

Original Research Article

S-adenosyl-L-methionine improves ventricular remodeling after myocardial infarction by regulating angiogenesis and fibrosis

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Abstract

Purpose: To investigate the effect of S-adenosyl-L-methionine (SAM) on angiogenesis and fibrosis in the heart of rats with myocardial infarction (MI), and to determine the mechanism of action.

Methods: Sprague Dawley rats with MI received SAM treatment (15 mg/kg) intraperitoneally. The cumulative survival (%) of rats was recorded to determine their rate of survival. Hematoxylin-eosin staining, echocardiography, and hemodynamics were also performed. In addition, the effects of SAM vascular regeneration in the rats were analyzed by determining the expression of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and hypoxia-inducible factor 1- α (HIF1- α) in rats.

Results: The 8-week survival rate of the MI group was significantly lower than that of the sham group, while SAM significantly improved the survival rate of the rats. In addition, SAM improved the contractile and diastolic heart function in the rats and also increased the ventricular pressure change. Furthermore, SAM elevated the expressions of VEGF, bFGF and HIF1- α in rat myocardium and serum. In myocardial tissues of SAM-treated rats, the expressions of collagen I, collagen III and α -sma were reduced, indicating that SAM inhibited myocardial fibrosis. In addition, SAM promoted cardiac angiogenesis by activating Jagged1/Notch1 signaling pathway.

Conclusion: SAM promotes angiogenesis of the myocardium by activating Jagged1/Notch1 signaling pathway and inhibiting fibrosis in rat myocardium. Therefore, SAM effectively inhibits ventricular remodeling in rats after MI, thereby improving the rats' heart structure and function. The results may provide new targets for the treatment of myocardial infarction.

Keywords: S-adenosyl-L-methionine, Ventricular remodeling, Angiogenesis, Fibrosis, Myocardium, Myocardial infarction

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INTRODUCTION

Heart failure (HF) is the leading cause of death and disability worldwide [1]. It is mainly caused by myocardial infarction (MI), and most of them are among elderly patients, so the number of patients is on the increase year on year [2]. Heart

repairing after MI includes a series of complicated processes. Strong aseptic inflammatory response is first initiated, immune inflammatory cells are infiltrated, and necrotic cells and extracellular matrix are removed. The inflammatory response during the repair phase involves the proliferation of fibroblasts and

myofibroblasts, leading to scarring and the induction of subsequent vascularization [3,4]. New blood vessels can compensate for the lack of coronary blood flow caused by coronary occlusion to a certain extent. However, excessive scar repair after MI leads to fibrosis of the myocardium, which further exacerbates loss of cardiac function [5,6]. Therefore, promoting blood vessel formation after MI and inhibiting myocardial fibrosis are the keys to the treatment of ventricular remodeling after MI.

S-adenosyl-L-methionine (SAM) is a common physiological active substance in the human body, which can play the role of transsulfur, transmethyl and transaminopropyl [7]. SAM is also a precursor of taurine, coenzyme A and cysteine, which play an important role in the physiological functions of the human body [8]. Recent studies have found that SAM is effective in the clinical treatment of degenerative joint disease, cholestasis, spinal cord deformation, fibromyalgia and abnormal liver function [9]. A study has found that SAM reduced cirrhosis and liver fibrosis caused by alcoholic liver disease [10]. In addition, SAM has been found to reduce oxidative stress-induced cell damage and promote cell regeneration through polyamines [11]. Therefore, SAM has potential therapeutic effects in many diseases.

However, the effect of SAM on myocardial fibrosis and myocardial tissue angiogenesis after MI has not been studied. Therefore, a rat MI model was obtained, and SAM was used to treat the MI rats in order to determine the effect of SAM on ventricular remodeling after MI.

EXPERIMENTAL

Animals

A total of 60 8-week-old male rats (300-400 g) were used in this study. The rats were housed in specific pathogen-free animal rooms. The temperature of the animal room was set at 25 °C and the relative humidity was set at 60-80 %. The animal experiments in this study were approved by Northern Theatre General Hospital Animal Experiment Ethics Committee (17-AEEC-No.021). All procedures were conducted in accordance with the 'Animal Research: Reporting in Vivo Experiments guidelines 2.0' [12].

