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Gastrodin alleviates myocardial infarction by inhibiting inflammation, and apoptosis and promoting endothelial cell proliferation

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ABSTRACT

Gastrodin, a bioactive ingredient extracted from the Chinese herb Gastrodia gastrodia, has shown potential therapeutic effects in cardiovascular diseases, but its specific role in myocardial infarction is unclear. This study investigated the protective effect of gastrodin on myocardial infarction and its potential mechanism. By clamping the left coronary artery, we created a model of myocardial infarction in C57BL/6J mice. For 14 days, mice in the control and myocardial infarction groups received a daily dose of 100 mg/kg gastrodin. Gastrodin significantly improved cardiac dysfunction in mice with myocardial infarction, decreased heart weight/body weight (HW/BW) and heart weight/tibial length (HW/TL) ratios, and inhibited mRNA expression levels of cardiac fibrosis biomarkers (Collagen Type I (Col1), Collagen Type III (Col3), Matrix Metalloproteinase-2 (MMP-2), Matrix Metalloproteinase-9 (MMP-9)). In addition, gastrodin also inhibited the activity of apoptosis marker Caspase 3, decreased the Bax/Bcl2 mRNA ratio, decreased the expression of pro-inflammatory factors (Interleukin-1 beta (IL-1β), Tumor Necrosis Factor-alpha (TNF-α), Interleukin-6 (IL-6)) and pro-inflammatory adhesion molecule Monocyte Chemoattractant Protein-1 (MCP1), and promoted the expression of angiogenesis marker Cluster of Differentiation 31 (CD31). RNA sequencing and Rt-qPCR analysis showed that gastrodin treatment significantly up-regulated the expression of genes related to cell proliferation (Cyclin-Dependent Kinase 1 (CDK1), Threonine Tyrosine Kinase (TTK), Cyclin B2 (CCNB2), Polo-Like Kinase 1 (PLK1)), and promoted the proliferation of human aortic endothelial cells (HAECs). These findings suggest that gastrodin can effectively reduce the pathological changes of myocardial infarction by inhibiting inflammation, reducing apoptosis, and promoting endothelial cell proliferation, thus providing a new strategy for the prevention and treatment of myocardial infarction.

1. Introduction

Myocardial Infarction (MI) is characterized by the sudden death of heart muscle tissue due to ischemia, typically triggered by the partial or complete obstruction of coronary arteries. This blockage is often caused by plaques that have become vulnerable and prone to rupture or erosion. On a global scale, MI is associated with significant incidence and mortality rates, predominantly impacting industrialized countries, although it is also increasingly prevalent in developing nations. [1,2] Reports indicate that in China, shifting lifestyles and the progression of an aging society have led to a notable rise in myocardial infarction mortality rates in recent years. Moreover, there is an increasing trend of myocardial infarction fatalities among the younger population, positioning it as a predominant reason for emergency hospital admissions. [3]After myocardial infarction, inflammation, apoptosis, and insufficient regenerative capacity of cardiomyocytes are the key pathological mechanisms leading to further deterioration of myocardial injury and heart failure. [4] Therefore, finding therapeutic strategies that can inhibit

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inflammation, reduce apoptosis, and promote the proliferation of cardiomyocytes is the top priority in improving myocardial infarction.

The Chinese plant gastrodia is the source of the phenolic glycoside known as gastrodin (GAS). Also known as 4-hydroxybenzyl alcohol-4-*O*-β-D-glucopyranoside. [5] GAS has a variety of pharmacological effects, including neuroprotective, anti-inflammatory, antioxidant and microcirculatory improvement. [6] Its neuroprotective effect is mainly achieved by inhibiting neuronal apoptosis and reducing oxidative stress. Studies have shown that GAS can activate the PI3K/Akt signaling pathway and up-regulate the activity of antioxidant enzymes (such as SOD and GSH-Px), thereby reducing nerve damage. [7] In addition, GAS exerts significant anti-inflammatory effects by inhibiting the NLRP3/caspase-1, NF-κB, and MAPK pathways and reducing the production of pro-inflammatory factors such as TNF-α, IL-1β, and IL-6. [8-10] In terms of antioxidants, GAS protects cells from oxidative damage by scavenging free radicals and inhibiting lipid peroxidation. [11]GAS can also significantly improve microcirculation by promoting the expression of angiogenic factors (CD31 and VE-Cadherin), thereby promoting angiogenesis, inhibiting endothelial cell pyrodeath, platelet aggregation and reducing blood viscosity. [8,12] In addition, there is evidence that gastrodia also has a certain protective effect on the heart, such as Shu et al. found that GAS can effectively block the ERK1/2 signaling pathway, thereby preventing cardiac hyperplasia and fibrosis caused by stress overload. [13] Cheng and Han et al. found that GAS can reduce myocardial ischemia-reperfusion injury by reducing the release of inflammatory factors, inhibiting apoptosis, and protecting cardiomyocytes. [14,15] In summary, gastrodin can exert its pharmacological effects through multi-target and multi-pathway, and has important clinical application value in the treatment of cardiovascular diseases.

The mechanism of action of GAS in myocardial infarction is unclear, therefore, We systematically investigated the mechanism by which GAS mitigated myocardial infarction damage to heart tissue by using in vivo myocardial infarction mouse models and in vitro HAECs. Our study demonstrated that GAS exerts cardioprotective effects by mitigating cardiac fibrosis, suppressing apoptosis, and inhibiting the release of inflammatory factors. Furthermore, GAS promotes endothelial cell proliferation and enhances CD31 expression, thereby offering a novel therapeutic strategy for both the treatment and prevention of myocardial infarction.

2. Materials and methods

2.1. Animal

C57BL/6J background mice were used in this study. Mice had unrestricted access to standard chow and tap water and were housed under specific pathogen-free conditions: 22±1 °C temperature and 55 % humidity with a 12-h light/dark cycle. All procedures were approved by the Animal Care and Use Committee of Nanjing Medical University (IACUC-2112034). Animals were randomly assigned using a random number generator for experiments in which drugs were administered. At the end of the experiment, all mice were anesthetized with 2 % isoflurane inhalation and underwent cervical dislocation to relieve pain in the mice.

