Modified Taohong Siwu Decoction Improved Heart Function After Myocardial Infarction by Inhibiting Inflammatory Factor and Promoting Chemotactic and Pro-Angiogenic Factors

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Research

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Abstract

Background

Traditional Chinese medicine has been applied to prevent and treat myocardial infarction (MI) in the clinic for a long history. We recently found that Taohong Siwu decoction (THSWD) exerted a beneficial effect on heart function after MI through improving the local hostile microenvironment. However, the improvement of cardiac function after THSWD administration was moderate. In this study, four Chinese medicine herbs, which have the properties of warming *Yang* or removing phlegm, were added into THSWD to constitute a new and multifunctional Chinese herbal compound, named modified THSWD (MTHSWD).

Methods

A rat model of MI was established by the ligation of left anterior descending coronary artery and MTHSWD was intragastrically administered for 2 weeks. The heart function was examined by echocardiography, cell apoptosis was detected by TUNEL staining and the infarct size was determined by Masson's trichrome staining. The expressions of cytokines, including chemotactic and inflammatory factors, were examined by ELISA in the infarcted myocardium and serum. The level of p-Akt and VEGF in the damaged myocardial tissues was further detected by Western blot.

Results

MTHSWD improved heart function and decreased infarct size and collagen deposition in the infarcted area. In addition, MTHSWD increased the expression of cTnT and Cx43, reduced cell apoptosis in the infarcted area by activating Akt signal and promoted angiogenesis by increasing the expression of VEGF. Interestingly, MTHSWD significantly increased the level of IGF-1, SDF-1 and TNF-α, and significantly reduced the expression of IL-1β in the infarcted myocardium. MTHSWD could also significantly increase the expression of IGF-1, SDF-1 and SCF in the serum.

Conclusions

MTHSWD reduced cell apoptosis, promoted angiogenesis and improved heart function after MI probably through the downregulation of inflammatory factor IL-1β, upregulation of chemotactic factors SDF-1 and SCF as well as pro-angiogenic factor VEGF, and activation of Akt signaling pathway.

Background

Cardiovascular disease has become the most common cause of morbidity and mortality worldwide, leading to enormous suffering and burden to the families and society. Myocardial infarction (MI), one of the most serious types of cardiovascular disease, is the condition of irreversible necrosis and loss of cardiomyocytes mostly due to myocardial ischemia and hypoxia after the occlusion of coronary artery. The necrotic cardiomyocytes are gradually replaced by fibroblasts and collagen tissue, causing the

deposition of collagen and formation of fibrosis scar, resulting in the decline of regional myocardial contractility, even heart failure and death.

Myocardial perfusion with percutaneous coronary intervention (PCI) plays a major role in the prevention of left ventricular remodeling and cardiovascular events in post-MI patients. However, the incidence of heart failure remains high even after the PCI treatment. Although several clinical trials have shown that the benefits of prevention pharmacological agents, such as angiotensin-converting-enzyme inhibitors/angiotensin II receptor blockers, mineralocorticoid receptor antagonists, beta blockers, aspirin and statins aimed at preventing post-MI remodeling, these interventions remain underutilized in the clinical practice [1]. Emerging data of animal experiments and clinical trials has indicated that stem cell transplantation has potential benefits in inhibiting left ventricular remodeling and improving heart function after MI [2, 3]. According to our previous studies, transplantation of mesenchymal stem cells (MSCs) after MI reduced scar size and improved cardiac function probably via paracrine mechanisms [4, 5]. However, its clinical application has been significantly hampered by many problems, such as the low survival, differentiation and integration rate after transplantation.

Traditional Chinese medicine (TCM) has a long history in China and has gained multiple clinical applications with multi-target, multi-pathway, low side effects and low cost. In recent years, more and more TCM has been applied to prevent and treat MI in the clinic whether it was used alone or as a complementary treatment [6, 7]. Early administration of TCM reduced the rates of in-hospital bleeding and the risk of hyperlipidemia. It was reported that *Salvia miltiorrhiza*, also known as *Danshen*, was most commonly used in the post-MI patients [7]. The formulation ShenZhuGuanXin Granules dose-dependently attenuated cardiac dysfunction and enhanced angiogenesis in MI rats through upregulating the expression of PECAM-1/CD31 and vascular endothelial growth factor (VEGF) [8]. A double-blind, randomized, placebo-controlled, pilot study has demonstrated that Danlou tablets significantly reversed adverse left ventricular remodeling in patients with MI [9]. However, the sample sizes for most clinical trials were small and the beneficial and detrimental effects of TCM in post-MI patients should be critically evaluated with more studies in the future.

Recently, we found that Taohong Siwu decoction (THSWD) exerted a beneficial effect on heart function after MI through improving the local hostile microenvironment and decreasing the expression of Fis1 [10]. However, the improvement of heart function after THSWD administration was moderate. Further investigations are needed to evaluate if the change in the utilization of Chinese herbal formulations impacts the effectiveness of the treatment. The main effects of THSWD on the patients with MI are “enhancing qi and accelerating the blood circulation” according to TCM theory. Nevertheless, the TCM syndrome typing of MI includes not only “*Qi* stagnation and blood stasis”, but also “*Yang* deficiency and cold coagulation, *Qi* stagnation and phlegm obstruction”. Therefore, the combination of THSWD with other TCM herbs, which have the properties of warming *Yang* or removing phlegm, may be more conducive to the improvement of heart function after MI.

Given the above, four TCM herbs including *Salvia miltiorrhiza*, *Radix Astragali,Epimedium* and *Notopterygii Rhizoma et Radix*, were added into THSWD according to the understanding of etiology and pathogenesis of MI in TCM to constitute a new and multifunctional Chinese herbal compound, named modified THSWD (MTHSWD). The purpose of this study was to evaluate whether MTHSWD has a better therapeutic effect in a rat MI model. The expressions of cytokines, including chemotactic and inflammatory factors, were examined by ELISA in the infarcted myocardium and serum. In addition, the level of p-Akt and VEGF in the damaged myocardial tissues was further detected to elucidate the possible mechanisms underlying the cardioprotective effects of MTHSWD.

Materials And Methods

Animals

Thirty-five healthy male SD rats (200 ± 20 g) were obtained from the Experiment Animal Center of Shanghai University of Traditional Chinese Medicine. The animals were received humane care and kept on a 12-hours light/dark cycle with free access to water and food. Temperature and relative humidity in the animal breeding room ranged between 23~25℃ and 50~70%, respectively. The protocol was approved by the animal ethics committee of Shanghai University of TCM and the Animal Research Committee of Shanghai. Every effort was made to minimize animal suffering and the number of rats used during experimental manipulation.