Establishment of MI model and grouping

The rats were divided into sham group, MI group and MI + SAM group. Pentobarbital sodium (2%, 40 mg/kg) was used for the anesthesia via

intraperitoneal injection. The breathing frequency was set to 70 times/min. The tidal volume was 0.7 mL. After disinfection using iodophor, a 4 cm longitudinal incision was made in the left chest of the rat. After blunt separation of the skin and muscles, the chest cavity in the fourth intercostal space was opened and the pericardium was separated. Then the left anterior coronary artery under the left atrial appendage and the arterial cone was ligatured using sterile sutures. The paleness of the anterior heart area indicated successful operation. Then the muscles and skin were sutured layer by layer. The rats were put back into the animal room and kept for 8 weeks. The rats in the sham group did not receive the coronary artery ligation. Rats in the MI + SAM group were injected with SAM (15 mg/kg) (Sigma-Aldrich, St. Louis, MO, USA) intraperitoneally daily after modeling [13]. Rats in MI group were injected with the same volume of normal saline.

Echocardiography

A small animal ultrasound system (Visual Sonics, Toronto, Ontario, Canada) was used to detect the rat cardiac function. After 8 weeks of modeling, rats were anesthetized with isoflurane and were placed on the monitor station in a supine position. After using depilatory agent to remove the fur on the rat's chest, the probe of the doppler ultrasound diagnostic instrument was placed on the left chest of the rat and adjusted the depth to 2-2.5 cm to acquire two-dimensional image of the left ventricle of the heart. The indicators measured using echocardiography included left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), left ventricular ejection fraction (LVEF) and left ventricular fraction shortening (LVFS).

Invasive cardiac catheterization in rats

After the rat heart ultrasound finished, the rat was anesthetized with 2 % pentobarbital sodium (40 mg/kg) and was fixed on the operating table. A sterile blade was used to make a 2.5 cm longitudinal incision at the midline of the rat's neck. Then vascular forceps were used to bluntly separate the tissue to expose the common carotid artery. Sutures were used to ligate the distal end of the carotid artery and an arterial clip to close the proximal end.

Thereafter, a microcatheter guide wire soaked in heparin saline was used to insert the aorta from the distal end to the proximal end. When the insertion depth was 3 - 4 cm and the catheter vibrated with the heart beats, the catheter was close to the left ventricle. Then the catheter was

split into a multi-channel physiological recorder. When the blood pressure amplitude suddenly increases several times, it meant that the catheter has entered the left ventricle. Then the catheter was fixed and the maximum rate of ventricular pressure change (dP/dt max) and the minimum rate of ventricular pressure change (dP/dt min) were analyzed according to pressure-volume analysis software.

Histology and hematoxylin-eosin (HE) staining

The hearts were collected from the rats, immersed in 4 % paraformaldehyde solution for 24 h and then were put in gradient alcohol and xylene in turn. Then the heart tissue was put into paraffin solution to make a paraffin block. A microtome (LEICA RM2235, Koln, Germany) was used to make 5 μ m thick paraffin sections. All paraffin sections were baked in a 37 °C incubator for 3 days. The sections were successively put in xylene and alcohol for dewaxing and hydration.

After washing the sections with running water, the cell nucleus were stained with hematoxylin (Beyotime, Shanghai, China) and the excess hematoxylin stain was differentiated with 1% hydrochloric acid alcohol. Then the cytoplasm was stained with eosin stain (Beyotime, Shanghai, China), and the sections were then placed in alcohol and xylene in turn. Finally, the neutral gum was used to seal the sections and observe the staining results were observed using an optical microscope (LEICA, Koln, Germany).

Immunohistochemical (IHC) staining

Paraffin sections were put into xylene and alcohol for dewaxing and hydration. Then citrate buffer was used to repair the antigen. The sections were placed in citrate buffer and slowly heated to 95 °C for 10 minutes. After the sections cooled naturally, the sections were washed with phosphate buffered saline (PBS) and incubated with 3% H₂O₂ for 30 min. After washing the sections with PBS, non-specific antigens were blocked with 10 % goat serum (Beyotime, Shanghai, China). The primary antibodies were added, and incubated overnight at 4 °C (VEGF, ab53465; bFGF, ab92337; collagen I, ab34710; collagen III, ab7778; α -sma, ab5694; Jagged1, ab7771; Notch1, ab52627. Abcam, Cambridge, MA, USA). After washing the sections with PBS, secondary antibody (GeneTech, Shanghai, China) was used to incubate the myocardial tissues for 1 h.

Then DAB (GeneTech, Shanghai, China) was used for color development. Hematoxylin was

used to stain the cell nucleus. Finally, a neutral gum was used to seal the sections and the staining results were observed using an optical microscope (LEICA, Koln, Germany).