2.2. Mouse model

Regarding the construction of the mouse MI model, 8–10-week-old male or female mice were anesthetized with 2 % isoflurane by inhalation, the left thorax was moistened with saline and then shaved, followed by an approximately 1-1.2 cm opening cut with scissors. The pectoral muscle was dissected until the third rib space was exposed, and then a hole was opened in the pleura with pointed curved forceps, and the curved forceps were slightly turned while the heart was gently extruded out of the hole. The left coronary artery was permanently ligated with 7-0 nylon silk. The heart is then immediately placed back into the thoracic cavity and any air that may be present in the thoracic cavity is manually evacuated before the skin and muscle are closed with 4-0 sutures. Mice were monitored until full consciousness was restored. The sham group was treated the same as the MI group as described previously but without ligation of the left coronary artery.

Mice were administrated with GAS (MedChemExpress) by gavage at a dose of 100 mg/kg once daily for 14 days starting on the first postoperative day. (According to gastrodin concentration in Refs. [16,17]) GAS was dissolved in CMC-Na (5 %). The preparation and administration of the drug is done in one day. It must be redispersed by agitation if stratification occurs before administration. Mice in the control group received the same amount of solvent orally.

2.3. Echocardiography

Transthoracic echocardiography was performed on mice using the high-resolution Vevo 2100 system (VisualSonics, Canada). [18] In this study, to avoid bias, all procedures were performed by a researcher who did not know the experimental treatment.

2.4. Masson staining

Mouse heart samples obtained after dissection were fixed in 4 % paraformaldehyde for 24 h. After dehydration through a series of ethanol baths, samples were embedded in paraffin, paraffin sections were cut at 5 μm, and sections were deparaffinized and rehydrated sequentially through 100 %, 95 %, and 70 % alcohol and stained with Masson's trichrome staining kits (G1006, Servicebio, China) with minor modifications according to the manufacturer's instructions. Images were captured using a light microscope. A minimum of five random fields were acquired for each heart sample, and all images were quantified using ImageJ (V1.53).

2.5. Immunofluorescent staining

Hearts were dissected and fixed in 4 % paraformaldehyde for 24 h. Before antibody staining, paraffin-embedded hearts were cut at 5 μm thickness and deparaffinized. Slides were incubated in antigen repair solution (P0086, Beyotine, China) at 95 °C for 20 min according to the manufacturer's instructions, followed by incubation in blocking solution prepared with PBS plus 10 % BSA (ST203, Beyotine, China) and 0.1 % Triton X-100 (ST795, Beyotine, China) at room temperature for 1 h. Slides were stained at 4 °C with anti-CD31 antibody (AF3628, 1:200, R&D) in 1 % BSA. On the next day, the slides were washed three times with PBS and then stained with the secondary antibody TRITC-conjugated Donkey anti-Sheep IgG (H + L) (SA00007-3, 1:500, Proteintech, China), which was prepared with 1 % BSA, for 1 h at 37 °C. Slides were then washed three times with PBS, and nuclei were stained with DAPI (P0131, Beyotine, China) containing an anti-fluorescence quencher and the coverslipped. Images were taken with an Olympus BX53 microscope. The data analyzer was blinded to the group information for immunofluorescence staining experiments.

2.6. TUNEL staining

Apoptosis of cardiomyocytes was assessed with the TUNEL FITC Apoptosis Detection Kit (A111-03, Vazyme, China) according to the manufacturer's instructions. Briefly, mouse hearts were fixed with 4 % paraformaldehyde for 24 h and then paraffin-embedded and cut into 5 μm sections for subsequent experiments. Heart sections were incubated with the TUNEL assay mixture for 1 h and the nuclei were stained with DAPI. TUNEL-labeled cells were visualized by fluorescence microscopy.

2.7. Quantitative real-time PCR

VeZol Reagent (R411-01, Vazyme, China) was used to extract total RNA from heart samples or cells. cDNA was obtained by reverse transcribing 0.5 μg RNA samples using HiScript III Reverse Transcriptase (R323-01, Vazyme, China) according to the manufacturer's protocol. qRT-PCR was performed using SYBR Green Master Mix (11202ES08, Yeasen, China) on a QuantStudio 5 system (Thermo Fisher Scientific, USA). PCR conditions included pre-denaturation at 95 °C for 30 s, 95 °C for 10 s, 60 °C for 30 s, and 40 cycles. Gene expression levels were normalized to β-Actin. All samples were performed in triplicate and relative expression levels of the genes were calculated using the 2−ΔΔCT method. Primers for these genes are shown below.

