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Effects of Tongmai Yangxin pills on ventricular remodeling in myocardial ischemia-reperfusion rats

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Abstract

Objective: This study aimed to determine whether Tongmai Yangxin pills (TM) can attenuate ventricular remodeling (VR) and to explore the underlying mechanisms.

Methods: After the myocardial ischemia-reperfusion (I/R) injury model has been established, the rats were divided into seven groups: control, Sham, I/R, TM (1.0 g/kg), TM (2.0 g/kg), TM (4.0 g/kg), and Tongxinluo capsules, respectively. Experimental parameters were assessed on days 3 and 28 after drug administration. Cardiac structure and function were assessed by echocardiography. Myocardial ischemia was quantified using triphenyl tetrazolium chloride staining, and the cardiac pathology was evaluated using hematoxylin-eosin staining. Myocardial enzyme and oxidant activities were detected using an automatic biochemical analyzer and kit, respectively. Masson's trichrome staining was used to analyze the degree of collagen deposition. The expression levels of inflammation and fibrosis-related proteins were detected using enzyme-linked immunosorbent assays.

Results: After 3 days of administration, TM improved cardiac function and morphology. It effectively reduced the area of myocardial infarction in I/R rats, inhibited the abnormal activity of myocardial enzymes, and significantly reduced superoxide dismutase activity, as well as C-reactive protein, tumor necrosis factor-α, and interleukin-1β expression at the protein level. TM administration inhibited oxidative stress, inflammation, and myocardial pathological damage. After 28 d of administration, TM improved heart function; inhibited ventricular dilation and the thinning of the ventricular wall; significantly reduced the protein expression of connective tissue growth factor, matrix metalloproteinase 2, and matrix metalloproteinase 9; and decreased the degree of myocardial fibrosis.

Conclusions: TM can effectively reduce the infarct size, improve the cardiac structure and function, reduce myocardial collagen deposition, and attenuate VR. The underlying mechanisms involve the inhibition of inflammatory responses in the early stages and a reduction of myocardial fibrosis in the late stages.

Keywords: Myocardial ischemia-reperfusion, Tongmai Yangxin pills, Ventricular remodeling

Graphical abstract: http://links.lww.com/AHM/A60.

Introduction

Ischemic heart disease is characterized by myocardial ischemic damage caused by an imbalance in the coronary blood flow and myocardial demand. It is an important cause of death in patients with cardiovascular disease and the primary factor leading to heart failure. Acute myocardial ischemia can be effectively treated by thrombolysis, percutaneous coronary artery dilation, or coronary artery bypass surgery enabling reperfusion of the ischemic myocardium[1]. However, the process of myocardial reperfusion can lead to further death of the myocardial cells, called reperfusion injury[2]. In the late stage of myocardial injury, inflammatory and other factors accumulate, the morphological structure defined by the myocardial cells and intercellular matrix changes, the left ventricle expands progressively, and the heart changes structurally and functionally due to the activation of neurohumoral regulation mechanisms. These changes, including alterations in the shape, volume, and wall thickness of left ventricular, have been termed ventricular remodeling (VR)[3]. The main characteristics of VR after myocardial ischemia-reperfusion (I/R) are apoptosis, necrosis, and fibrosis of myocardial cells in the infarcted area. Ultimately, the damaged myocardium is replaced by scar tissue. Apoptosis, compensatory hypertrophy, and reconstruction of the extracellular matrix network structure in the non-infarcted area (the area at risk between the infarcted and unaffected areas) occur. VR can lead to progressive ventricular dilatation and increased stress of the myocardial wall, and it eventually leads to heart failure. Proinflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α,

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participate in cardiac remodeling and play an important role in the pathogenesis and progression of heart failure[4]. In the process of I/R, inflammatory factors such as TNF-α and IL-1β can damage cardiomyocytes, inhibit myocardial contraction by inducing myocardial hypertrophy, and mediate other mechanisms, such as oxidative stress, apoptosis, and calcium overload. Sustained release of inflammatory cytokines leads to the activation of matrix metalloproteinases (MMPs), which causes collagen slippage and expansion, ultimately furthering the process of myocardial remodeling. IL-1β and TNF-α can upregulate the expression levels of MMP-2 and MMP-9 in cardiomyocytes and tissues, and MMP-9 can, in turn, activate IL-1β, leading to a positive feedback mechanism. Furthermore, C-reactive protein (CRP) can stimulate TNF-α and IL-1β production and activate MMPs in endothelial cells and macrophages, thereby accelerating VR. By contrast, anti-TNF-α treatment can decrease the expression levels of MMPs; accordingly, collagen synthesis is inhibited, and ventricular diastolic function improves[5].

Tongmai Yangxin pills (TM) are composed of 11 herbs, namely, *Rehmanniae Radix*, *Spatholobi Caulis*, *Ophiopogonis Radix*, *Polygoni Multiflori Radix Preparata*, *Asini Corii Colla*, *Glycyrrhizae Radixet Rhizoma*, *Schisandrae Chinensis Fructus*, *Codonopsis Radix*, *Testudinis Carapax et Plastrum*, *Jujubae Fructus*, and *Cinnamomi Ramulus*. The traditional Chinese patent medicine TM tonifies *qi* and nourishes *yin*. Clinically, TM significantly affects the treatment of angina pectoris of coronary heart disease with *qi-yin* deficiency and blood stasis pattern. In patients with coronary heart disease, TM can alleviate arrhythmia and angina pectoris[6–7]. TM can mitigate atrial fibrillation (deficiency of *qi* and *yin* syndrome)[8], prevent the occurrence of cardiac insufficiency[9], interfere with the development of cardiac insufficiency[10], and alleviate the clinical symptoms of patients with chronic stable angina pectoris[11]. Moreover, TM combined with tirofiban hydrochloride can be used to treat unstable angina pectoris and improve cardiac function[12]. It can also effectively improve heart rhythms, increase the blood and oxygen supply to the ischemic myocardium, and protect cardiac function[13]. Pharmacological studies have shown that TM inhibits inflammation and anti-oxidative processes[14–16]. Specifically, it has anti-hypoxic effects on myocardial cells[17]. In our previous studies, TM effectively improved myocardial no-reflow and apoptosis[18]. However, whether TM affects VR after I/R and whether it affects early or late stages in myocardial I/R remains unclear.

In this study, we used network pharmacology to predict potential core targets of protein-protein interactions (PPIs), for example, IL-1β, MMP-2, MMP-9, and CRP, involved in the TM-induced improvement of VR. Furthermore, we investigated the mechanisms underlying the observed TM effects on VR from the perspectives of inflammatory reactions, oxidative mechanisms, cardiac structure and function, and myocardial fibrosis.

Materials and methods

Construction of drug and disease target networks

The TCMSP database (https://old.tcmsp-e.com/tcmsp.php) was utilized. The oral bioavailability (OB), blood-brain barrier, and drug-like (DL) properties in the TCMSP database were considered as important parameters of the drug's absorption, distribution, metabolism, and excretion (ADME). Information on the chemical compositions and targets of the 11 identified TM drugs (OB≥30; DL≥0.18) was obtained. Cytoscape 3.7 was used to generate a visual network diagram representing the "drug–component–target". Furthermore, the GENECARD website (https://www.genecards.org/) was used to search for the disease keyword "ventricular remodeling" to identify drug targets for the treatment of VR. The intersection of drug and disease targets was used to determine shared genes.

PPI network generation

The STRING database (https://string-db.org/) was used for the visual analysis of the PPI network. The identified shared drug and disease genes were input into the STRING database. The species was set to be "human" for this analysis. The results were entered into Cytoscape 3.71 for visual network analysis. The MCODE plug-in was used to set the degree cutoff to 2, the node score cutoff to 0.2, and the K-core to 2, respectively, for the analysis and extraction of core targets.