Production of MI model

The rats were fixed on the surgical plate in a supine position after being anesthetized by 1% sodium pentobarbital (40 mg/kg, i.p.). The rats were orally intubated and mechanically ventilated with a rodent ventilator (Harvard Apparatus, USA). Under sterile conditions, the heart was exposed through a lateral thoracotomy. After that, the pericardium was gently removed and the left anterior descending coronary artery was permanently occluded at 2 to 3 mm below the starting point which is located between the pulmonary arterial cone and the left atrial appendage. Acute MI was confirmed by electrocardiography with ST elevation appearance and visual inspection for a rapid whitish discoloration of anterior wall of the LV. The muscle layer and skin were sutured, and the rats were allowed to recover on a warm heating pad (World Precision Instruments Inc., FL, USA).

Intragastric administration of MTHSWD

There were 5 rats were died during and after the surgery. The survival MI rats were then divided into three groups randomly at the second day after modeling, including MI group, THSWD group and MTHSWD group with 10 rats in each group. The rats in THSWD group or MTHSWD group were intragastrically administered THSWD or MTHSWD respectively twice a day for 2 weeks (1 ml each time). The rats in MI group were intragastrically administered an equivalent amount of physiological saline for the same time. The composition and dosage of THSWD were the same as that used in our published article [10]. MTHSWD for each rat contained 10 components including *Semen Persicae* (0.16 g), *Flos Carthami* (0.16 g), *Angelica Sinensis* (0.22 g), *Radix Paeoniae Alba* (0.18 g), *Rhizoma Chuanxiong* (0.14 g), *Radix*

Rehmanniae Praeparata (0.27 g), *Salvia miltiorrhiza* (0.54 g), *Radix Astragali* (0.4 g),*Epimedium* (0.27 g) and *Notopterygii Rhizoma et Radix* (0.27 g), which were provided by Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine. The doses of MTHSWD for rat was converted from a human equivalent dose commonly used in the clinical practice based on the body surface area. THSWD and MTHSWD were prepared according to our reported method [10]. In brief, the crude herbal drugs were mixed and extracted with boiling water. The decoction was concentrated through rotary evaporation under vacuum to an equivalent 2.61 g/mL of the crude herbal drugs.

Echocardiography

Echocardiography was performed on isoflurane anesthetized rats with a Vevo 2100 system (Visualsonics, Toronto, Canada) by an operator in a blinded manner at 1 and 2 weeks after treatment. M-mode images of the left ventricle were captured in the parasternal long-axis view and the left ventricle internal diameter at end-diastole (LVIDd) and end-systole (LVIDs) were measured for three consecutive cardiac cycles. The left-ventricular end-systolic (LVESV) and end-diastolic volume (LVEDV) were calculated according to LVIDs and LVIDd, and the ejection fraction (EF) and fractional shortening (FS) of LV were calculated as (LVEDV-LVESV)/LVEDV (%) and (LVIDd-LVIDs)/LVIDd (%), respectively.

Terminal dUTP nick-end labeling assay

The heart of rat was rapidly excised under anesthesia after the echocardiographic assessment and was cut into two parts transversally along the center of the infarct zone. The tissue near cardiac base was fixed with 4% formaldehyde solution after removing the atrium. Then the sample was embedded in Tissue-Tek OCT (Sakura, USA) and cut into 10-μm sections through a freezing microtome (HM525, Thermo Fisher Scientific, USA). To investigate the role of MTHSWD administration on cardioprotection after MI, the cell apoptosis was detected by the terminal deoxynucleotidyl transferase mediated dUTP nick end-labeling (TUNEL) staining kit (Roche, Mannheim, Germany) according to the instruction recommended by the manufacturer. For each section, at least 5 high-power fields (HPFs) were photographed and the number of TUNEL-positive cells were counted in each field.

Western Blot

The tissue near cardiac apex was homogenized in RIPA lysis buffer supplemented with protease and phosphatase inhibitors (Roche, Mannheim, Germany) to extract protein. After being incubated on ice for 30 min, the sample was centrifuged at 13,000g at 4°C for 15 min. The supernatant was harvested and the concentration of total protein was measured by BCA protein assay (Pierce Biotechnology, USA). The samples were subsequently boiled for 8 min to denature the protein. For the detection, 30 μg of each protein was loaded into each well and separated on a 10% SDS-polyacrylamide gel electrophoresis. The proteins were then transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, USA) using a semidry transfer system. After that, the unoccupied sites were blocked in 5% skim milk for 1 h and immunoblotted with primary antibodies of p-Akt (1:1000, CST), cleaved caspase 3 (1:200, Sigma), Akt (1:2000, CST), matrix metallopeptidase-2 (MMP-2, 1:2000, Abcam), tissue inhibitor of metalloproteinases-

2 (TIMP-2, 1:500, Abcam) and VEGF (1:1000, Beyotime Biotechnology, China) overnight at 4°C. After the incubation with corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies for 2 h at room temperature, the protein bands were visualized by enhanced chemi-luminescence (Pierce Biotechnology, USA) and photographed. The optical densities of the reactive bands normalized to GAPDH were quantified by the image analysis software ImageJ (NIH, Bethesda, MD, USA).

ELISA assay

The infarcted myocardium tissues were weighed and homogenized in PBS by tissue grinder on the ice. The samples were then centrifugated at 3,000 rpm for 20 min at 4°C and the supernatants were harvested for the evaluation of cytokine levels through the specific enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Enzyme-linked Biotechnology, Shanghai, China) according to the manufacturer's instruction.

Masson's trichrome staining

Masson's trichrome staining was performed to detect the infarct size and collagen content in the infarcted area through the routine procedures. The blue area indicated fibrotic tissue and the viable myocardium was stained red. The infarction size and collagen content were measured by Image-Pro Plus software (Bethesda, USA), and respectively defined as the percentage of blue area in the cross-sectional area of whole myocardium in the LV wall and the percentage of fibrotic tissue area in each HPF covering the infarcted and surrounding area.