Gene	Species	Primer NO.	Primer Sequence (5′-3′)
CDK1	Human	Forward	GGATGTGCTTATGCAGGATTCC
		Reverse	CATGTACTGACCAGGAGGGATAG
TTK	Human	Forward	TCATGCCCATTTGGAAGAGTC
		Reverse	CCACTTGGTTTAGATCCAGGC
CCNB2	Human	Forward	TTGGCTGGTACAAGTCCACTC
		Reverse	TGGGAACTGGTATAAGCATTGTC
PLK1	Human	Forward	AAAGAGATCCCGGAGGTCCTA
		Reverse	GGCTGCGGTGAATGGATATTTC
β-ACTIN	Human	Forward	CCCCTGAACCCCAAGGCCAA
		Reverse	AGCACAGCCTGGATAGCAAC
Anp	Mouse	Forward	ACCTCCCGAAGCTACCTAAGT
		Reverse	CAACCTTTTCAACGGCTCCAA
Bnp	Mouse	Forward	GAGGTCACTCCTATCCTCTGG
		Reverse	GCCATTTCCTCCGACTTTTCTC
β-Mhc	Mouse	Forward	GAGGGTGGCTCTCACACATTC
		Reverse	TTGGCCTTCGTAAGCAAACTG
Col1	Mouse	Forward	GTAACTTCGTGCCTAGCAACA
		Reverse	CCTTTGTCAGAATACTGAGCAGC
Col3	Mouse	Forward	ACGTAGATGAATTGGGATGCAG
		Reverse	GGGTTGGGGCAGTCTAGTG
MMP-2	Mouse	Forward	CAAGTTCCCCGGCGATGTC
		Reverse	TTCTGGTCAAGGTCACCTGTC
MMP-9	Mouse	Forward	CTGGACAGCCAGACACTAAAG
		Reverse	CTCGCGGCAAGTCTTCAGAG
Bax	Mouse	Forward	CCAAGAAGCTGAGCGAGTGT
		Reverse	CACGTCAGCAATCATCCTCTG
Bcl2	Mouse	Forward	TGGCATCTTCTCCTTCCAGC
		Reverse	ACGTCCTGGCAGCCATGTC
Cdk1	Mouse	Forward	AGAAGGTACTTACGGTGTGGT
		Reverse	GAGAGATTTCCCGAATTGCAGT
Ttk	Mouse	Forward	GCAGTGTGACGATTGATTCCA
		Reverse	TCGGCACAGATTTTAGACAAGC
Ccnb2	Mouse	Forward	GCCAAGAGCCATGTGACTATC
		Reverse	CAGAGCTGGTACTTTGGTGTTC
Plk1	Mouse	Forward	CTAGCACACCAACACGTCGTA
		Reverse	ACCTCCAGATCCTCGTTCAGG

2.8. Cell culture

The Chinese Academy of Sciences Cell Bank (CTCC, Shanghai, China) provided human aortic endothelial cells (HAECs). Cells were cultured in a CO2 incubator (Thermo Fisher Scientific, USA) and M199 medium (G4510, Servicebio, China) containing 10 % fetal bovine serum (10099-141, Gibco, Australia).

2.9. ELISA assay

Caspase-3 substrate Ac-DEVD-pNA was used to detect the caspase-3 activities in the heart tissues with colorimetric analysis kits according to the manufacturer's instructions. What's more, the expression of IL-1β (H002), TNF-α (H052-1), MCP-1 (H115), and IL6 (H007-1-1) was monitored using different ELISA kits purchased from Nanjing Jianchen (Nanjing, China), according to the manufacturer's instructions.

2.10. RNA sequencing and analysis

RNA sequencing library was generated as described previously (Wuhan Frasergen Genomic Medicine Co., Ltd., China). The Poly(A) Purist™ MAG Kit (Thermo Fisher Scientific) was used to enrich Poly-A tailed mRNA, and then the mRNA was generated RNA-seq library preparation by using a NEBNext® Ultra™ Directional RNA Library Prep Kit (NEB) according to the manufacturer's instructions. After checking the quality of libraries using the Agilent High Sensitivity DNA kit (Agilent Technologies), the library was sequenced using an Illumina NextSeq 500 instrument (150 cycles, paired end).

After filtration of raw reads using trim-galore, clean reads were aligned to mm10 by Hisat2 (v2.1.0). The edgeR package was used to screen for differentially expressed genes with the strict criteria (abs (logFC) $>$ 2 and *p-value* $<$ 0.01). Gene Ontology (GO) analysis was conducted by WebGestalt with FDR $<$0.05.

2.11. Statistical analysis

Prism 8 software (GraphPad) was used for analyses. Data are presented as mean ± SEM. Differences between 2 groups were analyzed by unpaired two-tailed Student's t-test, and differences between 3 or more groups were analyzed by one-way ANOVA. Statistically significant was defined as $p < 0.05$.

3. Result

3.1. GAS prevents cardiac dysfunction following myocardial infarction

To explore the protective effect of GAS on myocardial infarction, we performed a myocardial infarction operation on mice. Echocardiography showed that the left ventricular ejection fraction (EF), left ventricular fractional shortening (FS), and interventricular septum (IVS) of infarcted mice given GAS were significantly lower in the end diastole (IVS; d) and systole (IVS; s) was significantly higher than that of the infarcted mice without GAS. (Fig.1A-C). Unadministered infarcted mice had significantly enlarged left ventricular internal diameter (LVID) at the end of diastole (LVID; d) and systole (LVID; s) and left ventricular posterior wallat (LVPW) at the end of diastole (LVPW; d) and systole (LVPW; s) significantly thinner (Fig_1D-E). Then HE staining was used to observe the pathological changes in myocardial tissue. The myocardial fibers in the sham group were densely arranged, while those in the MI group were atrophic, which could be reversed after GAS treatment (Fig_1F). The ratio of heart weight to body weight and heart weight to tibial length in infarcted mice after GAS administration was lower than that in the untreated group (Fig_1G). We also found that GAS inhibited mRNA levels of Anp, Bnp, and β-mhc, biomarkers of central force failure in mice (Fig_1H). These results suggest that GAS can prevent cardiac dysfunction after myocardial infarction.

3.2. GAS prevents cardiac fibrosis and apoptosis

To confirm whether GAS can attenuate cardiac fibrosis, we performed Masson staining on histological sections of mouse myocardial tissue. The percentage of myocardial fibrosis in mice with myocardial infarction was significantly decreased and the thickness of myocardial tissue was significantly increased in mice with myocardial infarction (Fig_2A). Next, we removed the heart tissue and performed a qPCR assay, which showed that the mRNA levels of cardiac fibrosis biomarkers (Col1, Col3, MMP-2, and MMP-9) in the tissue of mice in the sham group were significantly lower than those in the MI group, and GAS could prevent the increase of mRNA expression levels of cardiac fibrosis markers in the MI group (Fig_2B). To determine whether GAS can reduce apoptosis, TUNEL staining was performed on the heart tissue sections of each group, and the apoptosis level of myocardial infarction mice treated with GAS was inhibited, and the apoptosis level of