Animals

Male Sprague-Dawley rats [(250±20) g, SCXK 2016X0006] were purchased from Beijing Weitonglihua Experimental Animal Technology Co., Ltd., China. Animal housing and experimental procedures were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health and the Animal Ethical Committee of Tianjin University of Traditional Chinese Medicine.

TM preparation

TM was purchased from Tianjin Zhongxin Pharmaceutical Co., Ltd. (1070353, Tianjin, China). Tongxinluo capsules (TXL) were provided by Shijiazhuang Yiling Pharmaceutical Co., Ltd. (Z19980015, Shijiazhuang, China). A total of 11 TM components were considered in this study (Table 1). TM was prepared in accordance with *Chinese Pharmacopoeia* (2015 Edition). TM composition and quality standards were based on previous descriptions[18–19].

Experimental protocol

The rat myocardial I/R model was based on our previous study[19]. After the left anterior descending coronary artery had been ligated for 2 h, the extrathoracic ligature was cut to restore blood perfusion, and the rats were sutured. The rats were divided into 14 groups, which were evaluated at two-time points (3 and 28 days) with seven groups at each time point: control (Con), Sham, I/R, TM (1.0 g/kg), TM (2.0 g/kg), TM (4.0 g/kg), and TXL (2.0 g/kg). The Con, Sham, and I/R groups received an equal volume of 0.5% CMC Na (C8621, Solarbio Science & Technology Co., Ltd., Beijing, China). All other groups were given 10 mL/kg of either TM or TXL by gavage for 3 or 28d.

Measurement of the ischemic area

The heart was collected, cleaned, and frozen in the freezer (–80°C) for 30 min. The myocardium was quickly cut from

Table 1

Coincidence genes between the predicted target of TM and the therapeutic target of ventricular remodelling.

PGR	HSP90AA1	ICAM1	HAS2	PTGER3	RUNX2
NCOA2	CDK2	BIRC5	F7	PRKCB	E2F1
NR3C2	PRSS1	IL2	CACNA1S	DUOX2	CTSD
PTGS2	CALM1	CCNB1	KDR	NOS3	IGFBP3
RXRA	OPRM1	TYR	CYP1A2	HSPB1	IGF2
SCN5A	RELA	IL4	ALB	CYP1B1	ERBB3
ADRB2	EGFR	TOP2A	CAV1	PLAT	HK2
CHRM3	AKT1	GSTP1	CTNNB1	THBD	GSTM1
PTGS1	VEGFA	SLC2A4	MYC	SERPINE1	MAPK10
ADRA2A	CCND1	CD40LG	F3	COL1A1	OLR1
SLC6A3	BCL2L1	PTGES	GJA1	ALOX5	HSD3B1
AKR1B1	CDKN1A	MET	MMP10	IL1A	IKBKB
PLAU	CASP9	CA2	MMP3	MPO	MAPK8
MAOB	MMP-2	OPRD1	FOS	NCF1	PPP3CA
MAOA	MMP-9	NR3C1	EGF	ABCG2	MAPK3
CHRM1	MAPK1	MMP13	POR	NFE2L2	LDLR
ADRB1	RB1	MMP8	ODC1	NQO1	BAD
ADRA1A	CDK4	HTR3A	RAF1	PARP1	MTTP
CHRM2	JUN	DRD2	SOD1	COL3A1	APOB
NOS2	IL6	CYP3A4	HIF1A	CXCL11	HMGCR
ESR1	CASP3	KCNH2	STAT1	CXCL2	CYP19A1
AR	TP63	PDE10A	RUNX1T1	NR1I3	GSR
PPARG	NFKBIA	ADRA2C	CDK1	CHEK2	ABCC1
ACHE	MDM2	BCL2	HSPA5	CLDN4	GOT1
ADRA1D	APP	BAX	ACACA	PPARA	ABAT
SLC6A4	MMP1	CASP8	CYP1A1	PPARD	SOAT1
ESR2	PCNA	PRKCA	IL1B	CRP	RXRB
DPP4	ERBB2	PON1	CCL2	CXCL10	STAT3
MAPK14	HMOX1	MAP2	SELE	CHUK	PRKCE
GSK3B	CASP7	CAT	VCAM1	SPP1	PRKCD
VDR	CYP27B1				

TM: Tongmai Yangxin pills.

the apex to the ligation site with a scalpel. The part under the ligation line was evenly cut into 5 slices with approximately 1 mm thickness each. After the slices had slightly melted, they were placed into a 1% triphenyl tetrazolium chloride (TTC) solution (T8170, Solarbio Science & Technology Co., Ltd.) and incubated at 37°C. The slices were turned over every 5 min, and staining was observed after 10 to 15 min. The ratio of the infarcted area to the noninfarcted area was calculated using the image processing software Image-Pro Plus V6.0 (Media Cybernetics, USA).

Histology

The extracted heart was fixed in 10% neutral formalin for 24 h. The left anterior descending coronary artery was ligated 2 mm below the ligation line. Myocardial slices with approximately 2 mm thick were cut and dehydrated with alcohol gradients. The slices were stained either with Masson's trichrome or hematoxylin-eosin (HE). Myocardial cells and collagen fibers were observed using a light microscope (DM750, Leica, Germany).

Echocardiographic assessment of cardiac structure and function

Echocardiography (Vevo 2100, VisualSonics, Canada) was performed as described previously[18]. In short, the rats were fixed on a plate. The probe of the ultrasonic device was coated with the proper coupling agent (YY 0299, Jinya Technology Development Co., Ltd., Tianjin, China). M-mode echocardiography was performed by positioning the M-mode sample line perpendicular to the ventricular septum and the left ventricular posterior wall. Measurement indexes included the following parameters: ejection fraction (EF), fractional shortening (FS), left ventricular end-diastolic volume (LV Vold), interventricular septum thickness at end-diastole (IV Sd), interventricular septum thickness at end-systole (IV Ss), left anterior descending (LAD), left ventricular end-systolic volume (LV Vols), left ventricular posterior wall at end-diastole (LV PWd), and left ventricular posterior wall at end-systole (LV PWs).

Myocardial enzyme activity measurements

After anesthetizing the animal, blood was collected, kept 30 min in a water bath at 37°C, and subsequently centrifuged (Thermo Fisher Scientific, Waltham, USA) at 3,500 rpm for 10 min. The supernatant was extracted and transferred into 600 μL centrifuge tubes, with each tube containing 200 μL. The activities of creatine kinase (CK), creatine kinase-MB (CK-MB), and lactate dehydrogenase (LDH) in the serum were measured (Vertu, Netherlands).

Malondialdehyde (MDA) level and superoxide dismutase (SOD) activity measurements

After anesthesia, blood was collected from the abdominal aortas of the rats. MDA levels and SOD activities were detected using the MDA test kit (BC0025, Solarbio Science & Technology Co., Ltd.) and SOD activity test kit (BC0175, Solarbio Science & Technology Co., Ltd.), respectively.

Measurements of CRP, IL-1β, TNF-α, connective tissue growth factor (CTGF), MMP-2, and MMP-9 expression levels

After the rats were anesthetized, blood was drawn from their abdominal aortas. Serum was collected following high-speed centrifugation and 30 min in a water bath at 37°C. Enzyme-linked immunosorbent assay (ELISA) kits (all Anoric Bio-technology Co., Ltd., Tianjin, China) were used to determine the expression levels of the following proteins: TNF-α (TAE-1103), IL-1β (TAE-959), CRP (TAE-232r), CTGF (TAE-224r), MMP-2 (TAE-904), and MMP-9 (TAE-957). The tests were carried out according to the protocols of the manufacturer.