Immunofluorescence staining

The frozen sections of hearts were incubated with 5% normal goat serum for 1 h at room temperature to block non-specific protein-protein interactions followed by primary antibodies Cx43 (1:200; CST, USA), cTnT (1:100; Abcam, USA), CD31 (1:100; Abcam, USA) and α-SMA (1:200; Abcam, USA) overnight at 4°C. The sections were then washed with PBS for 3 times and probed with appropriate secondary antibodies (1:200; Invitrogen, USA) for 2 h at room temperature. The nuclei were counter-stained with 4',6-diamidino-2-phenylindole (DAPI) in the detection of cTnT and Cx43 expression. At least 5 HPFs in each section were randomly selected and the number of CD31 or α-SMA positive blood vessels per HPF was counted to determine the angiogenesis. The fluorescence intensity of 5 HPFs in each section was analyzed using Image-Pro Plus software for the semi-quantitative analysis of cTnT and Cx43 expression.

Statistical Analysis

All data were represented as means ± standard deviation. Multiple groups were compared using one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls (SNK) post hoc test through a statistical software (IBM SPSS Statistics for Windows, Version 23.0, IBM Corp., NY, USA). Differences were considered to be statistically significant when $P < 0.05$.

Results

MTHSWD improved heart function of MI rats

One week after gavage, THSWD and MTHSWD could improve the EF and FS values of MI rats, but the differences between the treatment groups and MI group were not statistically significant (Fig. 1A, B). At 2 weeks after treatment, the EF and FS values of MTHSWD group were higher than those of THSWD group, but there was no statistical difference. However, compared with the MI group, the EF and FS values in the MTHSWD group were significantly higher (Fig. 1C, D). There were no significant differences in the values of LVIDd and LVEDV among the three groups at 2 weeks after treatment (Fig. 1E, G). However, the values of LVIDs and LVESV in the MTHSWD group were significantly lower than those in the MI group (Fig. 1F, H).

MTHSWD reduced cell apoptosis in the infarcted area by activating Akt signal

TUNEL staining was used to detect the number of apoptotic cells in the infarcted area (Fig.2A). The number of apoptotic cells in the MI group was the largest, and THSWD and MTHSWD could obviously attenuate the cell apoptosis. Moreover, the number of apoptotic cells in the MTHSWD group was significantly lower than that in the THSWD group (Fig.2B). To further investigate the mechanism of cardioprotection by MTHSWD, Western blot was used to detect the level of p-Akt and the expression of cleaved caspase 3. MTHSWD could significantly increase the level of p-Akt and reduce the expression of cleaved caspase 3, compared with that in the MI group and THSWD group (Fig.2C-E).

Effects of MTHSWD on the level of cytokines in the infarcted myocardium and serum

The levels of insulin-like growth factor-1 (IGF-1), stromal cell-derived factor-1 (SDF-1), stem cell factor (SCF), vascular cell adhesion molecule-1 (VCAM-1), interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) in the infarcted myocardium and serum were measured by ELISA. THSWD had no significant effects on the levels of these cytokines, but MTHSWD could significantly increase the contents of IGF-1, SDF-1 and TNF-α, and significantly reduce the expression of IL-1β, in the infarcted myocardium, compared with that in the MI group. MTHSWD did not affect the levels of SCF and VCAM-1 in the infarcted myocardium. There were no significant differences in the levels of these cytokines between the MTHSWD group and THSWD group (Fig.3A-F). Similarly, THSWD had no significant effect on cytokines in the serum. However, compared with the MI group, MTHSWD could significantly increase the expression of IGF-1, SDF-1 and SCF. The levels of VCAM-1, IL-1β and TNF-α in the serum were not affected by MTHSWD. Interestingly enough, the level of SCF in the serum in the MTHSWD group was much higher than that in the THSWD group (Fig.3G-L).

MTHSWD decreased infarct size and collagen deposition in the infarcted area

According to the Masson's trichrome staining of heart tissues, the rats in the MI group showed a large area of infarction, while the animals in the MTHSWD group showed the smallest infarct size (Fig.4A). The infarct size in the THSWD group was reduced compared to that in the MI group, but the difference was not statistically significant. However, the infarct area in the MTHSWD group was significantly lower than that in the MI group and the THSWD group (Fig.4B). In the MI group, the deposition of collagen fibers in

the infarct area was very obvious and the myocardium was robustly replaced by collagen fibers. Intragastric administration of THSWD and MTHSWD could reduce the collagen deposition in the infarct area (Fig.4C). According to statistical analysis, the collagen deposition in the infarction area of the MTHSWD group was the lowest, which was significantly lower than that of the MI group and the THSWD group (Fig.4D). Western blot was used to further detect the expression of MMP-2 and TIMP-2 in the infarcted myocardium (Fig.4E). Both THSWD and MTHSWD could down-regulate the expression of MMP-2, and the expression of MMP-2 in the MTHSWD group was significantly lower than that in the THSWD group (Fig.4F). In addition, compared with the MI group and THSWD group, administration of MTHSWD could significantly up-regulate the expression of TIMP-2 (Fig.4G).

MTHSWD increased the expression of cTnT and Cx43 in the infarcted area

Immunofluorescence staining showed that the cardiac-specific markers cTnT and Cx43 were rarely expressed in the infarcted area of the MI group. The expression of cTnT and Cx43 in the infarcted area of the hearts could be obviously observed in the THSWD group and MTHSWD group (Fig.5A). The relative levels of cTnT and Cx43 in the THSWD group and MTHSWD group were significantly higher than those in the MI group. Compared with the THSWD group, the expression of cTnT and Cx43 in the MTHSWD group was significantly increased (Fig.5B, C), suggesting that MTHSWD administration played a definite role in reducing and preventing the loss of cardiomyocytes and electrical coupling between cardiomyocytes.

MTHSWD promoted angiogenesis by increasing the expression of VEGF

The vascular density in the infarcted and peri-infarcted area of rat hearts was determined by the immunofluorescence staining for CD31 and α-SMA (Fig. 6A, B), as the blood perfusion of the lesion was a key factor for the survival of cardiomyocytes and cardiac repair after MI. Both THSWD and MTHSWD could increase the number of microvessels and the number of α-SMA positive vessels in the infarcted and peri-infarcted area. Compared with the THSWD group, the number of microvessels was prominently elevated in the MTHSWD group, but there was no significant difference in the number of α-SMA positive vessels between the two groups (Fig. 6C, D). To further explore the possible mechanism underlying the roles of MTHSWD for the enhanced angiogenesis after MI, the expression of VEGF in the infarcted myocardium tissues was detected by Western blot (Fig. 6E). Compared with the MI group, the expression of VEGF in the THSWD group and MTHSWD group was significantly increased. Moreover, the expression of VEGF in the MTHSWD group was higher than that in the THSWD group, suggesting that MTHSWD administration may enhance angiogenesis by increasing the expression of VEGF in the infarcted tissues.

