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Atorvastatin improves the cardiac function of rats after acute myocardial infarction through ERK1/2 pathway

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Abstract. – OBJECTIVE: To study the regulatory effect of atorvastatin (ATV) on the extracellular signal-regulated kinase (ERK) 1/2 pathway and explore its effect on acute myocardial infarction (AMI) rats.

MATERIALS AND METHODS: The rat model of AMI was established, and the model rats were randomly divided into AMI group and ATV-AMI group, and Sham group was also set up. At 4 weeks after successful modeling, the cardiac function indexes of Sprague-Dawley (SD) rats were detected via magnetic resonance imaging (MRI) and echocardiography (ECG). After the rats were executed, the left ventricular weight index (LVWI) was measured, and the myocardial damage was detected via hematoxylin-eosin (HE) staining and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining. Moreover, the messenger ribonucleic acid (mRNA) expressions of collagen I and collagen III in myocardial tissues were detected via Real Time-Polymerase Chain Reaction (PCR), and the expressions of ERK1/2 pathway-related proteins in myocardial tissues were detected via Western blotting.

RESULTS: After administration of ATV for AMI, the fractional shortening (FS%) and ejection fraction (EF%) were significantly restored. Compared with that in ATV-AMI group, LVWI was significantly increased in AMI group ($p<0.05$), indicating that ATV could improve the cardiac function after AMI. The results of HE staining and TUNEL staining showed that ATV-AMI group had a slighter myocardial damage and significantly lower apoptosis rate than AMI group, indicating that ATV could reverse AMI through the ERK1/2 pathway. Besides, the mRNA expressions of collagen I and collagen III were higher in AMI group and ATV-AMI group than those in Sham group ($p<0.05$), while they were significantly lower in ATV-AMI group than those in AMI group ($p<0.05$). The expressions of ERK1/2 pathway-related proteins were also higher in AMI group and ATV-AMI group than those in Sham group ($p<0.05$).

CONCLUSIONS: ATV can significantly improve the cardiac function of SD rats after AMI, whose mechanism is related to the expression of the ERK1/2 pathway.

Key Words:
Atorvastatin, ERK1/2 pathway, Acute myocardial infarction.

Introduction

The acute myocardial infarction (AMI) is manifested as myocardial ischemic necrosis, inability to exert normal myocardial systolic and diastolic function, and blockage, dramatic decline or forced interruption of coronary blood flow, seriously affecting the normal myocardial function and leading to severe myocardial ischemia[1,2]. AMI is mainly caused by coronary sclerosis and stenosis, decline and blockage of blood flow, leading to a mottled rupture of the coronary artery, which will result in a variety of secondary reactive diseases in the severe stage and even necrosis finally[3]. AMI is characterized by the acute onset, serious consequences, and a high mortality rate, seriously threatening people's health[4,5]. Clinical studies[6,7] have demonstrated that statins are able to improve the myocardial cell function, resist thrombosis and stabilize atherosclerotic plaques

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after MI. Atorvastatin (ATV) is an effective statin lipid-lowering drug inhibiting the hydroxymethylglutaryl coenzyme A reductase, which not only resists high-lipid diseases, but also significantly reduces the production of inflammatory cytokines, affects the cell proliferation, protects the vascular endothelial function and prevents the occurrence and development of cardiovascular diseases[8,9].