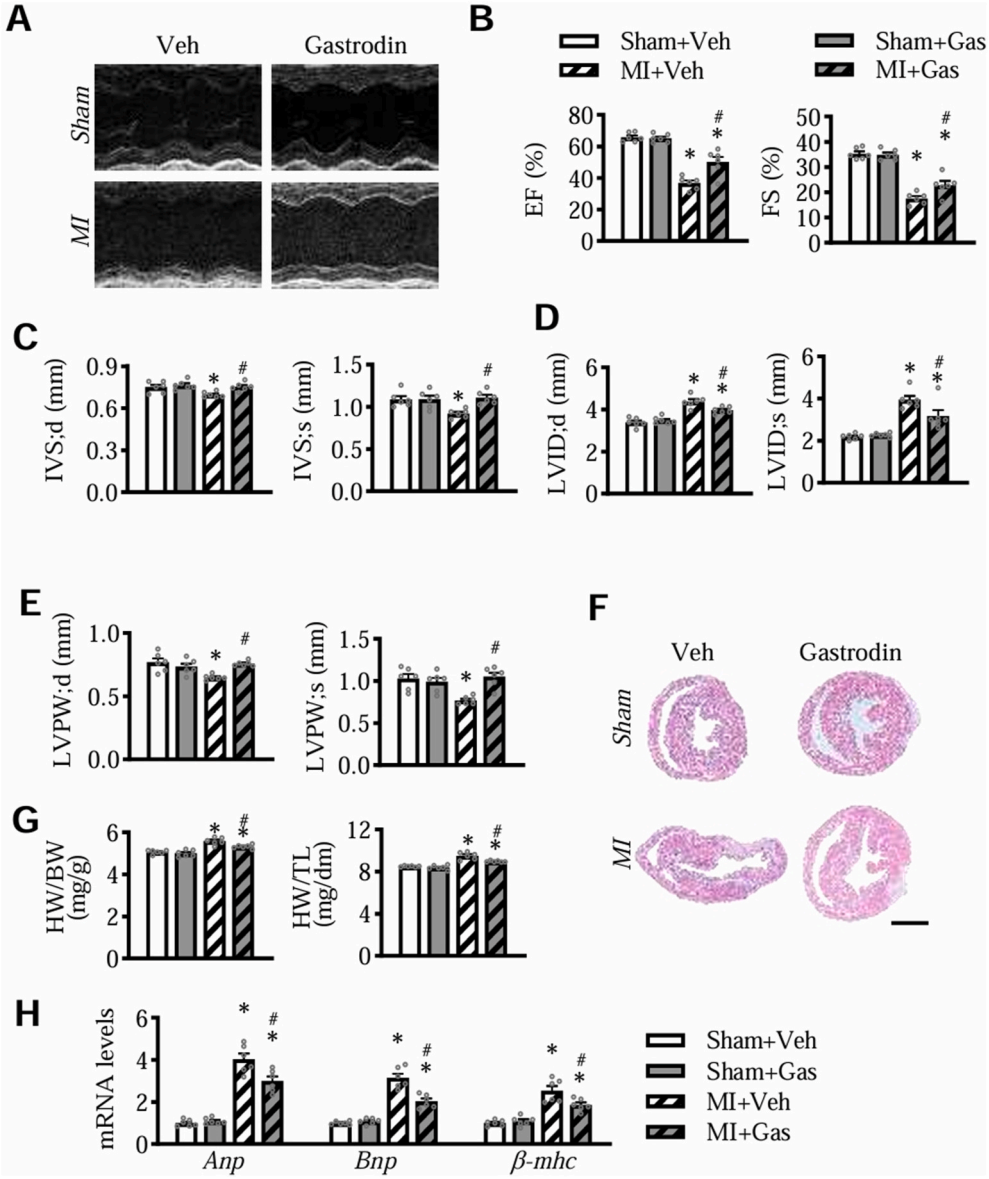

Fig. 1. Gastrodin prevents cardiac dysfunction following myocardial infarction. (A) Echocardiography analysis was performed, and M-model images were presented for each group. (B) Left ventricular ejection fraction (EF) and fractional shortening (FS) were presented in infarcted mice with/without administration of gastrodin. (C) Inter ventricular septum (IVS) at the end of diastole (IVS; d) and systole (IVS; s) was presented. (D) Left ventricular internal diameter (LVID) at the end of diastole (LVID; d) and systole (LVID; s) were presented in infarcted mice with/without administration of gastrodin. (E) Left ventricular posterior wall (LVPW) at the end of diastole (LVPW; d) and systole (LVPW; s). (F) Histological sections of myocardial tissues from infarcted mice with gastrodin administration were stained with HE staining. Scale bar, 200 μm. (G) Heart weight/body weight (HW/BW) and heart weight/tibia length (HW/TL) ratios were analyzed after gastrodin administration in mice suffered with LAD ligation. (H) The mRNA levels of Anp, Bnp, and β-mhc, biomarkers for heart failure were determined using qPCR analysis in mouse tissue. Male mice were subjected to infarction surgery, and then administrated with gastrodin (100 mg/kg per day) for 14 days (n = 6 each group). Data are mean ± SEM. $^{*}P < 0.05$ vs. sham group, $^{\#}P < 0.05$ vs. MI group.

myocardial cells was significantly increased in the MI group compared with the sham group (Fig_2C). In order to check whether the cells were apoptotic, Caspase 3 activity in cardiac tissue was detected. Caspase 3 activity was significantly activated in the MI group, and GAS could inhibit the activation of Caspase 3 activity in the MI group (Fig_2D). In the final analysis, we quantitatively assessed the Bax/Bcl-2 mRNA expression ratio, a critical indicator of apoptotic activity, in murine cardiac tissues. The MI group exhibited a significant elevation in this ratio compared to controls, whereas GAS administration effectively normalized this apoptotic index, demonstrating its anti-apoptotic efficacy. (Fig_2E). In summary, GAS can reduce cardiac fibrosis and apoptosis.