Discussion

MI is the most serious and life-threatening conditions among cardiovascular diseases. Current data suggest that TCM is emerging as a potential treatment for MI in clinical practice. TCM treatment may be beneficial in reducing the mortality of MI patients and improving their quality of life [11]. According to our previous study, THSWD was beneficial for the improvement of heart function after MI. However, this effect was modest although the local hostile microenvironment was improved and mitochondrial fission was decreased [10]. In this study, THSWD was modified by adding four more TCM herbs to constitute a

new Chinese medicine prescription, MTHSWD. Interestingly, MTHSWD effectively improved cardiac function, decreased infarct size and collagen deposition in a rat model of MI. More importantly, the infarct size and collagen deposition in the infarcted area were both significantly reduced in the rats of MTHSWD group compared with those in the THSWD group. These positive effects, to some extent, may be attribute to the addition of four TCM herbs with the properties of warming *Yang* and removing phlegm. However, whether these four TCM herbs could exert effects, or even be equivalent to the effects of MTHSWD, deserves further investigation. Besides, the long-term effects of MTHSWD on heart function and its side-effects should be explored in the future.

It has been reported that the supplemented four herbs have good effects on the patients with cardiovascular diseases. *Salvia miltiorrhiza*, belongs to the family *Labiatae*, has a relatively high safety profile and has been widely used in Asian countries for treating cardiovascular diseases [12]. As a promising candidate for treating cardiovascular diseases, has numerous cardioprotective effects, such as antioxidative, anti-inflammatory, inhibition of apoptosis and anti-cardiac fibrosis [13]. *Salvia miltiorrhiza* and *Carthamus tinctorius* extract has been documented to prevent myocardial fibrosis and adverse remodeling after MI by epigenetically suppressing Smad3 expression [14]. *Radix Astragali* is often used in a variety of Chinese herbal preparations as a representative drug for the treatment of *Qi* deficiency syndrome. *Radix Astragali* has numerous cardioprotective effects, including reducing inflammatory factor injury and myocardial apoptosis, promoting cardiac microvessel formation and relieving excessive oxidative stress [15-18]. A recent study showed that the combination of *Radix Astragali* and *Angelica Sinensis* not only promoted angiogenesis but also suppressed inflammation and cardiomyocyte apoptosis in a mouse model of MI [19]. The traditional Chinese herb *Epimedium* has the properties of warming *Yang* and has been used for the remedy of cardiovascular disease as it has anti-heart failure effect. The ethanol extract of *Epimedium* ameliorated LV dysfunction, cardiac remodeling and myocardial apoptosis in rats with congestive heart failure [20]. As a major component of *Epimedium*, Icariin attenuated myocardial infarct size and cell apoptosis by activating the PI3K/Akt/eNOS-dependent signal pathways [21]. In addition, Icariin ameliorated diabetic cardiomyopathy by preventing mitochondrial dysfunction through the Apelin/Sirt3 pathway [22]. *Notopterygii Rhizoma et Radix* has been widely used in clinical prescriptions to disperse cold and eliminate dampness in China. Modern pharmacological research indicated that *Notopterygii Rhizoma et Radix* has numerous beneficial effects, including antioxidant, anti-inflammatory and hepatoprotection [23]. *Notopterygii Rhizoma et Radix* was innovatively used in this study to improve the efficacy in consideration of its effect of dredging collaterals and relieving pain. Although MTHSWD reduced cell apoptosis and improve heart function, it is valuable to clarify the active ingredients of MTHSWD after administration. In addition, it's very interesting to investigate the effects of active ingredients on myocardial damage, thus providing reference data for new drug development.

The loss of cardiomyocytes due to ischemia and hypoxia is the characteristic feature of MI. Indeed, the cell apoptosis and fibrosis in the infarcted area extensively lead to heart dysfunction. In this study, we found that MTHSWD significantly attenuated cell apoptosis probably through activating Akt signaling pathway. PI3K/AKT has been well documented in mediating cell survival, growth, proliferation and

differentiation. A recent study indicated that Astragaloside IV inhibited cell apoptosis after MI via regulating PTEN/PI3K/Akt signaling pathway [24]. Meanwhile, we found that the expression of cleaved caspase 3 in the MTHSWD group was much lower than that in the MI group and THSWD group. Caspase-3 is a critical executioner of apoptosis and is responsible for the proteolytic cleavage of many apoptosis-related proteins. Lentivirus mediated interference of Caspase-3 expression could reduce the infarct size and cell apoptosis as well as improve the heart function in MI rats [25]. In addition, long-term infusion of caspase inhibitor reduced myocardial troponin-I cleavage and preserved myocardial contractile proteins after MI [26]. Interestingly, MTHSWD administration significantly increased the level of IGF-1, which is a hormone that promotes growth and prevents cell death. IGF-1 could activate intracellular Akt phosphorylation and decrease caspase activation by binding to its receptor, thus reducing cardiomyocyte apoptosis [27]. Local delivery of IGF-1 by biotinylated nanofibers activated Akt, decreased caspase-3 cleavage and improved systolic function after experimental MI [28]. Furthermore, the expression of cTnT and Cx43 in the infarcted area were significantly preserved in the hearts of MTHSWD treated rats, suggesting that MTHSWD played a definite role in preventing and reducing the loss of cardiomyocytes.

The inflammation is elevated and viewed as a major determinant of cardiac remodeling and function after MI. Therefore, the effects of anti-inflammatory drugs were evaluated in patients with MI [29]. MTHSWD could significantly reduce the expression of IL-1β in the infarcted myocardium compared with that in the MI group. However, administration of THSWD didn't decrease the levels of IL-1β in the infarcted tissues. As an ancient and evolutionary conserved cytokine, IL-1β is toxic for the cardiomyocytes [30]. The interleukin-1 receptor antagonist inhibited apoptosis and remodeling in experimental acute MI [31]. miR-132 inhibited cardiomyocyte apoptosis and myocardial remodeling after MI through downregulating IL-1β [32]. Interestingly, we found that MTHSWD significantly increased the level of TNF-α, which is an important proinflammatory factor, in the infarcted myocardium. It has been proposed that inflammation may have protective or detrimental effects on heart function with the progression of myocardial ischemia [33]. For example, although TNF-α was excessively elevated and contributed to cell apoptosis and cardiac dysfunction after myocardial ischemia-reperfusion, the application of TNF-α inhibitor markedly promoted myocyte apoptosis and reduced cardiac function after MI [34]. Therefore, the exact effect of TNF-α during the progression of myocardial ischemia should be further clarified through more investigations.