The extracellular signal-regulated kinase (ERK)1/2 pathway is involved in a variety of regulatory processes during the vital process, including the regulation of MI and ventricular remodeling, which is an important signal transduction pathway in myocardial regulation and plays a great role in the occurrence and development of MI[10,11]. Tiefenbacher et al[12] proved that fluvastatin can significantly reduce the MI area and alleviate the adverse reactions of MI through the partial inhibition of inflammation and endothelial dysfunction. Studies have demonstrated that statins can block the transduction of ERK1/2 signaling pathway in myocardial cells[13], thereby regulating MI. In the treatment of AMI, ATV can affect the RhoA/ROCK/ERK pathway through the improvement of the mesenchymal stem cell transplantation[8]. In addition, ATV can affect the expression of ERK1/2 in the genomic pathway, and play a role by regulating related genes or proteins in the pathway. Moreover, reports have shown that ATV inhibits the proliferation of cardiac fibroblasts[14], prevents the heart from being wrapped by excessive cardiac fibers and also prevents the increased heart burden, as well as the occurrence and deterioration of myocardial diseases. The expression of ERK1/2 in cardiac fibroblasts has an influence on MI, indicating its regulatory effect on MI. In the present study, the cardiac function indexes and the expressions of ERK1/2 pathway-related proteins were detected after the intervention in the AMI model, the effect of ATV on AMI rats through the ERK1/2 pathway was observed, the effect of statins in the ERK1/2 pathway on AMI and its regulatory mechanism were also explored to provide important experimental basis for the treatment of MI with statins, and offer theoretical and experimental references for the subsequent research on the treatment of MI with statins.

Materials and Methods

Establishment of Animal Model

A total of 30 male Sprague-Dawley (SD) rats weighing 220-280 g were purchased and fed adaptively for 1 week. Then 20 SD rats were randomly selected, the left anterior descending coronary artery was permanently ligated, and the operation was performed under aseptic conditions to establish the rat model of AMI. The above rats were randomly divided into AMI group and ATV-AMI group. In addition, the remaining 10 non-ligated SD rats were taken as Sham group. After the operation, rats in ATV-AMI group were gavaged with ATV solution (10 mg/kg), while those in AMI group and Sham group were administrated with the same amount of normal saline. This investigation was approved by the Animal Ethics Committee of the Shenzhen Longgang E.N.T. Hospital Animal Center.

Determination of Cardiac Function and Left Ventricular Weight Index (LVWI) of Rats

After routine feeding for 4 weeks, the left ventricular function of each rat was determined *via* magnetic resonance imaging (MRI) and echocardiography (ECG) under the probe frequency of 10 MHz, and the cardiac function indexes were recorded. Then, the rats were executed, and the body weight (BW) of rats was measured. The heart was quickly dissected under aseptic conditions, and the left ventricular weight (LVW) was measured: LVWI=LVW/BW (mg/g).

Hematoxylin-Eosin (HE) Staining

The heart dissected was soaked in formalin for 7 d, washed with running water for 24 h, dehydrated with gradient alcohol, routinely prepared into sections, deparaffinized and hydrated with 95%, 90%, 80%, 75%, and 50% ethanol, followed by transparentization and paraffin embedding. Then the paraffin blocks were prepared into pathological sections, baked dry, stained with HE and sealed, followed by observation under a light microscope.

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling (TUNEL) Apoptosis Assa

The myocardial apoptosis was detected using the paraffin sections according to the instructions of the TUNEL apoptosis assay kit (Roche, Basel, Switzerland). The sealed sections were labeled with the fluorescence developing agent, and the FITC-labeled TUNEL-positive cells were imaged at 530 nm under a fluorescence microscope. Finally, the TUNEL-positive cells were counted in 10 fields of view.

Table I. PCR primers.

mRNA	Sequence
Collagen I	F: 5'-ATCAGCCCAAACCCCAAGGAGA-3' R: 5'-CGCAGGAAGGTCAGCTGGATAG-3'
Collagen III	F: 5'-TGATGGGATCCAATGAGGGAGA-3' R: 5'-GAGTCTCATGGCCTTGCGTGTTT-3'
GAPDH	F: 5'-TGACTTCAACAGCGACACCCA-3' R: 5'-CACCCTGTTGCTGTAGCCAAA-3'

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The total RNA was extracted from the myocardial tissues of rats in each group using TRIzol (Invitrogen, Carlsbad, CA, USA) and reversely transcribed into complementary deoxyribose nucleic acid (cDNA), after the RNA purity and concentration were determined (note the use of isopropanol). The primer sequences of the target gene and internal reference glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Table I) were designed according to the GenBank, and the expression levels of target genes were detected *via* qRT-PCR. The mRNA expressions of collagen I and collagen III in myocardial tissues in each group were calculated by $2^{-\Delta\Delta Ct}$.