Enzyme-linked immunosorbent assay (ELISA)

ELISA kits (R&D Systems, Emeryville, CA, USA) were used to determine the concentration levels of VEGF and bFGF in the rat serum. The standard was then diluted in the ELISA kit to 5000, 2500, 1250, 625, 312 and 156 pg/mL. Then the concentration of the sample was calculated based on the absorbance of the sample, and then the standard curve was made.

RNA isolation and quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)

Myocardial tissues (30 mg) were collected and added into liquid nitrogen in a grinding bowl. Then 1 mL TRIzol (Invitrogen, Carlsbad, CA, USA) was added to dissolve myocardial tissue. Total RNA extracted by TRIzol were reversed to cDNAs by reverse transcription after the RNA concentration determination using a spectrophotometer (LASPEC, Shanghai, China). The reverse transcription system was shown below: 4 μ L 5 × Reaction Buffer + 1 μ L RiboLock RNase inhibitor + 2 μ L dNTP Mix + 1 μ L Reverted Aid reverse transcriptase + 1 μ g mRNA (Thermo Fisher Scientific, Waltham, MA, USA). The mixture was put into the PCR machine for 5 minutes at 25°C, 60 minutes at 42°C and 5 minutes at 72°C. The obtained cDNA was stored in a refrigerator at -20°C. Then SYBR Green Master Mix (Vazyme, Nanjing, Jiangsu, China) and corresponding primers (Generay, Shanghai, China) were used to amplify cDNA. GAPDH expression was used as reference. $2^{-\Delta\Delta Ct}$ was used to represent the relative expression of mRNA. RT-PCR primer sequences were shown in Table 1.

Statistical analysis

All data in this study were represented as mean \pm standard deviation. SPSS statistical analysis software (version 26.0) and Graphpad Prism 7.0 (La Jolla, CA, USA) were used to analyze the data. t-test was used to analyze the difference between the two groups. ANOVA was used to analyze the difference between multiple groups. The survival curve was analyzed by Log-Rank method. $P < 0.05$ was considered statistically significant. All experiments were repeated 3 times or more.

Table 1: RT-PCR Primers used

Variable	Sense/Anti-sense (S/AS)	Primer sequences (5'-3')
VEGF	S	ACAGGGAAGACAATGG GA
	AS	CTGGAAGTGAGCCAAC G
HIF1- α	S	TCCCATACAAGGCAGC A
	AS	GAAACCCACAGACAA CAA
collagen I	S	TGCAAGAACAGCGTAG CC
	AS	CAGCCATCCACAAGCG T
collagen III	S	TCCTGGTGCTAAGGGT GAG
	AS	CAGCGTGTCTTGTGG TC
α -sma	S	TCTATAACGAGCTTCGC GTGG
	AS	TGGCATGAGGCAGAGC ATAG
Jagged1	S	CAGGGATTGCCCACTT T
	AS	GCAGGTTTTGTTGCCAT T
Notch1	S	AGGCTCTGCCGACATC A
	AS	AGGAAGGGGTGCTCTG G
GAPDH	S	GTTGTGGCTCTGACAT GCT
	AS	CCCAGGATGCCCTTTA GT

RESULTS

SAM improved cardiac function in rats with MI

After inducing MI in rats by blocking coronary arteries, the rats were treated with SAM. The postoperative survivability of the rats was determined in each group. The survival rate of rats in the MI group after 8 weeks was 45 %, while the survival rate of the rats in the MI + SAM group was 75 %. The survival rate of rats in the sham group was 100 % (Figure 1 A). This showed that SAM reduces the mortality rate of rats after MI. The cardiac function of rats was assessed by echocardiography (Figure 1 B - E).

After MI was induced in the rats, LVEF and LVFS decreased significantly, while LVEDD and LVESD increased, indicating that the cardiac function of the MI rats decreased significantly. After treatment with SAM, LVEF and LVFS in the rats increased, while LVEDD and LVESD decreased, indicating that SAM improved their

cardiac function. The hemodynamic test results of their hearts showed that SAM increased the dP/dt max (Figure 1 F) and dP/dt min (Figure 1 G) of MI rats. After 8 weeks of modeling, the body weight (BW) and heart weight (HW) of the rats were determined. The HW/BW of the MI group was greater than that of the sham group, while the SAM decreased in HW/BW (Figure 1 H). H & E staining results showed that the arrangement of myocardial cells in the MI group was disordered, and the number of myocardial cells decreased, while the myocardial tissue in the MI + SAM group was significantly improved when compared to the MI group (Figure 1 I).