Besides the inflammatory factors, MTHSWD also significantly increased the level of SDF-1 in the infarcted myocardium and the levels of SDF-1 and SCF in the serum. SDF-1 is the most paramount chemokine that protects heart from ischaemic injury by recruiting stem/progenitor cells from bone marrow to the site of injury [35]. The interaction of SDF-1 with the receptor CXCR4 and the local concentration of SDF-1 is critical to induce chemotactic response. However, the concentration of SDF-1 in heart is declining by the following days after MI [36]. In addition, the systemic administration of SDF-1 failed to accumulate at the infarcted area. A growing body of evidence suggests that adenoviral delivery of SDF-1α post-infarction effectively improved retention of BM-derived stem-cells, reduced infarct size and improved heart function [37, 38]. Accumulating evidence suggests that SDF-1 could also promote angiogenesis after MI [38, 39]. The VEGF expression level was obviously increased after the injection of AdV-SDF-1 into the infarcted myocardium [38]. VEGF is well-known for its primary role in inducing and

promoting the angiogenesis [40], and MTHSWD obviously enhanced the expression of VEGF in the myocardium tissues. Therefore, the promotion of angiogenesis by MTHSWD may be related to the upregulation of SDF-1 and VEGF after MI. SCF is related to cell migration, proliferation, and survival by binding to the c-kit receptor. It is now accepted that SCF induces mobilization of stem cells from the bone marrow and plays an important role in cardiac repair after MI [41,42]. However, although MTHSWD increased the level of SCF in the serum, the expression of SCF in the infarcted myocardium was not changed obviously after MTHSWD administration.

The stem cell-based therapy is a potential therapeutic strategy for MI, but it was dramatically hampered by the low enrichment in the infarcted tissues after intravenous transplantation. Tanshinone IIA increased the mesenchymal stem cells (MSCs) migration to infarct region via up-regulating SDF-1/CXCR4 signaling pathway [43]. We have observed that Guanxin Danshen formulation promoted the migration and survival of the intravenous-injected MSCs after MI [44]. We proposed that MTHSWD could enhance the engraftment and survival of stem cells in the infarcted area after intravenous injection as it not only raised the expression of SDF-1 and SCF but also reduced the level of inflammatory factor. Therefore, more research is needed to validate effects of MTHSWD combined with the intravenous injection of MSCs or other types of stem cells for the treatment of MI.

Conclusions

The administration of MTHSWD reduced cell apoptosis, promoted angiogenesis and improved heart function after MI. More importantly, the effects of MTHSWD were superior to THSWD in improving heart function and reducing infarct size. This study also indicated that the improvement of cardiac repair after being treated by MTHSWD may be attributed to the downregulation of inflammatory factor IL-1β, upregulation of chemotactic factors SDF-1 and SCF as well as pro-angiogenic factor VEGF, and activation of Akt signaling pathway. Therefore, the novel Chinese medicine MTHSWD proposed in this study has practical significance and application potentials for the treatment of cardiovascular diseases such as MI.

Abbreviations

HPFs: High-power fields

IGF-1: Insulin-like growth factor-1

IL-1β: Interleukin-1β

LVEDV: Left-ventricular end-diastolic volume

LVESV: Left-ventricular end-systolic volume

LVIDd: Left ventricle internal diameter at end-diastole

LVIDs: Left ventricle internal diameter at end-systole

MI: Myocardial infarction

MMP-2: Matrix metallopeptidase-2

MSCs: Mesenchymal stem cells

MTHSWD: Modified THSWD

PCI: Percutaneous coronary intervention

SCF: Stem cell factor

SDF-1: Stromal cell-derived factor-1

TCM: Traditional Chinese medicine

THSWD: Taohong Siwu decoction

TIMP-2: Tissue inhibitor of metalloproteinases-2

TNF-α: Tumor necrosis factor alpha

TUNEL: Terminal deoxynucleotidyl transferase mediated dUTP nick end-labeling

VCAM-1: Vascular cell adhesion molecule-1

VEGF: Vascular endothelial growth factor

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Contributions

HG and LW conceived and designed the research. ZL, HL and YX performed most experiments and animal works. SX and HC assisted some experiments and result analysis. ZL, LW and HG drafted and wrote the manuscript.

Competing interests

The authors have no conflicts of interest to disclose.

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Figures

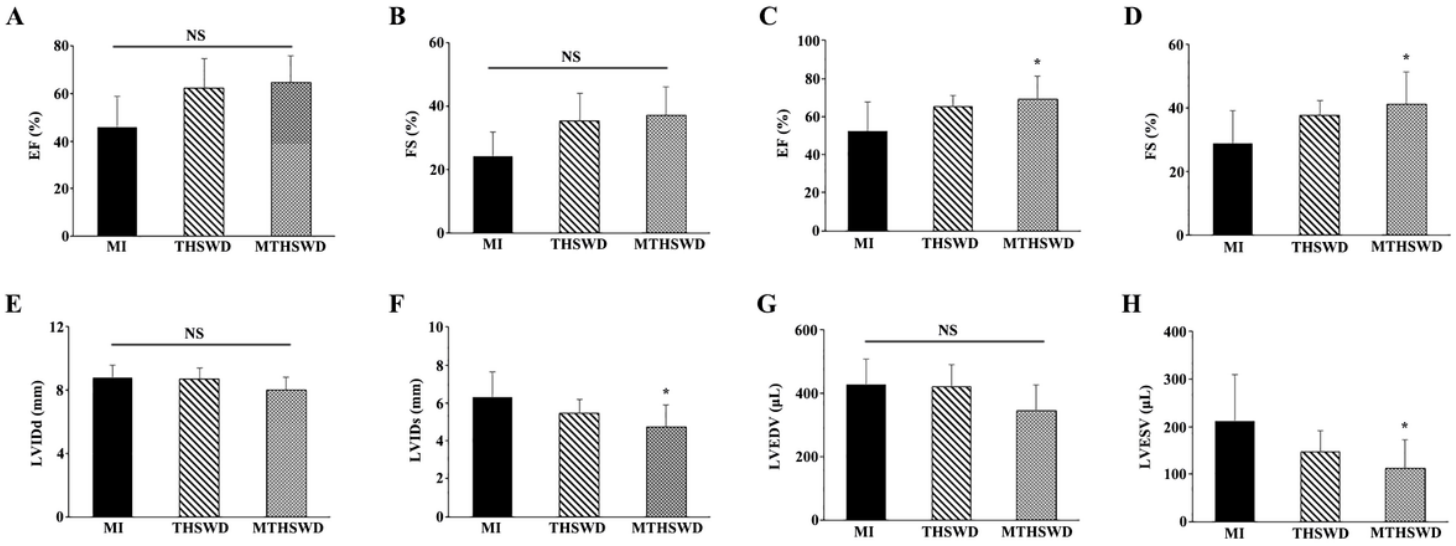

Figure 1

MTHSWD improved heart function in rats after MI. (A, B) The parameters of EF and FS were compared at 1 week after treatment. (C-H) The parameters of EF, FS, LVIDd, LVIDs, LVEDV, and LVESV were compared at 2 weeks after treatment. *P < 0.05 versus the MI group. n = 10.

