + SAC (10 mg/kg), and MI + SAC (20 mg/kg) groups. (A) The infarct size of the heart in rats was assessed through TTC staining. (B) Myocardial histopathological damage was evaluated by HE staining. ^{aaa} $p < 0.001$ vs the Sham group; ^b $p < 0.05$, ^{bbb} $p < 0.001$ vs the MI group HE, hematoxylin-eosin; MI, myocardial infarction; SAC, salvianolic acid C; TTC, triphenyltetrazolium chloride.

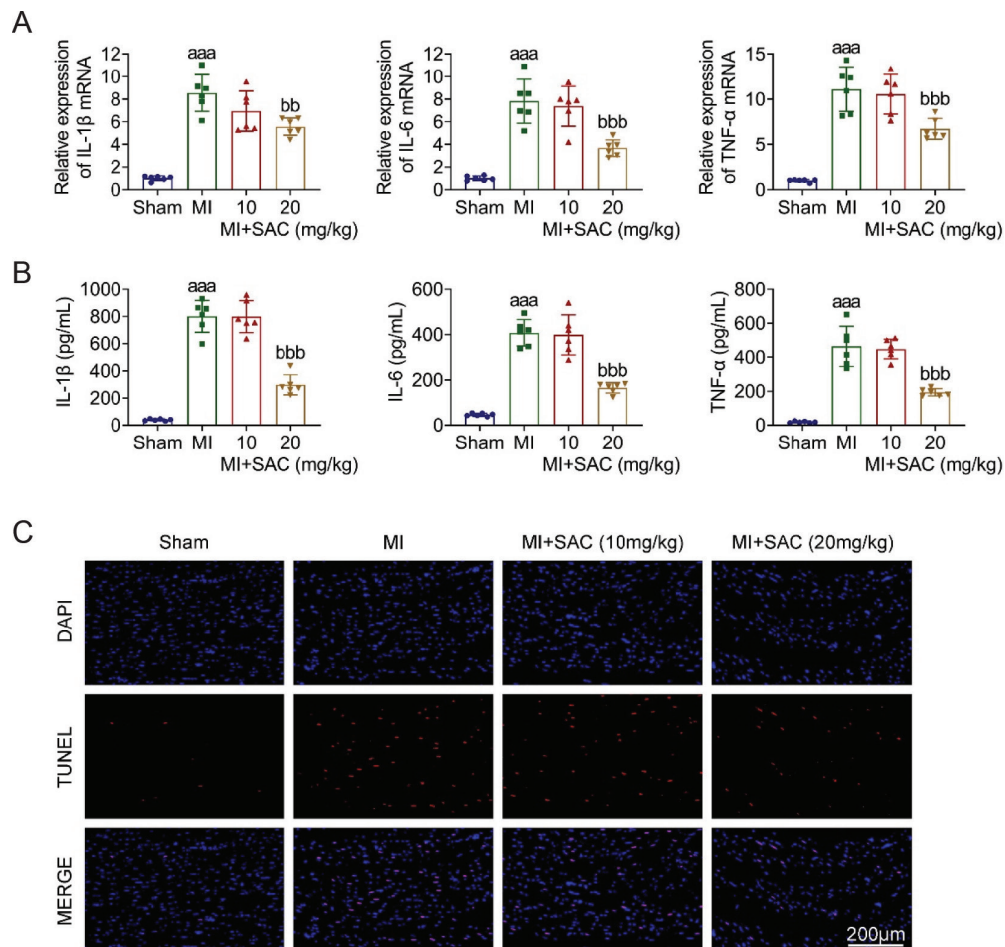


Fig 2. Groups were separated into the Sham, MI, MI + SAC (10 mg/kg), and MI + SAC (20 mg/kg) groups. (A) mRNA expressions of IL-1β, IL-6, and TNF-α were examined through RT-qPCR. (B) The levels of IL-1β, IL-6, and TNF-α were tested through ELISA. (C) Cardiomycocyte apoptosis in myocardial tissues was evaluated through the TUNEL assay.

significantly reduced IL-1 β , IL-6, and TNF- α levels after SAC administration (Figure 2B, $p < 0.001$). Furthermore, cardiomyocyte apoptosis was markedly increased in the MI group, whereas this effect was substantially neutralized after SAC treatment, as demonstrated by TUNEL staining (Figure 2C). Taken together, these results suggest that SAC effectively suppresses inflammation and cardiomyocyte apoptosis in MI rats.