Western Blotting

The heart tissues of rats were cut into pieces and added with the radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) for tissue homogenization. After the protein was extracted, the total protein concentration in myocardial tissues was determined using the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA). Then, the protein was loaded, subjected to electrophoresis, transferred onto the membrane, sealed and incubated with the rabbit anti-ERK1/2 primary antibody overnight and the secondary antibody for 1 h. After that, the protein band was scanned and quantified using the Odyssey scanner, and the level of protein to be detected was corrected using GAPDH. Finally, the Western blotting bands were quantified using the Image Lab software, and the expression levels of the corresponding proteins in each group were measured.

Statistical Analysis

All raw data obtained in the experiments were statistically analyzed using Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA), and expressed as mean ± standard deviation ($\bar{x}\pm s$). Univariate analysis was performed for the data between two groups. $p<0.05$ suggested that the difference was statistically significant.

Results

Cardiac Function of Rats in Each Group

According to the detection results of cardiac function indexes in AMI group, ATV-AMI group and Sham group (Table II and Figure 1), the fractional shortening (FS%) and the ejection fraction (EF%) were significantly lower in AMI group and ATV-AMI group than those in Sham group, while the left ventricular end-diastolic diameter (LVEDd) and left ventricular end-systolic diameter (LVESd) were significantly larger in AMI group and ATV-AMI group than those in Sham group ($p<0.05$), indicating that the AMI model was successfully established. After the intervention with ATV, both FS (%) and EF (%) were higher in ATV-AMI group than those in AMI group ($p<0.05$), indicating that ATV could improve the ventricular remodeling after AMI.

LVWI of Rats

The relative left ventricular weight in AMI group and ATV-AMI group was significantly increased compared with that in Sham group ($p<0.05$), and the left ventricular thickness was also increased in AMI group and ATV-AMI group. Both LVW and LVW/BW significantly declined in ATV-AMI group compared with those in AMI group ($p<0.05$) (Table III and Figure 2).

Table II. Hemodynamic and cardiac function indexes of rats determined via MRI&ECG ($\bar{x}\pm s$).

Group	MRI&ECG ($\bar{x}\pm s$).			
	LVEDd (mm)	LVESd (mm)	EF (%)	FS (%)
Sham	5.13±0.57	3.01±0.42	57±3.1	49.01±3.0
AMI	7.17±0.57*	6.27±0.72*	48±2.4*	36.4±2.3*
ATV-AMI	6.48±0.65#	5.41±0.75#	50±2.2#	41.2±1.8#

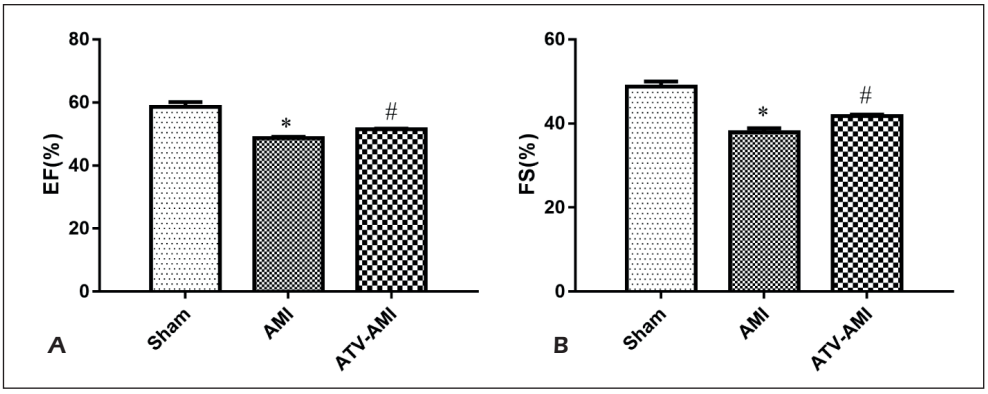

Figure 1. Cardiac function indexes EF *(A)* and FS *(B)* of rats detected via MRI&ECG. Both FS (%) and EF (%) are higher in ATV-AMI group than those in AMI group ($p<0.05$). *#$p<0.05$ *vs.* Sham group.