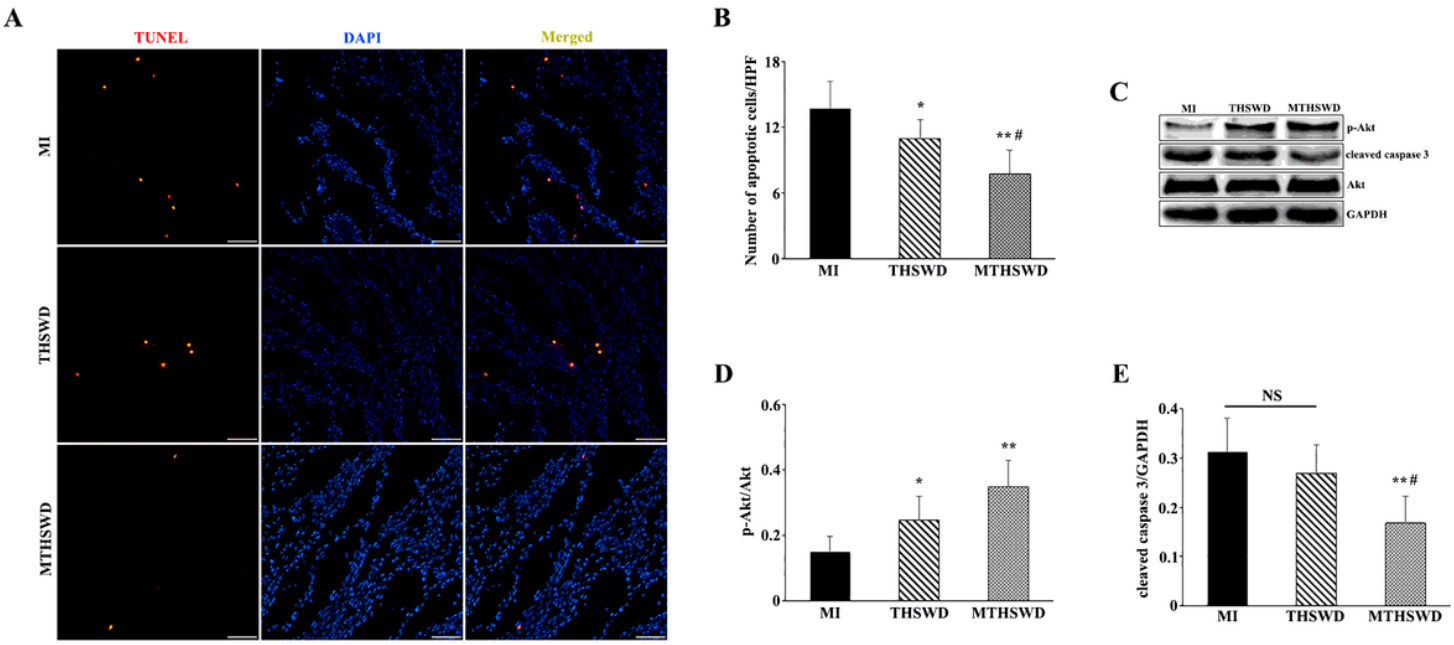

Figure 2

MTHSWD alleviated cell apoptosis in the infarcted area by activating Akt signal. (A) TUNEL staining was used to detect the number of apoptotic cells in the infarcted area. Scale bar: 50 μm. (B) The number of TUNEL-positive cells was calculated and analysized. *P < 0.05 and **P < 0.01versus the MI group. #P < 0.05 versus the THWSD group. n = 10. (C) The levels of p-Akt and cleaved caspase 3 in the infarcted myocardium were detected by Western blot. (D, E) The semiquantitative data of Western blots for p-Akt and cleaved caspase 3. *P < 0.05 and **P < 0.01versus the MI group. #P < 0.05 versus the THWSD group. n = 5.