Statistical analysis

All data are expressed as the mean±standard deviation and analyzed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Differences among groups were analyzed using the one-way ANOVA and were considered statistically significant at $P<0.05$.

Results

Generation of the drug and disease target networks

Based on relevant data from the TCMSP and GENECARD databases, the biological analysis information of traditional Chinese medicines, drugs, targets, and ingredients was imported into Cytoscape 3.7 to visualize them in a "drug–component–target" network diagram (Figure S1, http://links.lww.com/AHM/A16). Potential drug and disease targets were combined and analyzed, and their intersections were determined. A total of 182 overlapping genes between potential targets of TM and VR disease targets were obtained (Figure S1A and B, http://links.lww.com/AHM/A16).

PPI network assessment

The genes that were TM targets and involved in VR were input into the STRING database to analyze their protein-protein interactions, and this PPI network was visualized using Cytoscape 3.7. In Figure S2, http://links.lww.com/AHM/A17, the degree of node relevance is higher when its color is brighter. The MCODE module was used to analyze core targets, including IL-1β, MMP-2, MMP-9, CRP, and other related indicators of inflammation and fibrosis (Figure S2, http://links.lww.com/AHM/A17).

TM effects on the ischemic myocardial area in I/R rats

To study the effects of TM on myocardial infarction, hearts were stained for TTC on days 3 and 28 after drug administration (Figure 1A–C). Compared to that in the I/R group, the infarct size in the TM (4.0 g/kg) group was significantly decreased. However, no significant difference was found between the groups on days 3 and 28. This suggested that TM administration significantly reduced the ischemic areas in I/R rats.

Effects of TM administration on hemodynamic parameters of I/R rats

According to echocardiographic assessments, the EF and FS values were significantly decreased on days 3 and 28 in the I/R group compared with the sham group (Figure 2A and B), whereas LV Vold and LV Vols were significantly increased (Figure 2C and D). Compared to the echocardiographic measurements on day 3, EF and FS on day 28 showed a trend toward decreased values, whereas LV Vold and LV Vols on day 28 had a trend toward increased values. In the TM (1.0 g/kg), TM (2.0 g/kg), and TM (4.0 g/kg) groups, these I/R-induced effects were reversed, suggesting heart function improved.

TM effects on the cardiac structure of I/R rats

The structure of the rat heart was also assessed by echocardiography. The left ventricular internal end-systolic dimension (LV IDs) was significantly increased (Figure 3B), whereas IV Ss and LV PWs were significantly decreased in the 3-day group (Figure 3D and F). In the 28-day group, the parameters left ventricular internal end-diastolic dimension and LV IDs were significantly increased (Figure 3A and B), and IV Sd, IV Ss, LV PWd, and LV PWs were significantly decreased. After 3 or 28 days of continuous drug administration, TM (2.0 g/kg) significantly reduced LV IDs values. This indicated that TM treatment improved the cardiac structure of rats exposed to I/R injury.

Effects of TM on myocardial enzyme activity in I/R rats

Myocardial enzymes, such as CK, CK-MB, and LDH, are important indicators for evaluating myocardial injury. In the early stage of myocardial I/R injury, with these enzymes released from cells, myocardial enzyme activity increases. After a short time, reaching the peak, myocardial enzyme activity gradually returns to baseline values. On day 3, the CK, LDH, and CK-MB activity values were significantly increased in the I/R group (Figure 4), whereas they were significantly decreased in the TM (1.0 g/kg), TM (2.0 g/kg), TM (4.0 g/kg), and TXL groups.

TM effects on MDA levels and SOD activities

We analyzed the MDA levels and SOD activities to assess oxidative stress in rats exposed to I/R injury. Compared with the sham group, the SOD activities in the I/R group on days 3 and 28 were significantly decreased (Figure 5A and B), whereas the MDA levels showed a trend toward increased values. Compared with the I/R group, TM administration prevented these I/R-induced effects, especially at a dose of 2.0 g/kg.

Effects of TM on myocardial histomorphology

The histomorphological changes in I/R rats were observed using HE staining. In the 3-day I/R group, myocardial cell striations disappeared, and the nuclei became pyknotic, dissolved, and disappeared (Figure 6C). The muscle fibers were swollen and broken, and a large number of inflammatory cells infiltrated the tissue. The number of capillaries was reduced, and focal hemorrhage and necrosis were visible (Figure 6A and