3.3. SAC suppressed ferroptosis in MI rats

The ROS level was markedly increased in the MI group, whereas SAC treatment significantly reduced ROS accumulation (Figure 3A). Moreover, the SOD level was significantly decreased, while MDA and Fe²⁺ levels were markedly increased in the MI group. These alterations were reversed

after SAC treatment (Figures 3B–D, $p < 0.01$). In addition, the protein expression levels of the ferroptosis-related markers SLC7A11 and GPX4 were significantly reduced in the MI group. SAC administration effectively restored the expression of both proteins (Figure 3E, $p < 0.001$). Taken together, these findings demonstrated that SAC suppressed ferroptosis in MI rats.

3.4. SAC activated the AMPK signaling pathway

The protein level of p-AMPK/AMPK was decreased, and p-mTOR/mTOR was increased in the MI group. These changes were effectively reversed by SAC treatment (Figure 4, $p < 0.001$), indicating that SAC activates the AMPK pathway in MI rats.

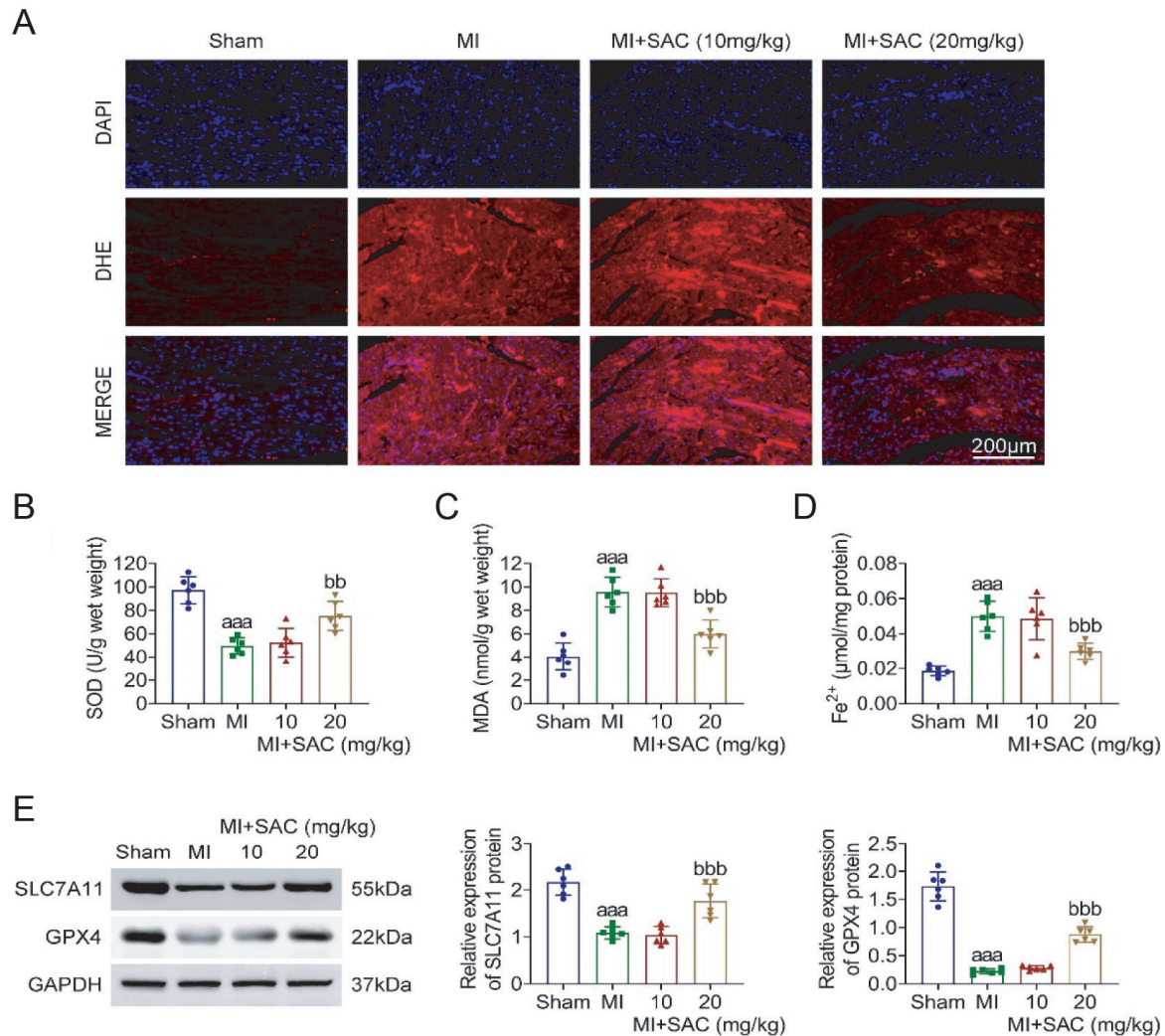


Fig 3. SAC suppressed ferroptosis in MI rats. Groups were separated into the Sham, MI, MI + SAC (10 mg/kg), and MI + SAC (20 mg/kg) groups. **(A)** The ROS level was measured through DHE staining. **(B–D)** The levels of SOD, MDA, and Fe²⁺ were examined through the corresponding kits. **(E)** The protein expressions of SLC7A11 and GPX4 were assessed through Western blot. ^{aaa} $p < 0.001$ vs the Sham group; ^{bbp} $p < 0.01$, ^{bbb} $p < 0.001$ vs the MI group DHE, dihydroethidium; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MDA, malondialdehyde; MI, myocardial infarction; ROS, reactive oxygen species; SAC, salvianolic acid C; SOD, superoxide dismutase.