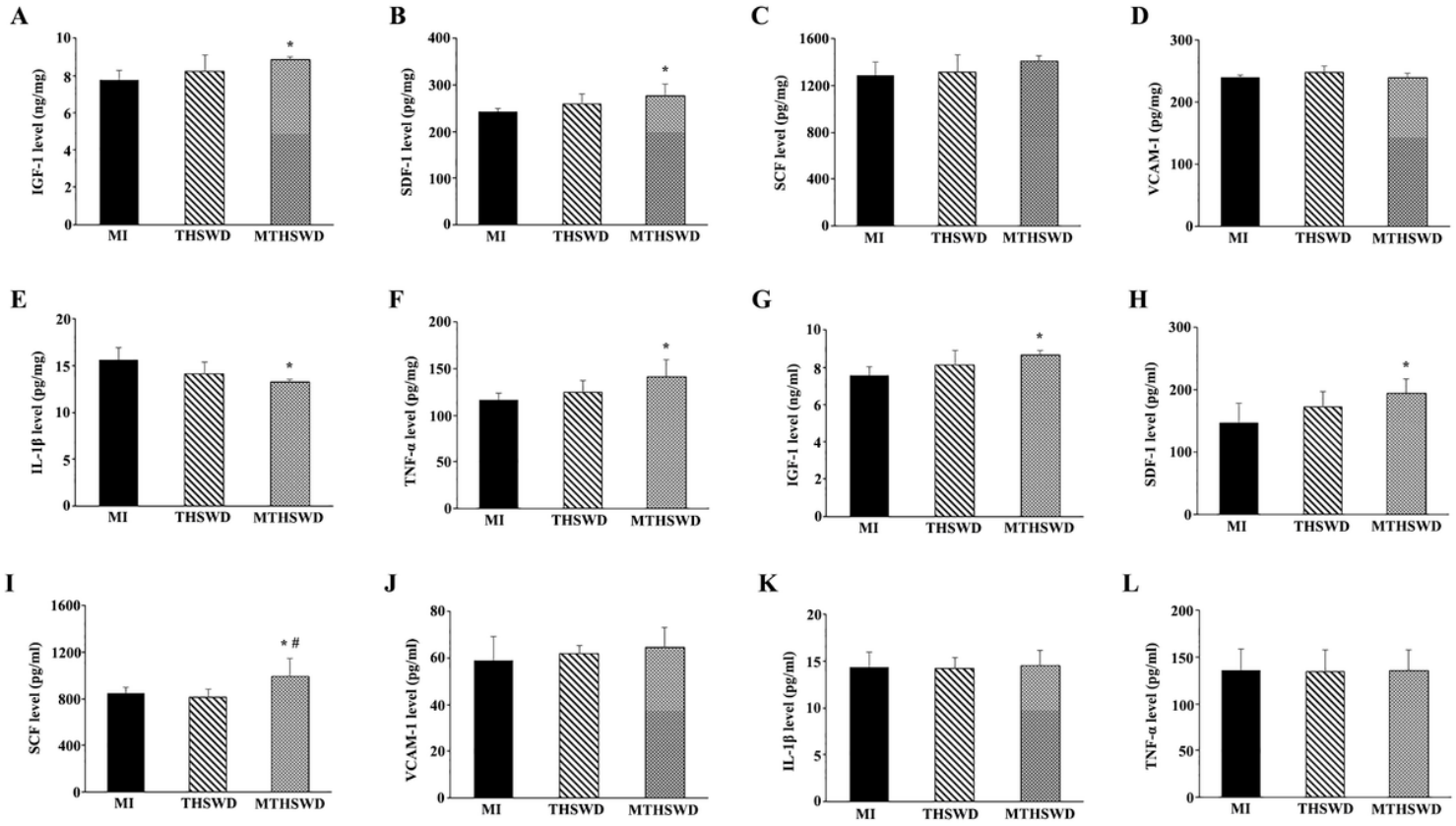

Figure 3

Effects of MTHSWD on the expression of cytokines in the infarcted myocardium and serum. (A-F) The levels of IGF-1, SDF-1, SCF, VCAM-1, IL-1β and TNF-α in the infarcted myocardium were compared among the different treatments. *P < 0.05 versus the MI group. n = 5. (A-F) The levels of IGF-1, SDF-1, SCF, VCAM-1, IL-1β and TNF-α in the serum were compared among the different treatments. *P < 0.05 versus the MI group. #P < 0.05 versus the THWSD group. n = 10.

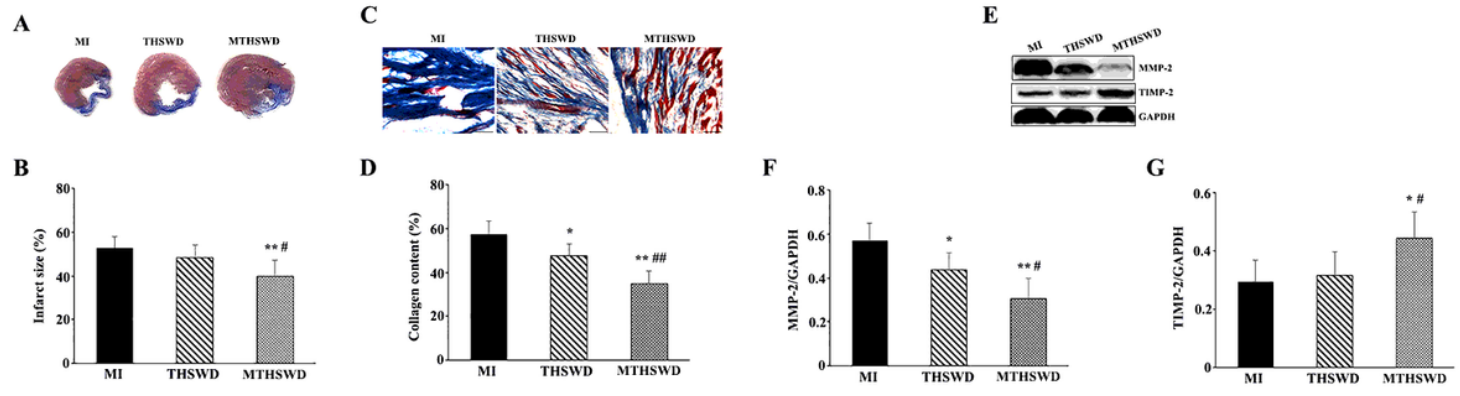

Figure 4

MTHSWD reduced infarct size and collagen deposition in the infarcted area. (A) The infarct area was detected by Masson's trichrome staining in each group. (B) Quantitative analysis of myocardial infarct area. (C) The collagen deposition in the infarcted area was determined after Masson's trichrome staining.

Scale bar: 50 μm. (D) Quantitative analysis of collagen deposition in the infarcted area. *P < 0.05 and **P < 0.01versus the MI group. #P < 0.05 and **P < 0.01 versus the THWSD group. n = 10. (E) The expressions of MMP-2 and TIMP-2 in the infarcted myocardium were detected by Western blot. (F, G) The semiquantitative data of Western blots for MMP-2 and TIMP-2. *P < 0.05 and **P < 0.01versus the MI group. #P < 0.05 versus the THWSD group. n = 5.