Table III. LVWI of rats ($\bar{x}\pm s$).

Group	LVW (mg)	LVW/BW (mg/g)
Sham	601.36±104.21	2.07±0.11
AMI	749.13±110.03*	3.11±0.13*
ATV-AMI	671.25±100.34#	2.56±0.31#

Note: LVWI significantly declines in ATV-AMI group compared with that in AMI group. *#$p<0.05$ vs. Sham group

HE Staining of Myocardial Tissues

The morphology and damage of myocardial tissues in each group were detected *via* HE staining. As shown in Figure 3, the normal myocardial cells were arranged orderly without fibrosis in Sham group (Figure 3A), the myocardial cells were arranged disorderly with muscle fiber thickening in AMI group (Figure 3B), and the myocardial damage after MI was alleviated after the administration of ATV (Figure 3C).

Myocardial Apoptosis of Rats Detected via TUNEL Stainin

The apoptosis level of myocardial tissues in each group was detected *via* TUNEL staining. As shown in Figure 4, there was almost no apoptosis in Sham group (Figure 3A), apoptosis occurred in a large number of myocardial cells in AMI group (Figure 3C), and myocardial apoptosis was significantly reduced in ATV-AMI group after the treatment with ATV (Figure 3B).

mRNA Expressions of Collagen I and Collagen III in Heart Tissues

The mRNA expressions of collagen I and collagen III in AMI group and ATV-AMI group were higher than those in Sham group ($p<0.05$), and the collagen deposition was increased after MI. Moreover, the mRNA expressions of collagen I and collagen III in ATV-AMI group were significantly lower than those in AMI group, and the collagen deposition was markedly weakened ($p<0.05$) (Figure 5).

Regulatory Effect of ATV on ERK1/2 Pathway Protein Level

The expression level of ERK1/2 pathway protein in myocardial tissues in each group was detected *via* Western blotting. It was found that the expression of ERK1/2 pathway protein in AMI group was remarkably higher than that in Sham group ($p<0.05$), and the ERK1/2 protein expression was increased after MI in AMI group. Compared with that in AMI group, the expression of ERK1/2 protein in ATV-AMI group was remarkably decreased ($p<0.05$) (Figure 6).

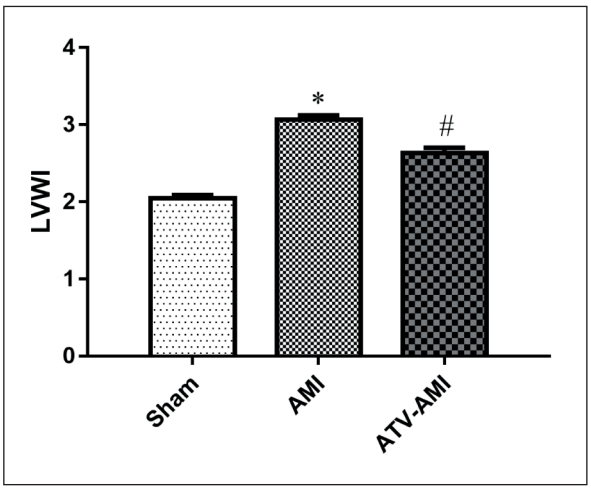

Figure 2. LVWI of rats in each group. LVW/BW is significantly increased in AMI group and ATV-AMI group. *#$p<0.05$ *vs.* Sham group.