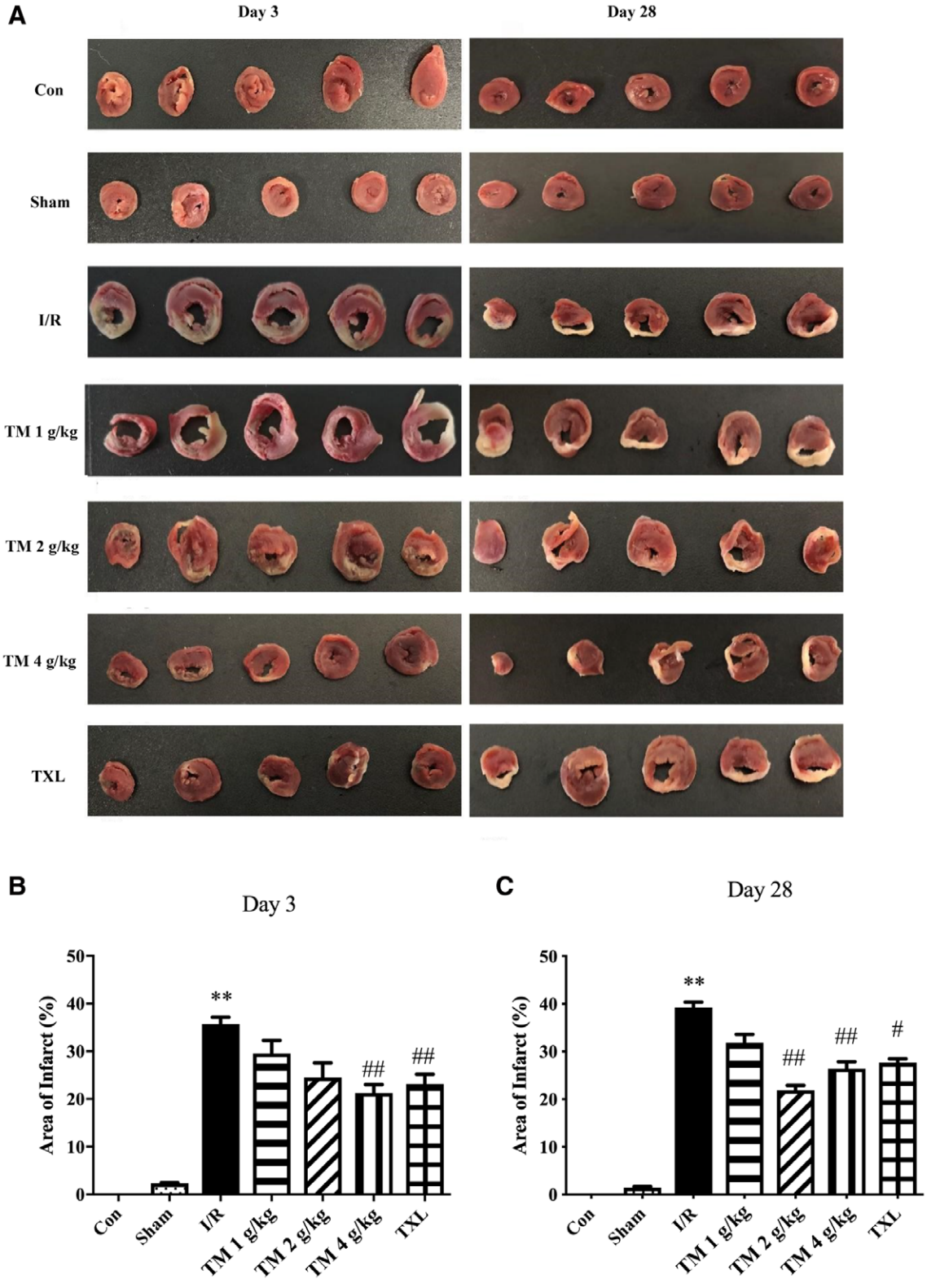

Figure 1 Effect of TM on the ischemic myocardial area in I/R rats. A: TTC staining was performed on days 3 and 28. B–C: Analysis of infarct sizes on days 3 and 28, n=5 rats for each group, $^{**}P<0.01$ versus Sham, $^{\#}P<0.05$, $^{\#\#}P<0.01$ versus I/R. Con: Control group; I/R: Ischemia-reperfusion injury group; TM: Tongmai Yangxin pills group; TTC: Triphenyl tetrazolium chloride; TXL: Tongxinluo capsules group.

B). Administration of TM reduced edema, as well as the density of inflammatory cell infiltration, especially at 2.0 and 4.0 g/kg.

In the 28-day group, inflammatory cells were reduced compared to the 3-day group, large numbers of connective tissues and fibroblasts were produced, and granulation tissue and fibrous tissue hyperplasia were observed (Figure 6B and D). Compared to that in the I/R group, the fibrosis degree in each TM administration group was decreased (Figure 6B and D).

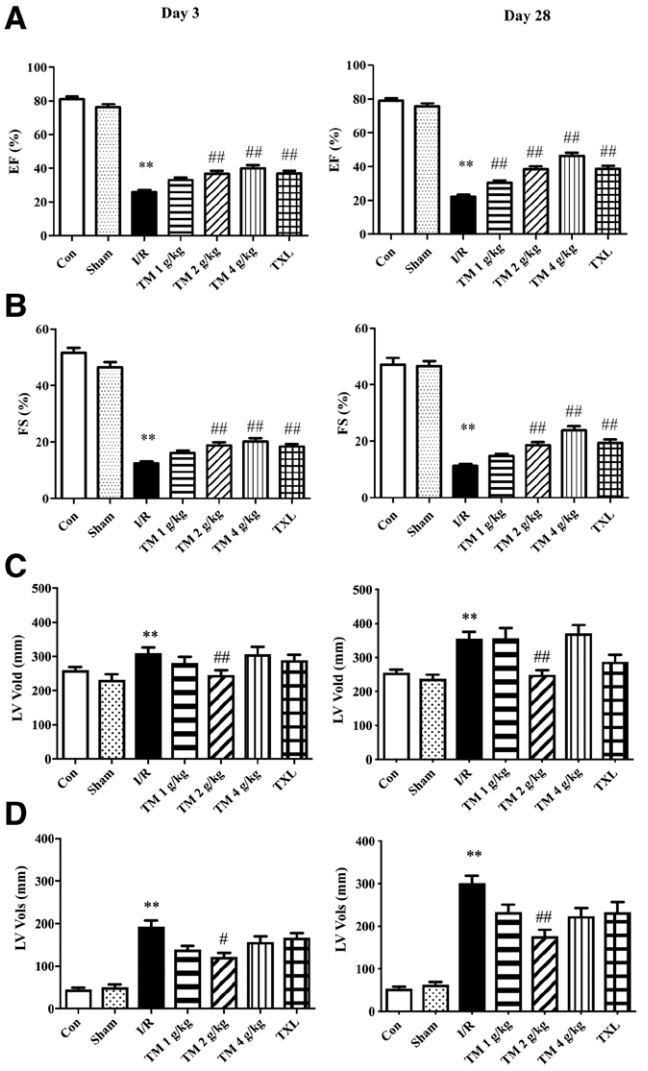

Figure 2 TM effects on hemodynamic parameters of I/R rats. n=18 rats for each group, $^{**}P$<0.01 versus Sham, $^{\#}P$<0.05, $^{\#\#}P$<0.01 versus I/R. Con: Control group; EF: Ejection fraction; FS: Fractional shortening; I/R: Ischemia-reperfusion injury group; LV Vold: Left ventricular end-diastolic volume; LV Vols: Left ventricular end-systolic volume; TM: Tongmai Yangxin pills group; TXL: Tongxinluo capsules group.

Effects of TM administration on myocardial collagen deposition in I/R rats

In the I/R group on day 3, large areas showed inflammatory cell infiltration (Figure 7A), bleeding, necrosis, and edema of myocardial cells were frequently observed (Figure 7C). All treatment groups, especially those with TM at 4.0 g/kg and TXL, exhibited reduced degrees of collagen deposition.

In the 28-day I/R group, large amounts of collagen were observed, and the myocardial tissue structure was seriously damaged (Figure 7B). The numbers of collagen fibers were increased, the myocardial fibers showed disordered shapes, and intercellular space was widened (Figure 7D). The administration of TM significantly improved the fibrosis degree, especially with doses of TM at 2.0 and 4.0 g/kg.