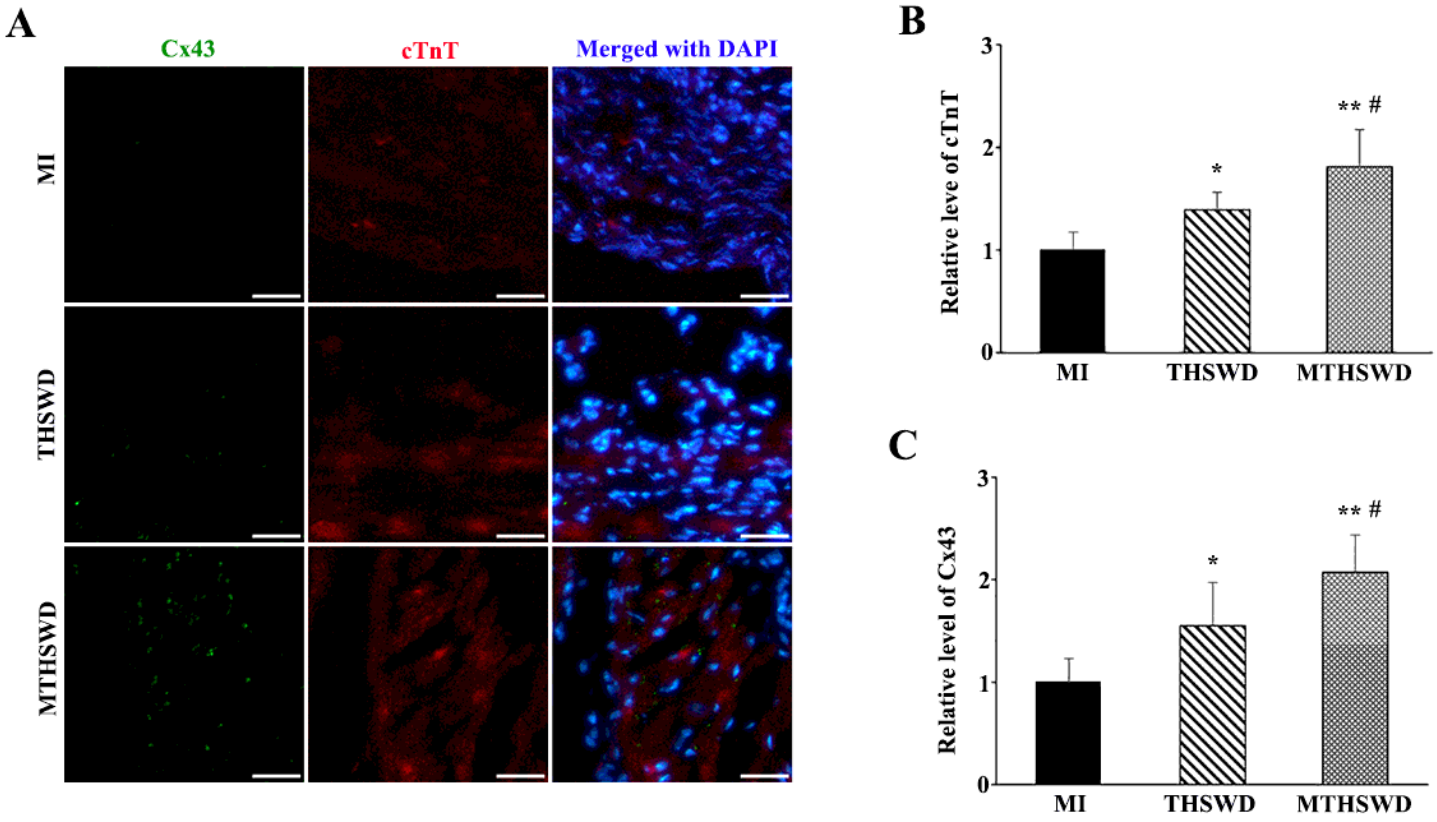

Figure 5

MTHSWD increased the expression of cTnT and Cx43 in the infarcted area. (A) The expression of cardiac-specific markers cTnT and Cx43 were detected by immunofluorescence staining in the infarcted and surrounding area. Scale bar: 20 μm. (B, C) The fluorescence intensity of cTnT and Cx43 in each group was analyzed. *P < 0.05 and **P < 0.01versus the MI group. #P < 0.05 versus the THWSD group. n = 10.

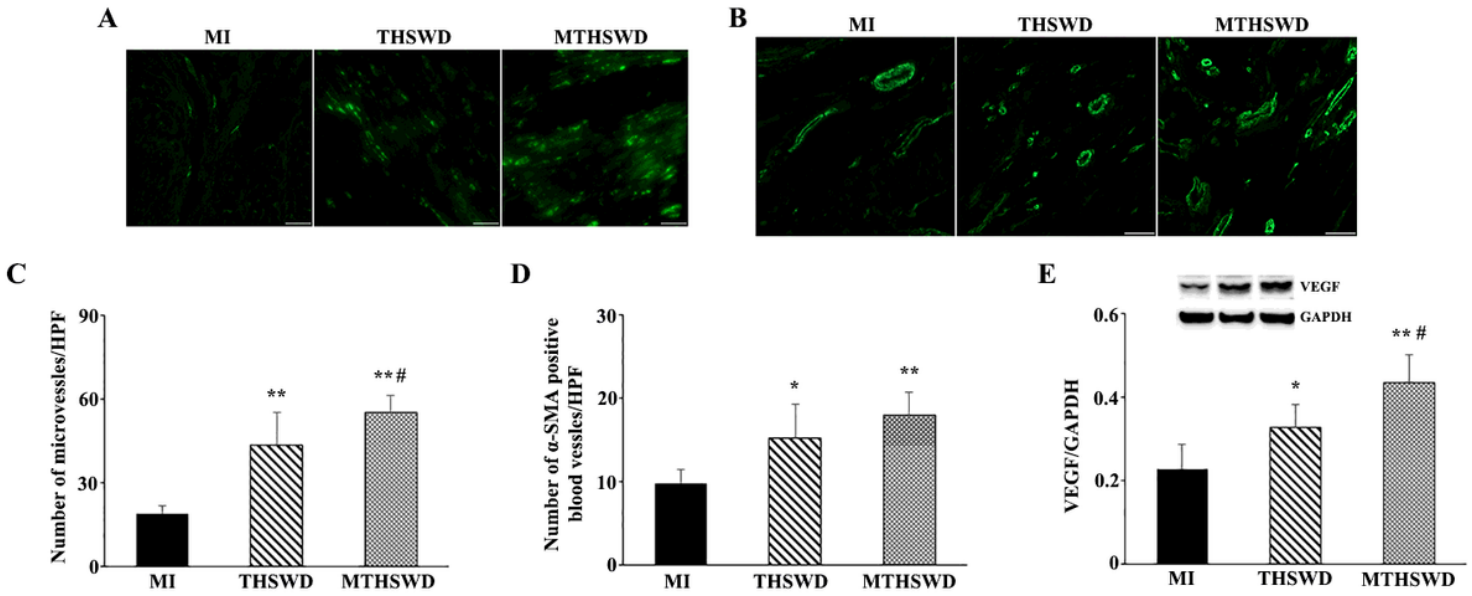

Figure 6

MTHSWD promoted angiogenesis by increasing the expression of VEGF. (A, B) The blood vessels in the infarcted and surrounding area of rat hearts was examined by immunofluorescence staining for CD31 and α-SMA. Scale bar: 25 μm (A) and 50 μm (B). (C, D) Quantification of blood vessels density. *P < 0.05 and **P < 0.01versus the MI group. #P < 0.05 versus the THWSD group. n = 10. (E) The expression of VEGF in the infarcted myocardium were detected by Western blot. *P < 0.05 and **P < 0.01versus the MI group. #P < 0.05 versus the THWSD group. n = 5.