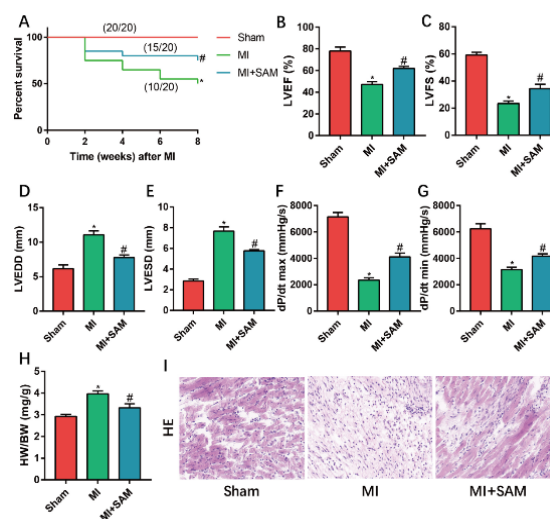


Figure 1: SAM improved the structure and cardiac function in MI rats. (A). Survivability of rats 8 weeks after MI; (B-E). Cardiac function (LVEF, LVFS, LVEDD and LVESD) in rats; (F, G). dp/dt max and dp/dt min of rat hearts; (H). HW/BW of rats; (I). H&E staining results of rat myocardium ($\times 200$). (* $p < 0.05$ vs. sham group; # $p < 0.05$ vs. MI group)

SAM promoted angiogenesis in MI rat myocardium

In order to clarify the protective effect of SAM on the myocardium of MI rats, the changes in angiogenesis in rat myocardium were detected. IHC staining detected the expression of angiogenic cytokines VEGF and bFGF in rat myocardium (Figure 2 A). The expressions of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in myocardium of MI group decreased, while SAM increased their expression. ELISA detected the concentration of VEGF (Figure 2 B) and bFGF (Figure 2 C) in rat serum, and SAM was also found to increase the expression of VEGF and bFGF in rat serum. The RT-PCR results also showed that SAM increased the expressions of VEGF (Figure 2 D) and hypoxia-inducible factor

(HIF)1- α (Figure 2 E) mRNA in myocardial tissue.

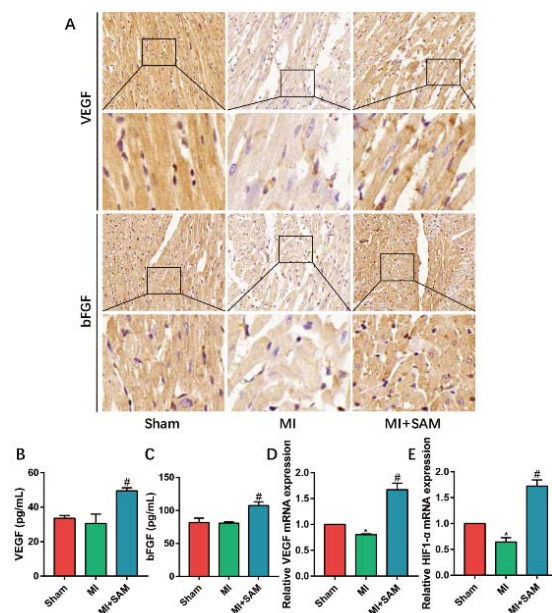


Figure 2: SAM promotes angiogenesis in MI rat myocardium. (A). Results of IHC staining of VEGF and bFGF in rat myocardium (200 \times); (B, C). ELISA results of VEGF and the bFGF in rat serum; (D, E). RT-PCR results of mRNA expression of VEGF and HIF-1 α in rat myocardium. (* $p < 0.05$ vs. sham group; # $p < 0.05$ vs. MI group)

SAM inhibits myocardium fibrosis in MI rats

The expressions of collagen I, collagen III and α -sma in rat myocardium were determined by IHC staining (Figure 3 A). The expression of collagen and α -smooth muscle actin (sma) in the myocardial tissue of the rats in the MI group increased significantly. After treatment with SAM, the expression of collagen and α -sma in the myocardial tissue of the MI + SAM group was significantly reduced. The RT-PCR results showed that SAM reduced the mRNA expressions of collagen I (Figure 3 B), collagen III (Figure 3 C) and α -sma (Figure 3 D) mRNA in the rat myocardium.