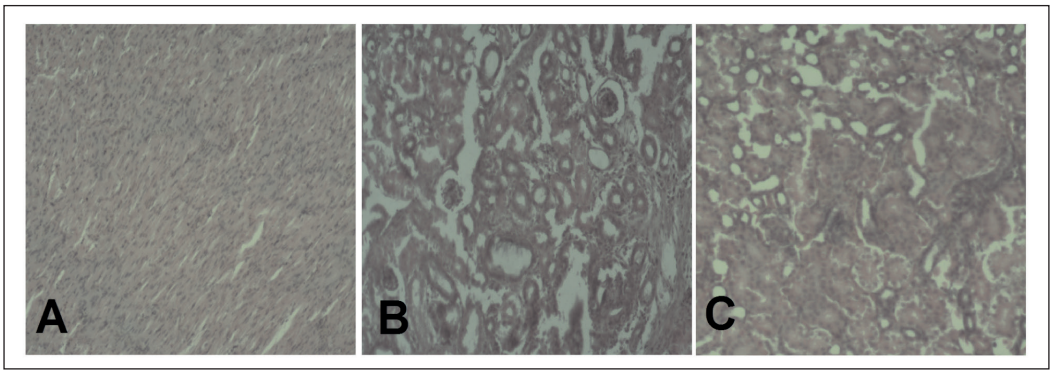

Figure 3. HE staining of rat's heart. ***A***, Sham group, ***B***, AMI group, ***C***, ATV-AMI group. The myocardial morphology is normal in Sham group, and the myocardial damage caused by AMI is reversed in ATV-AMI group (magnification: 200×).

Discussion

AMI seriously affects the normal myocardial function, which will lead to a variety of secondary reactive diseases in the severe stage, bringing the burden on individuals, families, and society, harming the development of social welfare for the aged. Therefore, an early and accurate diagnosis is the key to successful treatment and improvement of prognosis. Cardiovascular risk events are reduced by percutaneous coronary intervention and postoperative drug maintenance. In 1987, the introduction of hydroxymethylglutaryl coenzyme A reductase inhibitors (statins)[15] was a significant

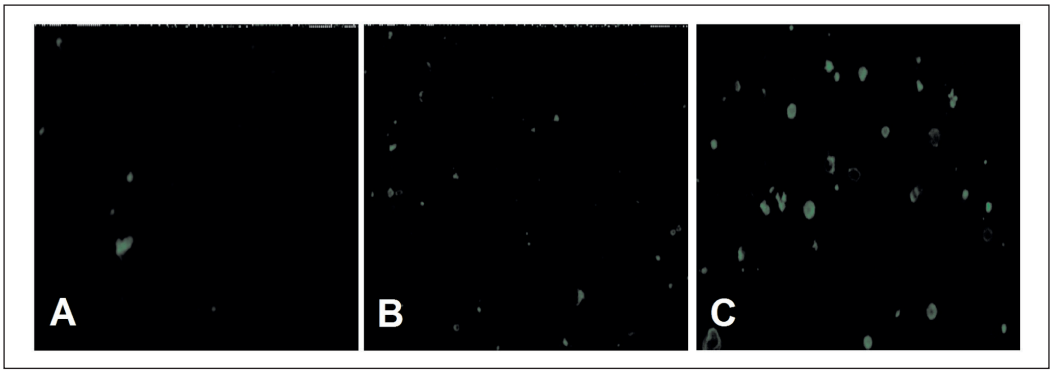

Figure 4. Apoptosis level detected via TUNEL staining, ***A***, Sham group, ***B***, ATV-AMI group, ***C***, AMI group. Apoptosis occurs in a large number of myocardial cells in AMI group, while it is significantly reduced in ATV-AMI group.