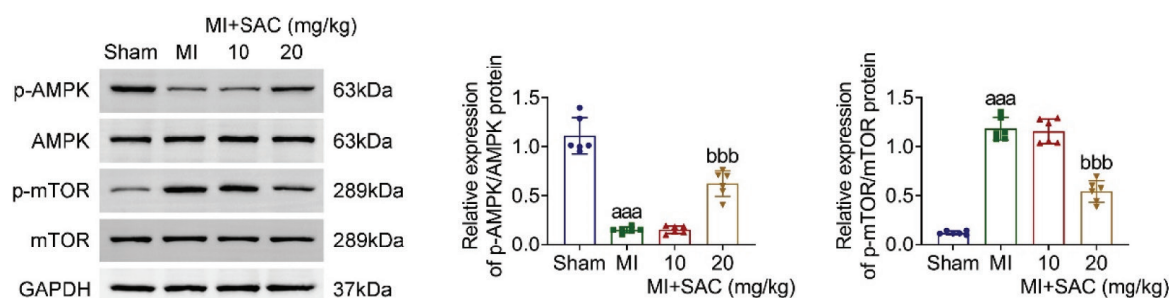


Fig 4. SAC evoked the AMPK pathway. Groups were separated into the Sham, MI, MI + SAC (10 mg/kg), and MI + SAC (20 mg/kg) groups. The protein expressions of p-AMPK, AMPK, p-mTOR, and mTOR were determined through western blot. ^{aaa} $p < 0.001$ vs the Sham group; ^{bbb} $p < 0.001$ vs the MI group. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MI, myocardial infarction; SAC, salivianolic acid C.

3.5. SAC activates the AMPK pathway to ameliorate OGD-induced cardiomyocyte injury

In vitro experiments revealed that the cell viability of H9C2 cells was significantly reduced after SAC treatment with high concentrations of SAC (40, 80, and 160 μ M) (Figure 5A, $p < 0.001$). Therefore, 20 μ M SAC was chosen for subsequent experiments. The decreased p-AMPK/AMPK protein level in the OGD group was reversed after SAC treatment, whereas this protective effect was abolished following AMPK inhibition (Figure 5B, $p < 0.001$). In addition, OGD-induced reductions in SOD levels and increases in MDA and Fe^{2+} levels were significantly reversed by SAC treatment. These effects were further abrogated after AMPK knockdown (Figures 5C–E, $p < 0.05$). Similarly, the elevated ROS levels induced by OGD were attenuated by SAC treatment, while AMPK suppression reversed this effect (Figure 5F). Furthermore, OGD-induced downregulation of SLC7A11 and GPX4 protein expression was significantly reduced by SAC treatment, and this rescue effect was further diminished after AMPK knockdown (Figure 5G, $p < 0.01$). Collectively, these results indicate that SAC alleviated OGD-induced cardiomyocyte injury through activation of the AMPK pathway.