SAM improves the structure and function of MI rats via Jagged1/Notch1 signaling pathway

The Jagged1/Notch1 signaling pathway is closely related to angiogenesis. Therefore, the changes of this pathway in rat myocardial tissue were determined. The results of IHC staining showed that the expression of Jagged1 and Notch1 in the myocardial tissue of the MI group decreased, while the expression of Jagged1 and Notch1 in the myocardial tissue of the MI + SAM group increased, indicating that SAM increased the

activity of Jagged1/Notch1 signaling pathway in the rat myocardial cells (Figure 4 A). The RT-PCR results showed that SAM increased the expression of Jagged1 (Figure 4 B) and Notch1 mRNA (Figure 4 C).

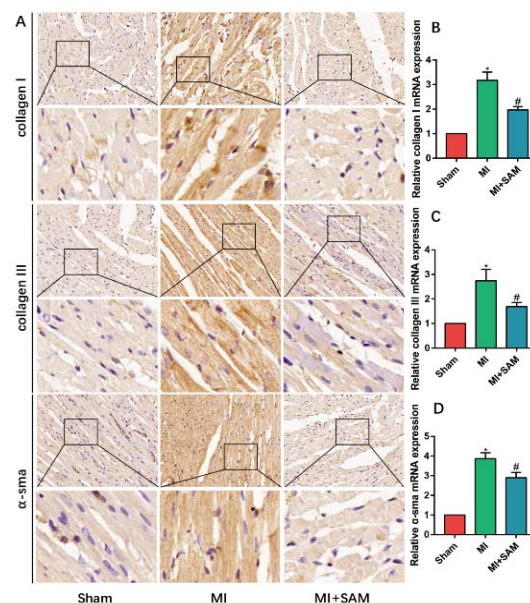


Figure 3: SAM-induced tissue fibrosis of MI rat myocardium. (A). IHC staining results for collagen I, collagen III and α -sma in rat myocardium (200 \times); (B-D). RT-PCR results of mRNA expression of collagen I, collagen III and α -sma in rat myocardium. (* $p < 0.05$ vs. sham group; # $p < 0.05$ vs. MI group)

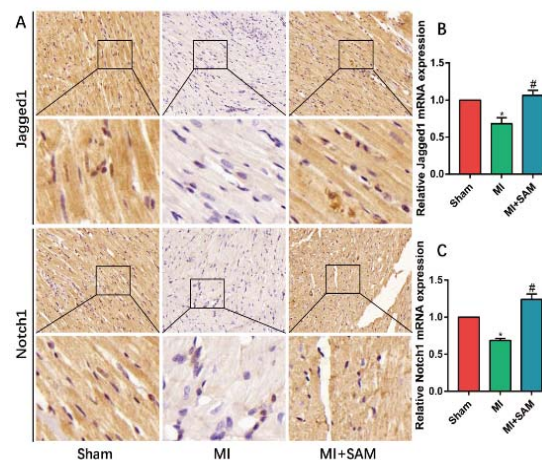


Figure 4: SAM improved the structure and function of MI rats through Jagged1/Notch1 signaling pathway. (A). IHC staining results of Jagged1 and Notch1 in rat myocardium (200 \times) showed that the expression of Jagged1 and Notch1 in the myocardial tissue of the MI + SAM group significantly increased; (B, C). RT-PCR results of mRNA expression of Jagged1 and Notch1 in rat myocardium. * $P < 0.05$ vs. sham group; # $p < 0.05$ vs. MI group

DISCUSSION

Myocardial Infarction (MI) is a cardiovascular disease with serious health consequences. It is one of the main diseases in the world that causes disability and death [14]. After the occurrence of MI, a large number of myocardial cells (including myocardial cells, fibroblasts, and endothelial cells) died due to acute ischemia and hypoxia. The state of oxidative stress, the formation of scar tissue and the remodeling of the ventricle after MI causes cardiac dysfunction and eventual progression to HF [15].

The present study found that SAM improved MI-induced ventricular remodeling in rats. The survivability of MI rats treated with SAM also significantly improved. The results of echocardiography also showed that SAM effectively improved the cardiac function of the rats. SAM also promoted the formation of blood vessels in rat myocardium and reduced the degree of fibrosis. In addition, results showed that these protective effects of SAM on myocardial tissue may be related to the activation of Jagged1/Notch1 signaling pathway.