3.3. GAS inhibits inflammatory response and promotes angiogenesis

ELISA assay was used to detect the inflammatory response of the heart. The results showed that compared with the sham group, the contents of pro-inflammatory factors IL-1β, TNF-α, IL-6, and pro-inflammatory adhesion molecule MCP1 in myocardial tissue were significantly increased in the MI group, and GAS treatment could inhibit the increase of these inflammatory factors (Fig_3A-D). To observe whether GAS can promote angiogenesis, immunofluorescence staining was performed on the heart tissues of each group, and it was detected that the expression of CD31 in the heart tissues of mice with myocardial infarction was significantly increased, and the expression was further increased after GAS treatment (Fig_3E). GAS can reduce the increase of

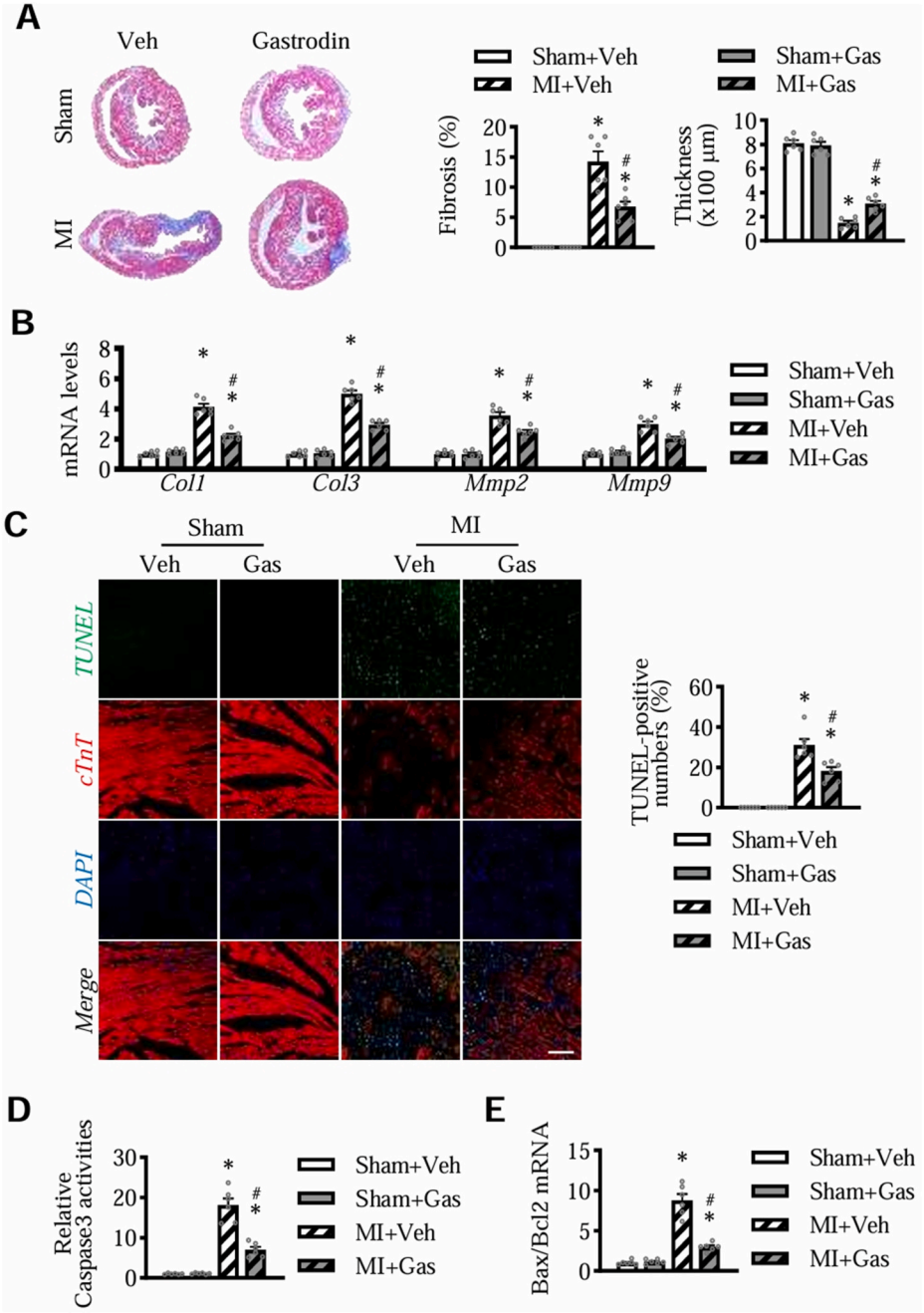

Fig. 2. Gastrodin prevents cardiac fibrosis and apoptosis. (A) Histological sections of myocardial tissues from infarcted mice with/without gastrodin administration were stained with Masson staining. The percent of cardiac fibrosis and thickness of heart tissues were quantified. (B) The mRNA levels of Col1, Col3, Mmp2, and Mmp9, biomarkers for cardiac fibrosis were determined using qPCR analysis in mouse tissue. (C) Representative images of heart tissues were presented with TUNEL staining. Scale bar, 100 μm. (D) Caspase 3 activities in heart tissues were monitored using ELISA assay. E, The ratio for Bax to Bcl2 mRNA was determined using qPCR analysis in mouse heart. Male mice were subjected to infarction surgery, and then administrated with gastrodin (100 mg/kg per day) for 14 days (n = 6 each group). Data are mean ± SEM. $^{*}P < 0.05$ vs. sham group, $^{\#}P < 0.05$ vs. MI group.

inflammatory factors and promote angiogenesis.

3.4. GAS promotes endothelial proliferation

To understand how GAS mitigated myocardial infarction, we sequenced RNA from mouse heart tissue, which was divided into three clusters on the volcano map, with red representing up-regulated genes, green representing down-regulated genes, and grey representing unaltered genes. Four genes (Cdk1, Ttk, Ccnb2, Plk1) with significant differences related to proliferation were selected by GSEA(Gene set enrichment analysis) using RNAseq results (Fig_4A-B). Then we analyzed by qPCR and found that the mRNA expression levels of Cdk1, Ttk, Ccnb2, and Plk1 in the heart tissues of GAS-treated mice were significantly increased compared with those of untreated mice (Fig_4C). In addition, we cultured HAECs in vitro with and without GAS in CoCl2 and observed the mRNA expression levels of CDK1, TTK, CCNB2, and PLK1 in HAECs. Compared with the untreated group, the mRNA expression levels of these four genes were significantly increased in the treated group (Fig_4D). We then used CCK8 to monitor the number of cells in each group at different time points and found that GAS promoted the proliferation of HAECs (Fig_4E).