3.6. SAC and Ferrostatin-1 exert similar protective effects against OGD-induced injury

Additional experiments demonstrated that cell viability was significantly reduced following OGD exposure, whereas this reduction was markedly reversed by treatment with SAC or Ferrostatin-1, a ferroptosis inhibitor (Figure S1A, $p < 0.01$). Similarly, OGD-induced decreases in SOD levels were alleviated by SAC or Ferrostatin-1 treatment (Figure S1B, $p < 0.05$). Moreover, the elevated MDA and Fe^{2+} levels observed after OGD treatment were significantly attenuated after SAC or Ferrostatin-1 (Figures S1C,D, $p < 0.01$). The GPX4 protein expression was also significantly reduced in the OGD group, whereas both SAC and Ferrostatin-1 restored GPX4 expression (Figure S1E, $p < 0.05$). These findings

further confirm that SAC exerts cardioprotective effects by inhibiting ferroptosis.

4. Discussion

SAC has been reported to exhibit anti-oxidant, anti-inflammatory, and anti-apoptotic properties in various diseases (Chien et al. 2021; Shen et al. 2022a; Wu et al. 2023a; Guo et al. 2024). However, its regulatory effects and underlying mechanisms in the progression of MI have remained largely unclear. In recent years, increasing attention has been directed toward the use of herbal extracts and natural compounds for MI treatment. For example, *S. miltiorrhiza* has been shown suppress cardiomyocyte ferroptosis after MI (Wu et al. 2024). In addition, latifolin relieves MI progression by attenuating myocardial inflammation (Lai et al. 2020), while polydatin stimulates Sirt3 to protect cardiomyocytes in MI injury (Zhang et al. 2017). Consistent with these previous studies, this study demonstrated that SAC significantly alleviates the infarct size in MI rats. Inflammation and cardiomyocyte apoptosis were markedly enhanced in the MI group, whereas these pathological changes were effectively attenuated following SAC treatment. Collectively, these findings indicate that SAC exerts a protective effect against MI progression.

Accumulating evidence indicates that ROS production triggered by MI plays a key role in myocardial injury (Cadenas 2018). It has been confirmed that ferroptosis participates in the pathogenesis of myocardial diseases (Fang et al. 2024). Ferroptosis is a distinct form of regulated cell death characterized by iron-dependent lipid peroxidation and excessive lipid ROS accumulation (Endale et al. 2023). Therefore, targeting ferroptosis has emerged as a promising strategy for alleviating myocardial injury after MI, attracting increasing attention from researchers. For example, epigallocatechin gallate (EGCG) suppresses ferroptosis by targeting the miR-450b-5p/ACSL4 axis, thereby alleviating acute MI (Yu et al. 2023). Additionally, *S. miltiorrhiza* activates the Nrf2 pathway to attenuate ferroptosis after MI (Wu et al. 2024). Ferrostatin-1