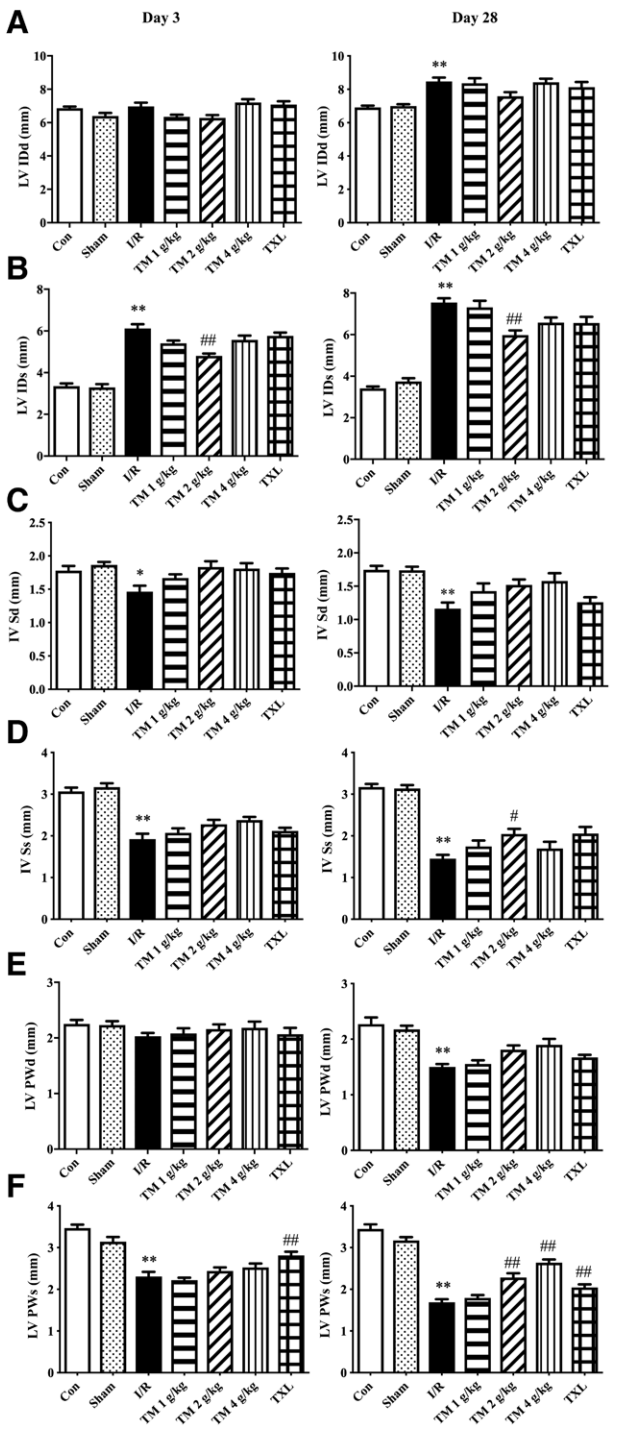

Figure 3 Effects of TM on the cardiac structure of I/R rats. A–F: Echocardiographic analysis of the left ventricular internal diameter at end-diastole (A), left ventricular internal diameter at end-systole (B), interventricular septum thickness at end-diastole (C), interventricular septum thickness at end-systole (D), left ventricular posterior wall at end-diastole (E), and left ventricular posterior wall at end-systole (F). n=18 rats for each group, $^{**}P$<0.01 versus Sham, $^{\#}P$<0.05, $^{\#\#}P$<0.01 versus I/R group. Con: Control group; I/R: Ischemia-reperfusion injury group; IV Sd: Intraventricular septum thickness at end-diastole; IV Ss: Intraventricular septum thickness at end-systole; LV IDd: Left ventricular internal end-diastolic dimension; LV IDs: Left ventricular internal end-systolic dimension; LV PWd: Left ventricular posterior wall at end-diastole; LV PWs: Left ventricular posterior wall at end-systole; TM: Tongmai Yangxin pills group; TXL: Tongxinluo capsules group.

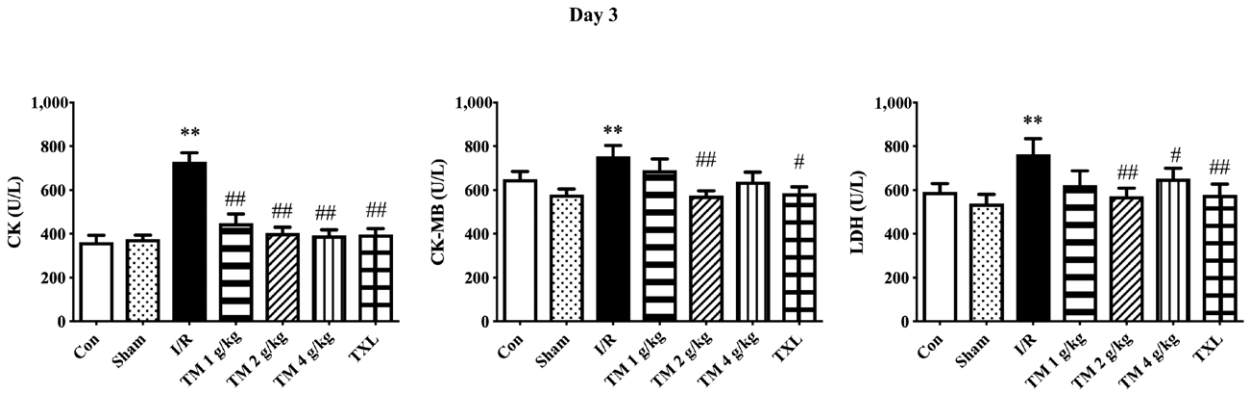

Figure 4 Effects of TM on myocardial enzyme activity in I/R rats. n=18 rats for each group, $^{**}P$<0.01 versus Sham, $^{\#}P$<0.05, $^{\#\#}P$<0.01 versus I/R. CK: Creatine kinase; CK-MB: Creatine kinase-MB; Con: Control group; I/R: Ischemia-reperfusion injury group; LDH: Lactate dehydrogenase; TM: Tongmai Yangxin pills group; TXL: Tongxinluo capsules group.

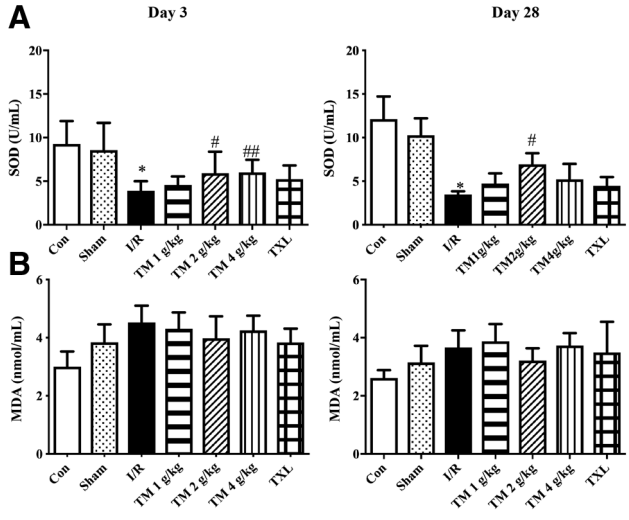

Figure 5 Effects of TM on MDA levels and SOD activities of I/R rats. n=18 rats for each group, $^{**}P$<0.01 versus Sham, $^{\#}P$<0.05, $^{\#\#}P$<0.01 versus I/R. Con: Control group; I/R: Ischemia-reperfusion injury group; MDA: Malonaldehyde; SOD: Superoxide dismutase; TM: Tongmai Yangxin pills group; TXL: Tongxinluo capsules group.

Effects of TM on inflammatory parameters in I/R rats

We measured CRP, TNF-α, and IL-1β levels (Figure 8A–C) to determine the influence of TM on I/R-induced inflammation. The expression levels of CRP, TNF-α, and IL-1β were significantly increased on day 3, but these increases were mitigated by the administration of TM or TXL. Compared to that in the 3-day group, the inflammation in the 28-day group was significantly decreased. These findings indicated that I/R rats had a strong inflammatory reaction on day 3 and that TM administration suppressed this inflammatory response.

TM effects on myocardial fibrosis in I/R rats

Next, we determined whether structural and functional remodeling of hearts from I/R rats was associated with the activation of myocardial fibrosis. The expression levels of CTGF, MMP-2, and MMP-9 in the 28-day I/R group were significantly increased (Figure 9A–C), whereas tissue inhibitor of metalloproteinase 1 (TIMP-1) expression was significantly decreased. Administration of TM or TXL reversed the I/R-induced changes in expression levels of these proteins. Compared to those in the 3-day group, the expression levels of fibrosis-related proteins in the 28-day group were significantly increased. These results implied that myocardial fibrosis was the main cause of I/R in the 28-day group, and TM administration reduced myocardial fibrosis.

Discussion

TM, a traditional Chinese patent medicine, tonifies *qi* and nourishes *yin*. It promotes blood circulation, inhibits inflammation and anti-oxidative processes, and has anti-hypoxic effects on myocardial cells[17]. TM treatment can significantly improve myocardial no-reflow and reduce apoptosis[18–19]. However, the TM effects on VR after myocardial I/R remain unclear. Our study findings suggest that TM can improve VR after myocardial I/R by inhibiting early inflammatory responses and myocardial fibrosis at a later stage.

VR is caused by acute myocardial injury, which includes progressive changes in the infarcted and non-infarcted myocardium. In the early stage of acute myocardial infarction, a large number of inflammatory cells, including macrophages and other antigen-presenting cells, destroy the structure of the collagen scaffold[20]. To maintain the ventricular shape, fibroblasts begin to appear at the site of myocardial injury, and a new collagen matrix is deposited; ultimately, a scar is formed after infarction. Moreover, the left ventricle expands, and wall stress increases, which increases myocardial end-systolic and end-diastolic volumes, the dependence of myocardial preload, and myocardial oxygen demand; finally, myocardial ischemia is aggravated, and the risk area increases[21].