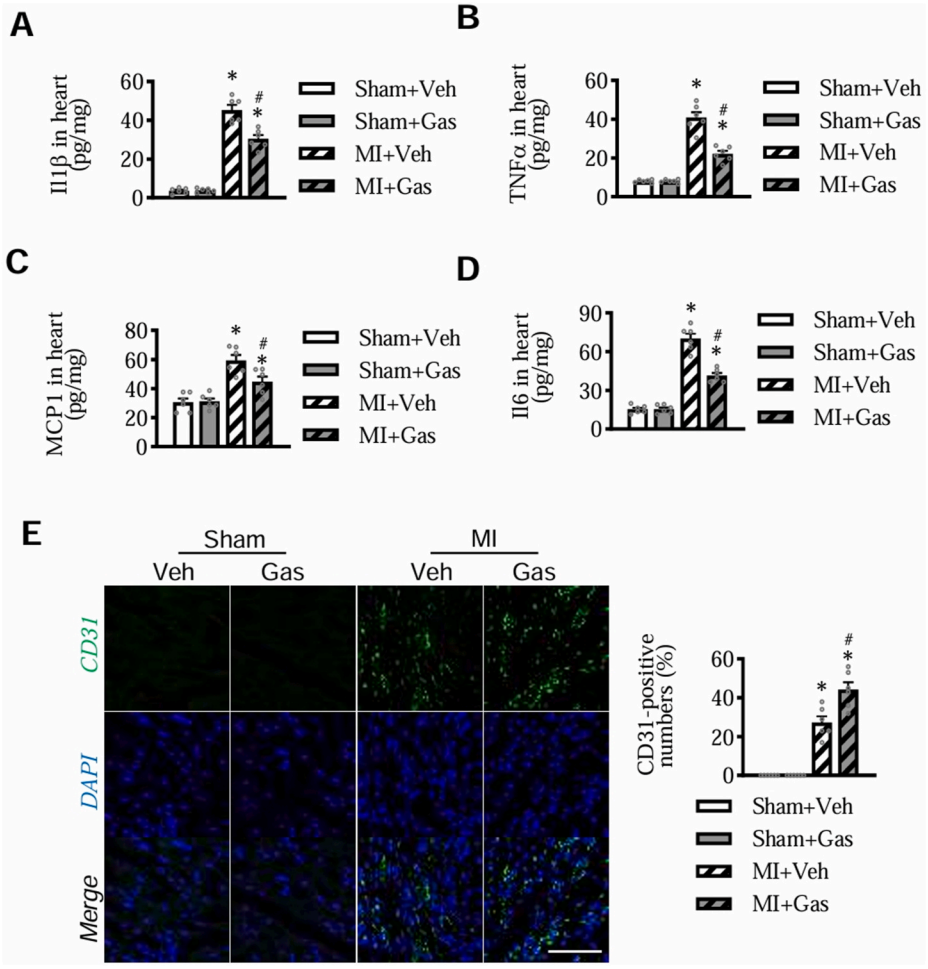

Fig. 3. Gastrodin inhibits inflammatory response and promotes angiogenesis.(A-D) The inflammatory response was elevated using ELISA kits in infarcted hearts with/without gastrodin administration, such as IL1β (A), TNFα (B), MCP1 (C) and IL6 (D). (E) Representative images of heart tissues stained with CD31 (green) and DAPI (blue for nuclei). Male mice were subjected to infarction surgery, and then administrated with gastrodin (100 mg/kg per day) for 14 days (n = 6 each group). Data are mean ± SEM. $^{*}P < 0.05$ vs. sham group, $^{\#}P < 0.05$ vs. MI group.

4. Discussion

Extensive research has elucidated a sophisticated and highly regulated cascade of reparative processes following myocardial infarction. This intricate sequence initiates with the recruitment of inflammatory immune cells to confine the inflammatory response, progressing through the phagocytic clearance of necrotic cells and tissue debris. Subsequently, the inflammatory phase transitions into regression, accompanied by fibroblast activation, neovascularization, and ultimately scar tissue formation, leading to ventricular wall thickening. However, this compensatory remodeling process results in ventricular dilatation, consequently impairing systolic function. [4,19] One of the active components in the rhizome of Gastrodia, called GAS, has been demonstrated to reduce inflammation and alleviate chronic pain brought on by Complete Freund's adjuvant (CFA). [20] GAS has been reported in neurological diseases, and it has also been applied to cardiovascular diseases. For example, GAS can reduce the inflammatory response of H9c2 cardiomyocytes by activating PI3-K/Akt signaling and inhibiting the activation of NF-κB and MAPKs. [21] GAS can help reduce foam cell production and the inflammatory response, hence improving atherosclerosis. [22] Atherosclerosis is the main underlying disease process leading to myocardial infarction. [23] In conclusion, GAS may play a role in myocardial infarction by inhibiting inflammatory response. Therefore, by establishing a mouse myocardial infarction model and administering the drug, we found that the contents of inflammation-related factors IL-1β, TNF-α,IL-6 and MCP1 in myocardial tissue of mice with myocardial infarction were significantly reduced after GAS treatment. These results indicate that GAS can improve myocardial infarction by inhibiting the release of inflammation-related factors.