Angiogenesis is involved in the development of various cardiovascular diseases such as HF, atherosclerosis, and aortic dissection [16]. One of the important biological processes after MI is microvascular angiogenesis. This refers to the generation of blood vessels based on previously existing blood vessels, and pro-angiogenic factors play an important role in this process [17]. The angiogenic factors directly act on endothelial cells and regulate their proliferation and activation. Post-MI hypoxia activates HIFs (such as HIF1- α), thereby promoting the expression of a series of pro-angiogenic genes such as VEGF, bFGF, endothelial nitric oxide synthase, placental growth factor, and angiopoietin [18].

VEGF and bFGF are the most widely studied pro-angiogenic factors in ischemic tissues. VEGF is considered to be the most important vascular stimulating factor which induced multiple signaling pathways, and these signaling pathways coordinate various biological processes, such as endothelial cell maturation, angiogenesis and arterioles formation [19]. In addition, VEGF stimulates angiogenesis by activating VEGF receptor 2 signaling pathway [20]. The treatment of angiogenesis may protect against HF. Therefore, for patients with HF, alternative angiogenesis therapy is of great significance. However, Stewart *et al* [21] found no significant difference in the cardiac capillary density and blood flow between the VEGF group and the placebo group in the clinical study of MI

patients. Other studies have also found that MI patients treated with bFGF alone did not significantly recover blood flow around the infarcted myocardium, and did not significantly recover cardiac function [22]. It was discovered that VEGF or bFGF alone does not significantly improve the blood flow of myocardium. However, the combination of these two growth factors will produce different therapeutic effects. A study found that combined application of VEGF and bFGF in porcine MI models increased blood flow to ischemic myocardium and increased ejection fraction [23,24]. This current study also demonstrated that SAM promoted the expression of VEGF and bFGF in rat serum, and improved the function of rat myocardium. In addition, SAM promotes the expression of HIF1- α in rat myocardium.

Myocardial fibrosis is one of the main pathological features of myocardial remodeling. The activation of cardiac fibroblasts produces a large amount of extracellular matrix, including collagen I and collagen III, which causes excessive deposition of interstitial proteins and in turn leads to decreased compliance of myocardial tissue [25]. The purpose of fibrosis is to maintain the structural integrity of the heart and cope with excessive stress. However, the activation of myofibroblasts significantly promote the pathological remodeling of the heart [26]. Although this early adaptive change temporarily maintains the structure and function of the heart, it will eventually lead to adverse changes in the structure and compliance of the ventricle [27]. Therefore, inhibiting fibrosis is an effective way to relieve ventricular remodeling after MI.

A study investigated the effect of dapagliflozin on myocardial fibrosis. By regulating the STAT3 signaling pathway, dapagliflozin attenuated myocardial fibrosis and relieved MI in rats [28]. Another study also found that microRNA-34a regulated myocardial fibrosis by targeting Smad4, thereby alleviating myocardial damage caused by MI [29]. The present study found that the extracellular collagen fibers (collagen I and collagen III) and α -sma in myocardial tissues of SAM-treated rats decreased, suggesting a fall in the degree of myocardial fibrosis. Jagged1/Notch1 is an important signaling pathway in the regulation of angiogenesis. Jagged1 is widely expressed in endothelial cells during angiogenesis [30]. Zimrin *et al* [31] found that the Jagged1/Notch1 signaling pathway regulated bFGF-induced endothelial cell migration, which is early changes in vascularization. Therefore, the Jagged1/Notch1 signaling pathway is essential for angiogenesis *in vivo*.

In our study, the expression of Jagged1/Notch1 signaling pathway in the MI group was significantly reduced, and treatment with SAM effectively promoted the expression of Jagged1 and Notch1 in rat myocardium. This may be an important mechanism by which SAM promoted myocardial angiogenesis and inhibited myocardial fibrosis. To our knowledge, this is the first study to investigate the effect of SAM on myocardial remodeling in rats, and the results may provide new targets for clinical treatment of MI.

CONCLUSION

Ventricular remodeling after MI is an important factor in the pathogenesis of irreversible myocardial injury. SAM promotes myocardial angiogenesis and inhibits myocardial fibrosis by activating Jagged1/Notch1 signaling pathway. It also improves the structure of rat myocardium and the rate of ventricular pressure change. Therefore, SAM may also play an important role in protecting against MI-induced ventricular remodeling in clinical practice, but this has to be investigated in clinical studies.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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