Myocardial enzymes, such as CK, CK-MB, and LDH, are important parameters for the evaluation of myocardial injury. Their activities can reflect the degree of myocardial injury to a certain extent. Early myocardial I/R injury can promote the release of myocardial enzymes from cells, and myocardial enzyme activity increases. After the peak has been reached shortly after disease onset, myocardial enzyme levels gradually return to normal values. After 3 days of myocardial I/R, myocardial enzyme levels were significantly increased in the I/R

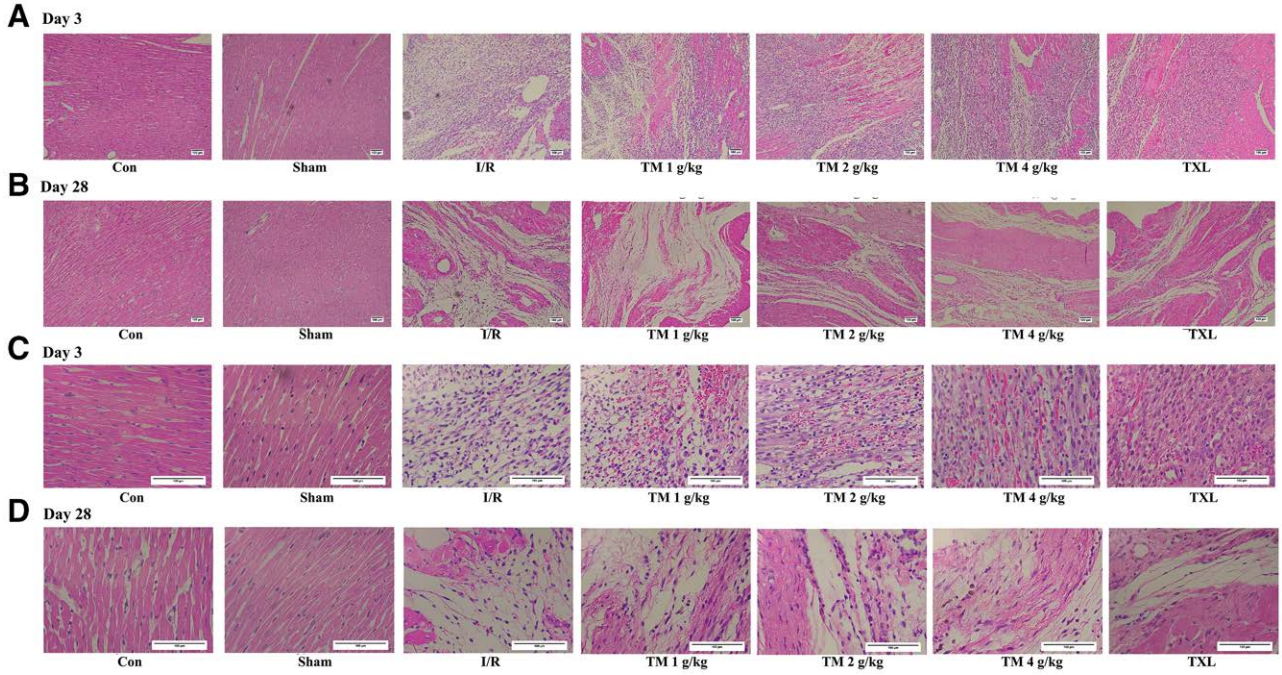

Figure 6 TM effects on the myocardial tissue of I/R rats. A, B: Representative HE staining showing the myocardial morphology of I/R rats on days 3 and 28 (HE ×100). Scale bars: 100μm. C, D: Representative HE staining displaying the myocardial morphology of I/R rats (HE ×400). Scale bars: 100μm. Con: Control group; HE: Hematoxylin-eosin; I/R: Ischemia-reperfusion injury group; TM: Tongmai Yangxin pills group; TXL: Tongxinluo capsules group.

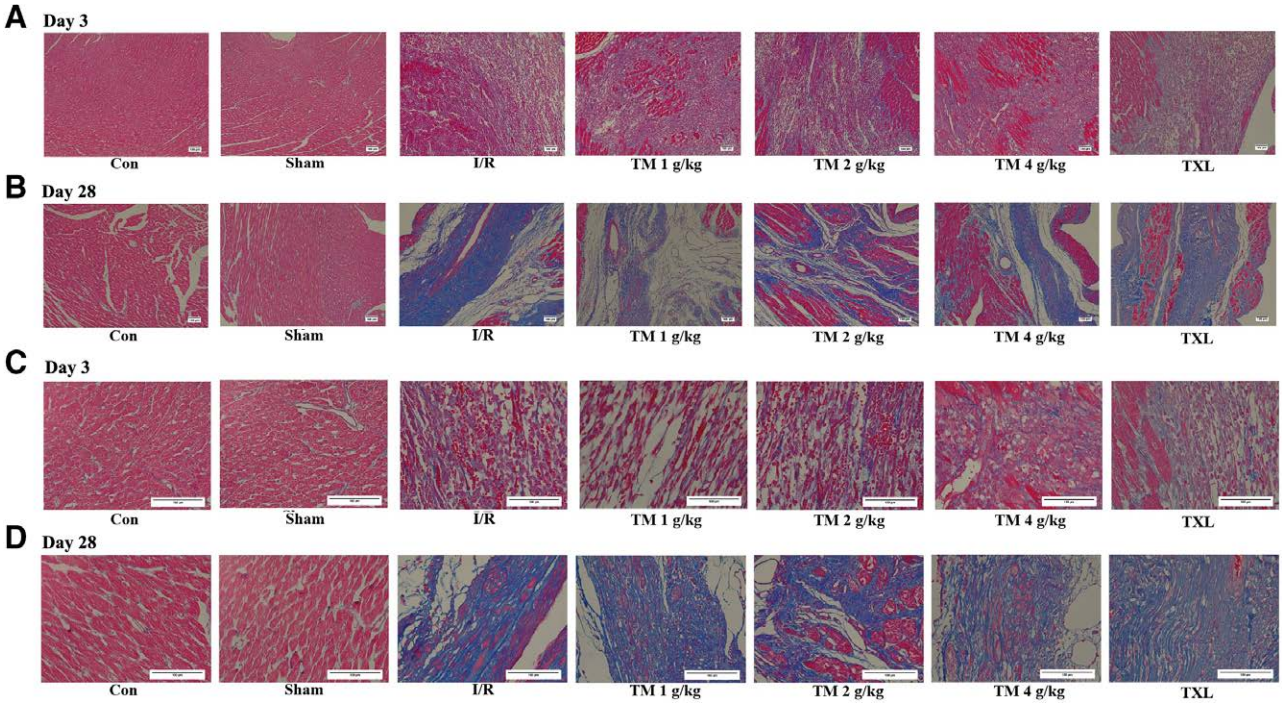

Figure 7 Effects of TM on myocardial collagen deposition in I/R rats. A, B: Representative Masson's trichrome stain showing the myocardial morphology on days 3 and 28 in I/R rats (Masson ×100). Scale bars: 100μm. C, D: Representative Masson's trichrome stain displaying the myocardial morphology of I/R rats (Masson ×400). Con: Control group; I/R: Ischemia-reperfusion injury group; TM: Tongmai Yangxin pills group; TXL: Tongxinluo capsules group.

group, whereas those in each drug group were inhibited to a certain extent. Thus, TM effectively reduced myocardial enzyme activity after 3 days of drug administration.