GAS has been demonstrated to reduce apoptosis, which improves cardiac ischemia-reperfusion injury and subacute phase cerebral ischemia-reperfusion injury. [15,24] Our experimental results demonstrated significant elevation in apoptotic markers in myocardial infarction mice, as evidenced by increased TUNEL-positive cells, enhanced Caspase-3 activity, and elevated Bax/Bcl-2 mRNA expression ratio. Notably, GAS treatment effectively attenuated these apoptotic indicators. Furthermore, GAS exhibited cardioprotective properties through its ability to suppress both cardiac hypertrophy and fibrosis, suggesting its therapeutic potential in myocardial infarction management. [13,25] Our experimental findings further revealed that pharmacological intervention significantly attenuated pathological alterations in myocardial infarction mice, as manifested by a marked reduction in both cardiac fibrosis and the expression levels of heart failure-related biomarker mRNAs compared to untreated controls. Importantly, these molecular improvements were accompanied by significant amelioration of cardiac function parameters.

To repair the damaged tissue after myocardial infarction, the angiogenic response will begin from the infarct margin area and extend to the infarct center area. [26] Evidence suggests that CD31 is a critical

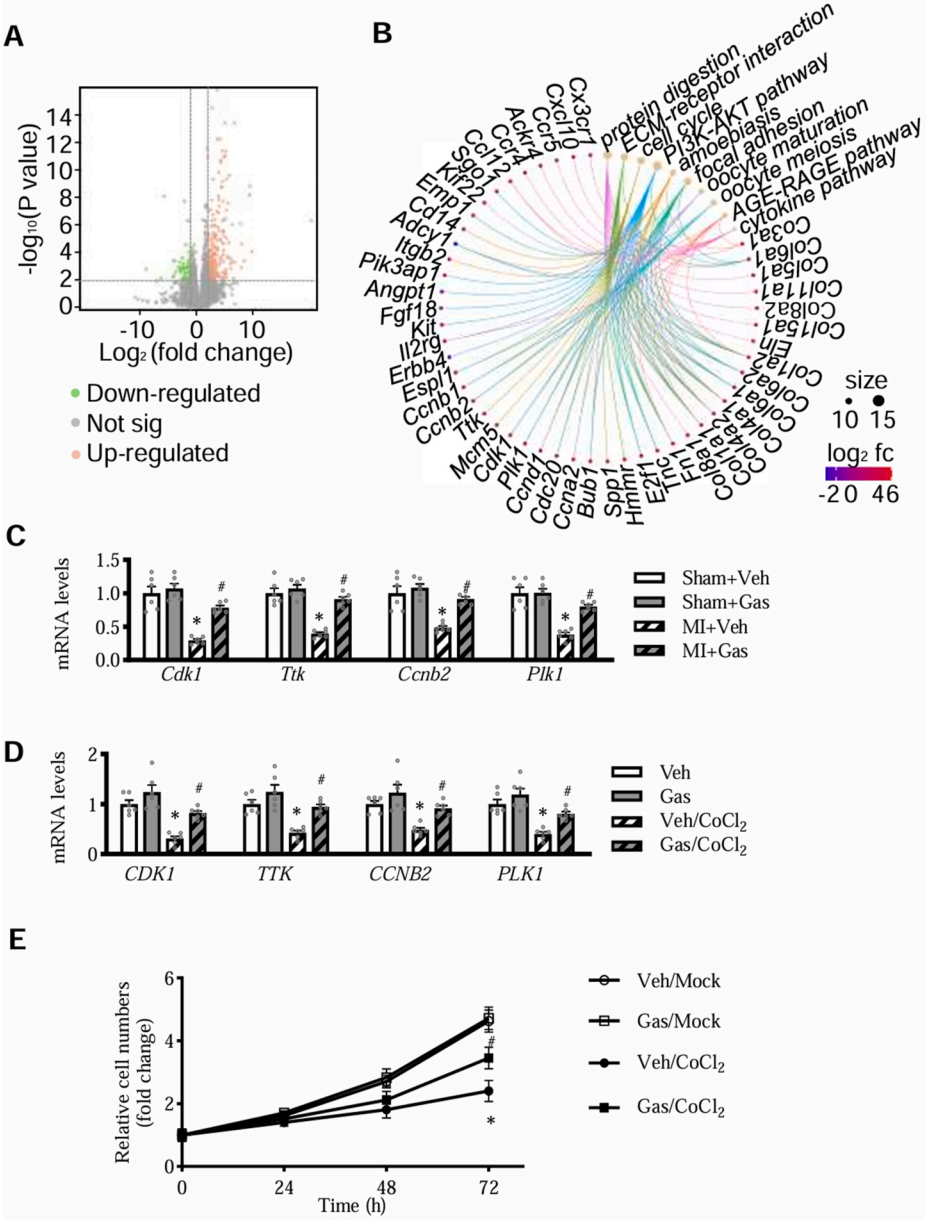

Fig. 4. Gastrodin promotes endothelial proliferation. (A) RNA sequencing using heart tissues from infarcted mouse administrated with/without gastrodin administration. The volcano plot was presented to show the three clusters, including upregulated genes (red), down-regulated genes (green), and no-changed genes (grey). (B) GSEA (gene set enrichment analysis) was performed with RNAseq result. (C) The mRNA levels of Cdk1, Ttk, Ccnb2, and Plk1 expression in heart tissues were analyzed using qPCR assay. (D-E) Human aortic endothelial cells (HAECs) were incubated with CoCl2 with/without gastrodin treatment. (D) The mRNA levels of CDK1, TTK, CCNB2, and PLK1 expression in treated HAECs were analyzed using qPCR assay. (E) The cell numbers were monitored using CCK8 (cell counting kit 8) at different time points. Male mice were subjected to infarction surgery, and then administrated with gastrodin (100 mg/kg per day) for 14 days (n = 6 each group). Data are mean ± SEM. $^{*}P < 0.05$ vs. sham group, $^{\#}P < 0.05$ vs. MI group.

molecule for maintaining endothelial cell junctional integrity and vascular homeostasis. Through its intracellular domain, it transmits signals to regulate endothelial cell migration, proliferation, and lumen formation, thereby promoting new blood vessel formation. In addition, it also mediates intercellular adhesion and maintains endothelial barrier function. [28,29] Therefore, CD31 is closely related to both endothelial cells and angiogenesis. [27] In our experiment, CD31 was found to increase significantly in the heart tissue of mice with myocardial infarction, and the increase was even greater after GAS treatment. We examined four differential genes, all linked to cell proliferation, by sequencing RNA from mouse cardiac tissue in order to better understand how GAS stimulates angiogenesis to treat myocardial infarction. Through both in vitro and in vivo experiments, the mRNA expression levels of CDK1, TTK, CCNB2, and PLK1 were considerably raised in the heart tissue and HAECs of GAS-treated mice with myocardial infarction. Furthermore, as time went on, there was an increasing discrepancy in the number of cells proliferating between HAECs treated with GAS and untreated HAECs. These results suggest that GAS may induce endothelial cell proliferation and ultimately promote angiogenesis by promoting CD31 elevation.