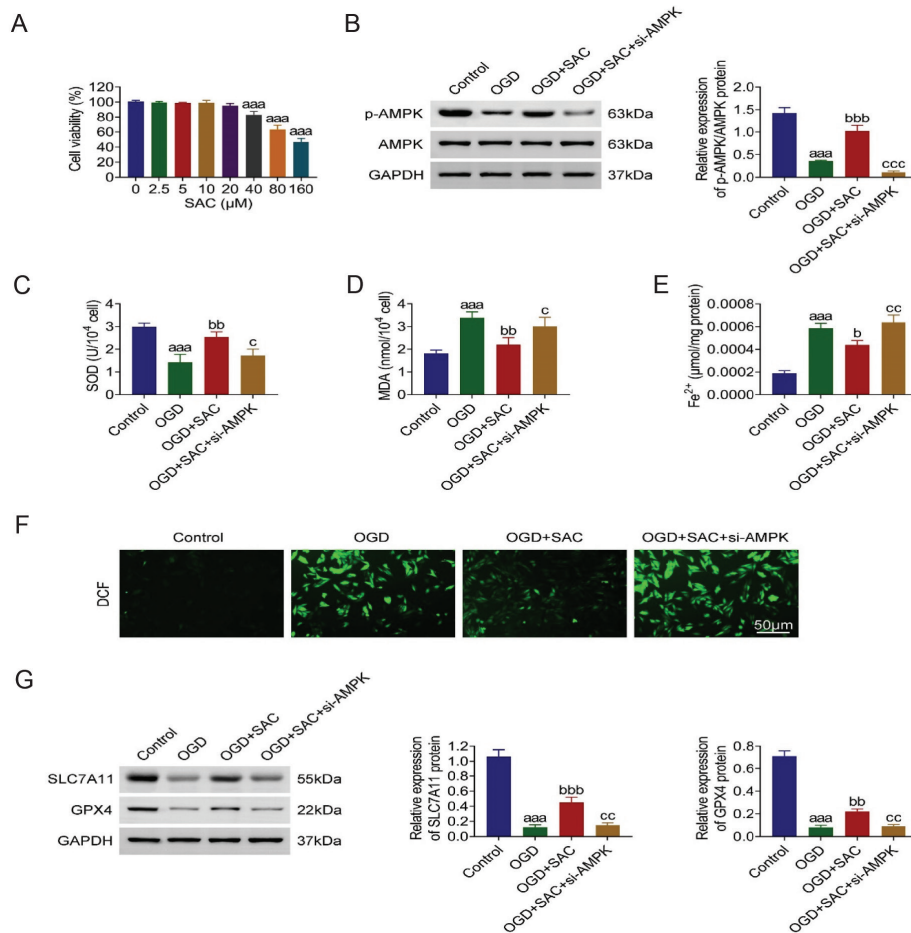


Fig 5. SAC triggered the AMPK pathway to ameliorate cardiomyocyte damage mediated by OGD. **(A)** The cell viability was inspected through CCK-8 assay in the 0, 2.5, 5, 10, 20, 40, 80, and 160 μ M SAC groups. **(B)** The protein expressions of p-AMPK and AMPK were assessed through western blot in the control, OGD, OGD + SAC, and OGD + SAC + si-AMPK groups. **(C–E)** The levels of SOD, MDA, and Fe²⁺ were examined through the corresponding kits in the control, OGD, OGD + SAC, and OGD + SAC + si-AMPK groups. **(F)** The ROS level was tested through DCF staining in the control, OGD, OGD + SAC, and OGD + SAC + si-AMPK groups. **(G)** The protein expressions of SLC7A11 and GPX4 were measured through western blot in the control, OGD, OGD + SAC, and OGD + SAC + si-AMPK groups. ^{aaa}*p* < 0.001 vs the control group; ^{bp} *p* < 0.05, ^{bbp} *p* < 0.01, ^{bbbp} *p* < 0.001 vs the OGD group; ^{cp} *p* < 0.05, ^{ccp} *p* < 0.01, ^{cccp} *p* < 0.001 vs the OGD + SAC group CCK-8, cell counting kit-8; DCF, dichlorofluorescein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MDA, malondialdehyde; OGD, oxygen-glucose deprivation; ROS, reactive oxygen species; SAC, salivianolic acid C.

has also been reported to attenuate ferroptosis after MI (Wu et al. 2023b). Furthermore, fraxetin targets the AKT/Nrf2/HO-1 pathway to relieve ferroptosis in MI (Xu et al. 2021). Similarly, our study demonstrated that SAC suppressed ferroptosis in MI rats, highlighting its potential role in regulating oxidative cell death.

AMPK is a key modulator of lipid metabolism and glucose metabolism, and exhibits crucial roles in controlling cellular energy homeostasis (Carling 2017; Herzig and Shaw 2018). The impaired AMPK phosphorylation has been reported to affect the absorption and metabolism of glucose in the heart during myocardial ischemia (Lu et al. 2022), suggesting that AMPK represents a strategic therapeutic target for MI. Importantly, activation of the AMPK pathway has been reported to inhibit ferroptosis in cardiomyocytes, thereby

alleviating myocardial ischemia–reperfusion (I/R) injury in mice (Liu et al. 2024). Many studies have emphasized the significance of the AMPK pathway in MI progression. For instance, Rap1GAP aggravated the MI progression by modulating the AMPK/NF- κ B pathway (Shan et al. 2024b). Geniposide activates the AMPK pathway to suppress pyroptosis and improve MI outcome (Li et al. 2022). Moreover, remote cyclic compression has been shown to alleviate MI injury via AMPK-dependent mechanisms (Xu et al. 2022). In addition, SERPINB1 targets the AMPK/mTOR pathway to ameliorate acute MI-triggered myocardial damage (Wang et al. 2022). Consistent with these reports, our study confirmed that SAC activates the AMPK signaling pathway *in vivo*. Furthermore, *in vitro* experiments demonstrated that SAC alleviates OGD-induced cardiomyocyte injury by activating

the AMPK pathway, providing mechanistic evidence for its cardioprotective effects.