Under physiological conditions, oxygen free radicals and their scavenging systems maintain a dynamic balance. During myocardial injury, reactive oxygen species are generated in myocardial tissue and accumulate in large quantities. The intracellular content of antioxidant enzymes decreases substantially, and scavenging systems for oxygen free radicals exhibit decreased functionality. These changes increase the levels of MDA and lipid peroxide metabolites and

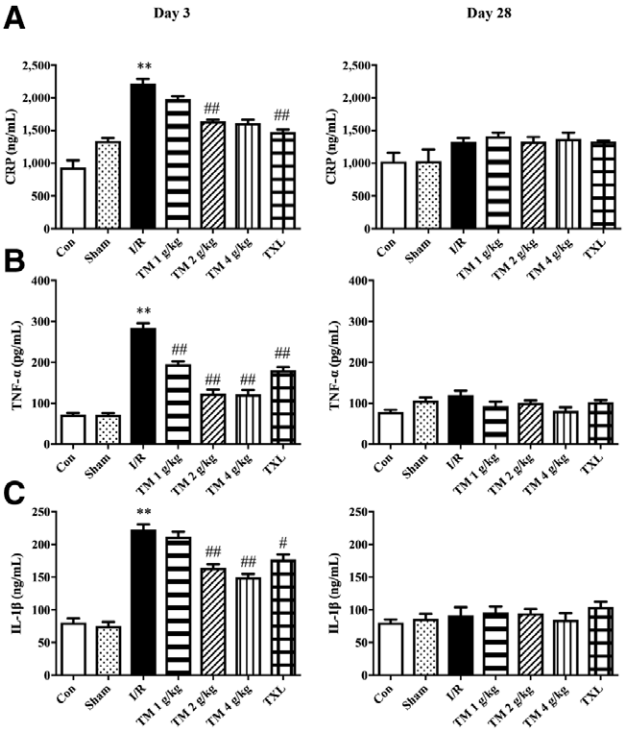

Figure 8 TM effects on inflammatory responses in I/R rats. A–C: Representative quantifications of the inflammation-related proteins CRP (A), TNF-α (B), and IL-1β (C). n=18 rats for each group, $^{**}P$<0.01 versus Sham, $^{\#}P$<0.05, $^{\#\#}P$<0.01 versus I/R. Con: Control group; CRP: C-reactive protein; IL-1β: Interleukin-1β; I/R: Ischemia-reperfusion injury group; TM: Tongmai Yangxin pills group; TNF-α: Tumor necrosis factor-alpha; TXL: Tongxinluo capsules group.

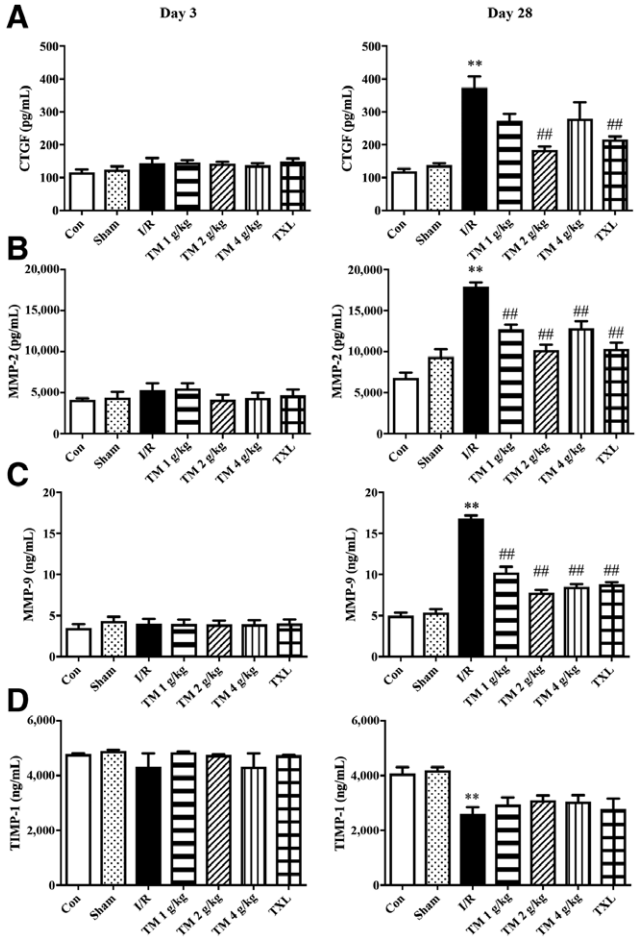

Figure 9 Effects of TM on myocardial fibrosis in I/R rats. A–D: Representative quantification of the myocardial fibrosis-related proteins CTGF (A), MMP-2 (B), MMP-9 (C), and TIMP-1 (D). n=18 rats for each group, $^{**}P$<0.01 versus Sham, $^{\#}P$<0.05, $^{\#\#}P$<0.01 versus I/R. Con: Control group; CTGF: Connective tissue growth factor; I/R: Ischemia-reperfusion injury group; MMP: Matrix metalloproteinase; TIMP-1: Tissue inhibitor of metalloproteinase 1; TM: Tongmai Yangxin pills group; TXL: Tongxinluo capsules group.

damage vascular endothelial and myocardial cells[22]. These changes also lead to a disorder of myocardial energy metabolism. In the current study, TM administration increased SOD activities and decreased MDA levels on days 3 and 28.

During I/R, inflammatory factors like TNF-α and IL-1β can damage cardiomyocytes and mediate other mechanisms, such as oxidative stress, apoptosis, and calcium overload. TNF-α can induce left ventricular hypertrophy and dilation and upregulate MMP-9 and MMP-2 expression[23]. In cardiomyocytes and cardiac tissue, IL-1β can activate and induce the expression of MMPs, including MMP-1, MMP-3, MMP-8, and MMP-9. In turn, MMP-9 can activate IL-1β, thereby forming a positive feedback mechanism[24]. CRP is an acute-phase protein that can stimulate the production of TNF-α and IL-1β and activate MMPs in endothelial cells and macrophages; these effects accelerate VR processes[25]. Compared to the TNF-α, IL-1β, and CRP levels of the 28-day group in the present study, those of the 3-day group were significantly increased, and the administration of TM or TXL reversed these I/R-induced effects. Thus, the main inflammatory reaction was in the early stage of I/R, and TM had a substantial anti-inflammatory effect.

CTGF can promote cell mitosis and induce cytokine chemotaxis, adhesion, and proliferation[26]. Its overexpression can increase the expression levels of MMP-2 and MMP-9[27–28], accelerate the process of cell fibrosis, and increase the secretion of extracellular matrix proteins. Blocking the expression of CTGF can effectively inhibit the secretion of extracellular matrix components[29]. MMP-2 and MMP-9 can rapidly precipitate and degrade various proteins in the myocardium, resulting in disordered collagen in the extracellular matrix and accelerated remodeling of the extracellular matrix. This phenomenon plays an important role in VR[30]. Downregulating the expression levels of MMP-2 and MMP-9 can inhibit left VR and improve cardiac dysfunction. TIMP-1, an inhibitor of matrix metalloproteinases, plays an important role in VR. In patients, the expression levels of MMPs and TIMP-1 were significantly correlated with mortality and the hospitalization rate, as well as heart failure[31]. In our study in rats, CTGF, MMP-2, and MMP-9 levels were significantly increased and TIMP-1 levels were significantly decreased on day 28. Administration of TM or TXL reversed these effects. No significant differences in fibrosis-related parameters were found in the 3-day groups. Therefore, myocardial fibrosis occurred in the late stage of I/R, and TM treatment effectively inhibited myocardial fibrosis.