Our study has several limitations. First, it did not delve into GAS's inhibition of apoptosis, cardiac fibrosis, and inflammatory response, as well as the targets of these differential genes. Subsequently, we can combine network pharmacology to predict the target of GAS and conduct experimental demonstration. Secondly, although GAS is widely used and has a promising prospect in myocardial infarction, the

pharmacokinetics show that the action time is short, and follow-up experiments can further study the delivery method of GAS. Finally, the combination of traditional Chinese and Western medicine has been shown to be more effective with fewer side effects in some aspects, such as GAS combined with phenytoin sodium (PHT) to enhance the anticonvulsant effect in mice and reduce the side effects of the drug in balance and memory function. [30] Next, we can further investigate which approach is more helpful in improving myocardial infarction by combining GAS with other drugs.

Although our study has some limitations, GAS offers significant advantages over conventional treatment for MI. Traditional treatment modalities mainly include early reperfusion therapy (such as thrombolysis and percutaneous coronary intervention) [31] and drug therapy (such as antiplatelet, anticoagulant, and statins) [32,33]. Although these methods can effectively restore blood flow and reduce myocardial damage, there are still some limitations. For example, reperfusion therapy may be accompanied by reperfusion injury, triggering a series of pathological reactions, including free radical explosion, inflammatory cell infiltration, and apoptosis, leading to further damage to cardiomyocytes. [34] In our experiment, GAS was found to significantly reduce the damage caused by myocardial infarction and protect cardiac function through its powerful anti-apoptotic and anti-inflammatory effects. In addition, traditional drug therapy is usually only targeted at a single pathological link, such as antiplatelet drugs mainly inhibit thrombosis, [32], statins mainly regulate blood lipids, [35], GAS has a multi-target and multi-pathway mechanism of action, and GAS has a multi-pathway mechanism. In our study, we found that it can simultaneously inhibit inflammation, reduce apoptosis, promote angiogenesis and endothelial cell proliferation, improve myocardial fibrosis and cardiac dysfunction, and then more comprehensively deal with the complex pathological process of myocardial infarction. In addition, traditional treatment may have certain side effects, such as anticoagulants may increase the risk of bleeding, and statins may cause liver dysfunction or muscle damage. [32,33] In contrast, GAS as a natural compound, toxic side effects are small, has good safety and tolerance, [36], suitable for long-term use.

In conclusion, our study has made a novel discovery that GAS exerts multifaceted cardioprotective effects through distinct molecular mechanisms. Primarily, GAS upregulates the expression of cell cycle regulators (CDK1, TTK, CCNB2, PLK1) and enhances HAECs proliferation coupled with CD31 release, thereby promoting angiogenesis and myocardial repair. Additionally, GAS demonstrates potent anti-inflammatory and anti-apoptotic properties by suppressing inflammatory factor release, reducing TUNEL-positive cells and Caspase-3 activity, and modulating the Bax/Bcl-2 mRNA ratio. Importantly, GAS attenuates myocardial fibrosis and cardiac remodeling, as evidenced by downregulation of cardiac stress markers (Anp, Bnp, and β-mhc), ultimately preserving cardiac function and preventing heart failure progression. Therefore, GAS provides a new therapeutic idea for myocardial infarction with its multi-effect and unique myocardial protective effect.

5. Conclusions

Our study shows that GAS can improve myocardial infarction by inhibiting inflammation and apoptosis, promoting endothelial cell proliferation and angiogenesis. The innovation of this project is the discovery that GAS can induce cell proliferation by promoting the expression of CDK1, TTK, CCNB2, and PLK1. These findings provide new molecular mechanisms and therapeutic options for the prognosis of clinical myocardial infarction.

CRediT authorship contribution statement

Ruochen Du: Writing – original draft, Validation, Methodology, Data curation, Conceptualization. **Ying Guo:** Writing – original draft, Validation, Methodology, Data curation, Conceptualization. **Wanting Zhong:** Investigation, Formal analysis. **Yuantao Gao:** Investigation, Formal analysis. **Miaomiao Xu:** Software, Project administration. **Chunfang Wang:** Writing – review & editing, Supervision. **Yitong Yuan:** Writing – review & editing, Supervision.

Data availability

All materials and data are available to the corresponding author upon reasonable request. Due to company and laboratory policies, we are unable to provide Web links or login numbers to the sequencing database. But we can provide some of the sequencing data (Supplementary Tables S1–S2).

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Declaration of competing interest

The authors declare that they have no conflict of interest with the contents of this article.

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Abbreviations

GAS	Gastrodin
EF	Ejection Fraction
FS	Fractional Shortening
IVS	Inter Ventricular Septum
LVID	Left Ventricular Internal Diameter
LVPW	Left Ventricular Posterior Wallat
HAECs	human aortic endothelial cells
Bax	BCL2-Associated X Protein
Bcl2	B-cell lymphoma 2
TNF-α	Tumor Necrosis Factor-alpha
IL-6	Interleukin-6
IL-1β	Interleukin-1 beta
Cdk1	Cyclin-dependent kinase 1
Ttk	Threonine tyrosine kinase
Ccnb2	Cyclin B2
Plk1	Polo-like kinase 1

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2025.102014.

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