In conclusion, his study is the first to demonstrate that SAC activates the AMPK pathway to inhibit ferroptosis and alleviate cardiomyocyte injury following acute MI. These findings highlight the therapeutic potential of SAC as a novel cardioprotective agent for MI treatment. Nevertheless, several limitations should be acknowledged. This study was limited to animal models and cell lines, and no experiments were performed using human samples. In addition, other relevant cellular phenotypes and clinical treatment scenarios were not explored. These limitations may restrict the direct translation of our findings to human patients. Future studies will focus on validating the protective effects of SAC in clinical settings and further elucidating its role in MI progression.

5. Conclusion

This study demonstrates that SAC protects against acute MI by inhibiting ferroptosis through activation of the AMPK signaling pathway. These findings suggest that SAC may represent a promising therapeutic candidate for MI. Further studies are needed to validate its clinical applicability.

Acknowledgments

Not applicable.

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Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Competing Interests

The authors state that there are no conflicts of interest to disclose.

Ethics Approval

This work was approved by the Institutional Animal Care and Use Committee (IACUC), Zhejiang Provincial Laboratory Animal Center (Approval No. ZJCLA-IACUC-20011035), with the application submitted through Shaoxing Central Hospital.

All animal experiments should comply with the ARRIVE guidelines and conducted in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

Author's Contribution

Conceptualization, Methodology, and Writing – Original Draft were performed by Yanping Zeng; Formal analysis, Resources, and Investigation were performed by Lin Chen; Formal analysis, Visualization and Data Curation were performed by Zhenhua Qian and Weixing Ma; Project administration, Supervision, and Validation were performed by Cheng Ma and Yan Wang; Validation, Supervision, and Writing – Review and Editing were performed by Guanfeng Zhu. All authors read and approved the final manuscript.

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Supplementary Figure

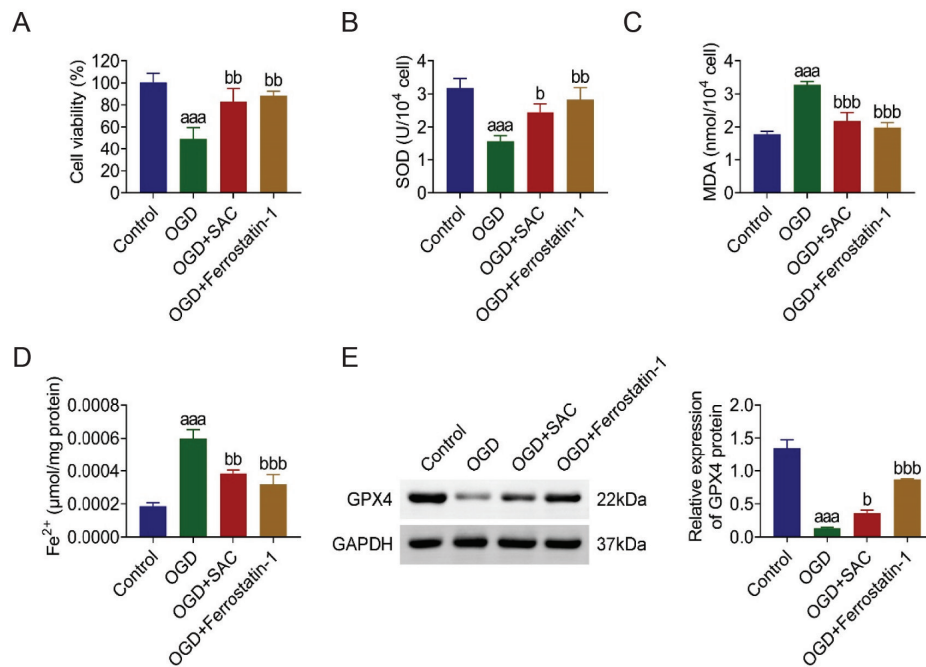


Fig S1. SAC and Ferrostatin-1 both can affect MI progression. Groups were separated into the control, OGD, OGD + SAC, and OGD + Ferrostatin-1 groups. **(A)** The cell viability was inspected through the CCK-8 assay. **(B–D)** The levels of SOD, MDA, and Fe²⁺ were examined through the corresponding kits. **(E)** The protein expression of GPX4 was measured through western blot. ^{aaa}*p* < 0.001 vs the control group; ^b*p* < 0.05, ^{bb}*p* < 0.01, ^{bbb}*p* < 0.001 vs the OGD group CCK-8, cell counting kit-8; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MDA, malondialdehyde; MI, myocardial infarction; OGD, oxygen-glucose deprivation; SAC, salvianolic acid C; SOD, superoxide dismutase.