This study has some limitations. Although we showed that the cardioprotective actions of TM were accompanied by changes in I/R-induced inflammation and fibrosis, improved inflammatory responses, and reduced myocardial fibrosis, mechanistic studies are required to determine the specific pathways in which TM plays an anti-inflammatory role and reduces myocardial fibrosis. We also defined only two time points in our study to clarify the mechanism of TM at different stages of I/R development. Therefore, the precise mechanisms of TM in VR should be elucidated in further studies.

Conclusions

TM can attenuate reperfusion injury-induced VR by inhibiting inflammatory responses in the early stages and myocardial fibrosis in the late stages.

Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

Yi Wang designed the research. Caijun Wang, Qingbo Lyu, Di Jiang, Xinya Ding, Jinpeng Xu, Lin Wang, Yujing Wang, and Kun Zhou performed the experiments and analyzed the data. Rui Chen and Ke Meng wrote the paper. All authors read and approved the final manuscript.

Ethical approval of studies and informed consent

Animal housing and experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and Animal Ethical Committee of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2020023).

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None.

References

[1] Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med* 2007;357(11):1121–1135.
[2] Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. *J Clin Invest* 2013;123(1):92–100.
[3] Florea VG, Mareyev VY, Samko AN, et al. Left ventricular remodelling: common process in patients with different primary myocardial disorders. *Int J Cardiol* 1999;68(3):281–287.
[4] Zornoff LA, Paiva SA, Duarte DR, et al. Ventricular remodeling after myocardial infarction: concepts and clinical implications. *Arq Bras Cardiol* 2009;92(2):150–164.
[5] Nian M, Lee P, Khaper N, et al. Inflammatory cytokines and postmyocardial infarction remodeling. *Circ Res* 2004;94(12):1543–1553.
[6] Hou ZL, Rui X. Clinical observation on 60 cases of angina pectoris of coronary heart disease with deficiency of qi and yin and blood stasis treated by Tongmai Yangxin pill. *World Latest Med Inform* 2019;19(26):132–134.
[7] Xu H. Observation on effect of Tongmai Yangxin pill combined with metoprolol on angina pectoris in patients with coronary heart disease. *Doctor* 2018;3(5):50–51.
[8] Wang YQ, Liu JL, Xu PG, et al. Clinical observation on the treatment of atrial fibrillation with Tongmai Yangxin pill. *Chin J Evid Based Cardiovasc Med* 2020;12(8):988–995.
[9] Di J, Liu L, Huang SH, et al. Tongmai Yangxin Wan for atrial arrhythmias of coronary heart disease: a clinical study. *Chin J Evid Based Cardiovasc Med* 2019;11(7):856–858.
[10] Wang Y, Wang X, Wang J, et al. Tongmai Yangxin intervening in myocardial remodeling after PCI for coronary heart disease: study protocol for a double-blind, randomized controlled trial. *Trials* 2020;21(1):287.
[11] Wu LH, Chen Q, Zhang B. Clinical observation of Tongmai Yangxin pills and compound Danshen tablets in treating 54 cases with chronic stable angina qi-deficiency and blood-stasis syndromes. *Chin J Exp Tradit Med Formul* 2015;21(15):172–175.
[12] Huang XT, Bai BQ, Li ZH, et al. Clinical study of Tongmai Yangxin pills combined with tirofiban in treatment of unstable angina pectoris. *Drugs Clinic* 2021;36(1):59–63.
[13] Tian YJ, Di J. Therapeutic effects of Tongmaiyangxin pill combined with beta blocker on coronary heart disease arrhythmia. *Hebei Med J* 2021;43(1):71–77.
[14] Cui Y, Li C, Zeng C, et al. Tongmai Yangxin pills anti-oxidative stress alleviates cisplatin-induced cardiotoxicity: network pharmacology analysis and experimental evidence. *Biomed Pharmacother* 2018;108:1081–1089.
[15] Cai X, Du J, Li L, et al. Clinical metabolomics analysis of therapeutic mechanism of Tongmai Yangxin pill on stable angina. *J Chromatogr B Analyt Technol Biomed Life Sci* 2018;1100-1101:106–112.
[16] Guo R, Liu N, Liu H, et al. High content screening identifies licoisoflavone A as a bioactive compound of Tongmaiyangxin pills to restrain cardiomyocyte hypertrophy via activating Sirt3. *Phytomedicine* 2020;68:153171.
[17] Wang Y, Zhang L, Xiao Y. The effect of Tongmai Yangxin pill on the inflammatory factors and oxidative stress induced by hypoxia-induced myocardial cells. *Chin J Tradit Chin Med* 2011;52(4):326–328.
[18] Chen R, Chen T, Wang T, et al. Tongmai Yangxin pill reduces myocardial no-reflow by regulating apoptosis and activating PI3K/Akt/eNOS pathway. *J Ethnopharmacol* 2020;261:113069.
[19] Chen R, Chen T, Wang T, et al. Tongmai Yangxin pill reduces myocardial No-reflow via endothelium-dependent NO-cGMP signaling by activation of the cAMP/PKA pathway. *J Ethnopharmacol* 2021;267:113462.
[20] Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. *Circulation* 1990;81(4):1161–1172.
[21] McKay RG, Pfeffer MA, Pasternak RC, et al. Left ventricular remodeling after myocardial infarction: a corollary to infarct expansion. *Circulation* 1986;74(4):693–702.
[22] Gao ZH, Lu SH, Zhao QT. Active ingredients and mechanism of traditional Chinese medicine for protection of myocardial ischemia. *Chin J Tradit Chin Med Pharm* 2015;30(1):170–173.
[23] Siwik DA, Colucci WS. Regulation of matrix metalloproteinases by cytokines and reactive oxygen/nitrogen species in the myocardium. *Heart Fail Rev* 2004;9(1):43–51.
[24] Iyer RP, de Castro Brás LE, Jin YF, et al. Translating Koch's postulates to identify matrix metalloproteinase roles in postmyocardial infarction remodeling: cardiac metalloproteinase actions (CarMA) postulates. *Circ Res* 2014;114(5):860–871.
[25] Tron K, Manolov DE, Röcker C, et al. C-reactive protein specifically binds to Fcgamma receptor type I on a macrophage-like cell line. *Eur J Immunol* 2008;38(5):1414–1422.
[26] Toda N, Mukoyama M, Yanagita M, et al. CTGF in kidney fibrosis and glomerulonephritis. *Inflamm Regen* 2018;38(1):14.
[27] Song B, Zhang ZZ, Zhong JC, et al. Loss of angiotensin-converting enzyme 2 exacerbates myocardial injury via activation of the CTGF-fractalkine signaling pathway. *Circ J* 2013;77(12):2997–3006.
[28] Au CG, Butler TL, Sherwood MC, et al. Increased connective tissue growth factor associated with cardiac fibrosis in the mdx mouse model of dystrophic cardiomyopathy. *Int J Exp Pathol* 2011;92(1):57–65.

[29] Koitabashi N, Arai M, Kogure S, et al. Increased connective tissue growth factor relative to brain natriuretic peptide as a determinant of myocardial fibrosis. *Hypertension* 2007;49(5):1120–1127.
[30] Tarin C, Lavin B, Gomez M, et al. The extracellular matrix metalloproteinase inducer EMMPRIN is a target of nitric oxide in myocardial ischemia/reperfusion. *Free Radic Biol Med* 2011;51(2):387–395.
[31] Ferreira JM, Ferreira SM, Ferreira MJ, et al. Circulating biomarkers of collagen metabolism and prognosis of heart failure with reduced or mid-range ejection fraction. *Curr Pharm Des* 2017;23(22):3217–3223.

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