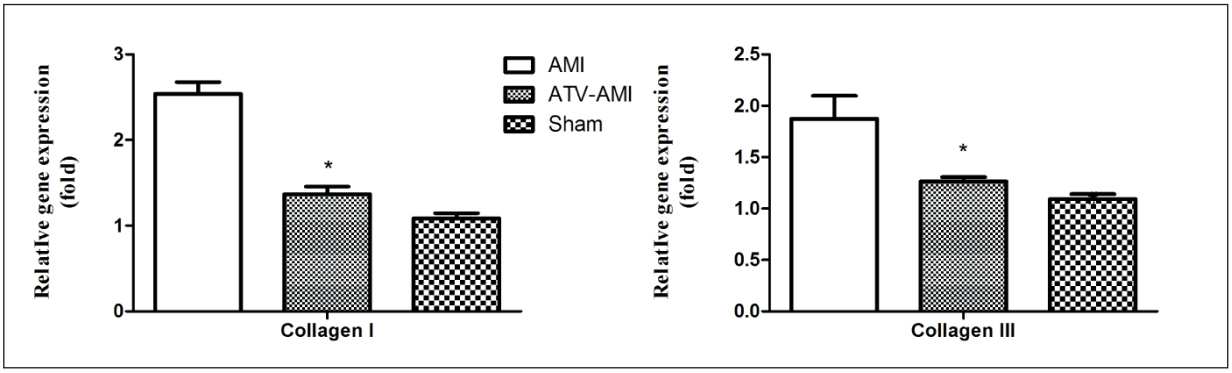

Figure 5. mRNA expressions of collagen I and collagen III in heart tissues detected via RT-PCR. The collagen deposition is significantly weakened in ATV-AMI group (*p<0.05).

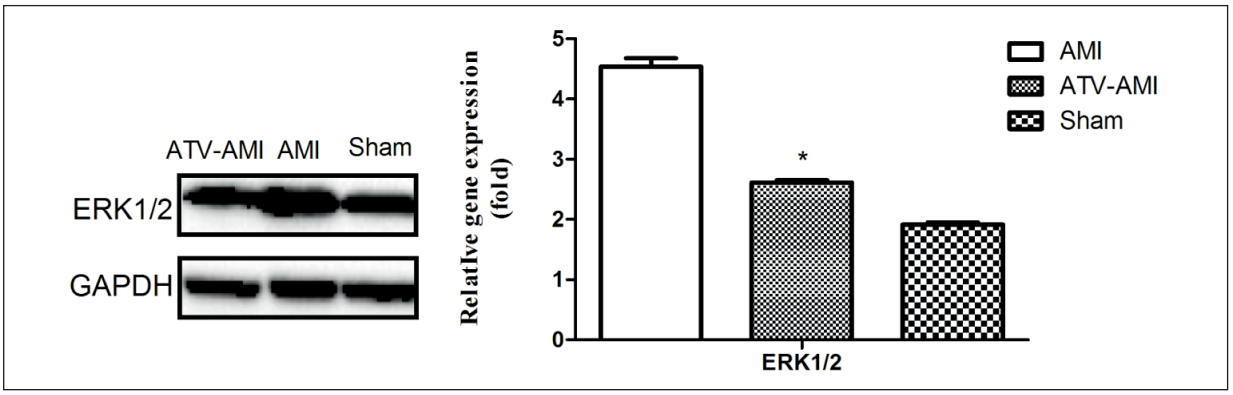

Figure 6. ERK1/2 protein level in heart tissues of MI rats detected via Western blotting. The expression of ERK1/2 protein in ATV-AMI group is remarkably decreased compared with that in AMI group.

progress in the prevention and treatment of cardiovascular diseases, bringing good news for patients and their families. Statins are cholesterol-lowering drugs with a variety of biological activity, which have been proved to possess various cardiovascular effects and can significantly improve the cardiac function and prognosis of patients with heart failure[16], but its regulatory mechanism remains unclear, hindering the improvement in treatment and prognosis of patients, so it is necessary to further study its potential mechanism. Merla et al[17] have manifested that the excessive proliferation of cardiac fibroblasts plays an important role in the myocardial remodeling, and may help to prevent heart failure inhibiting the proliferation of cardiac fibroblasts. During this process, ERK1/2 plays a key role, which can effectively inhibit heart failure. Some scholars[14] argued that ATV greatly blocks the aldosterone-induced ERK1/2 expression in the genomic pathway, and it may interfere in the ERK1/2 phosphorylation in cardiac fibroblasts. In addition, according to the studies on statins in adherent human monocytes, statins block the C-reactive protein-induced ERK1/2 phosphorylation[18]. Similarly, ATV treatment can also inactivate the MYC dephosphorylation-related signaling pathways (Ras and ERK1/2)[19]. In primary pulmonary microvascular endothelial cells of rats, simvastatin induces activation of caspase-3 and expression of Rac1, inhibits RhoA and reduces the phosphorylation of protein kinase B and ERK[20]. In this paper, the effect of ATV in the ERK1/2 pathway on AMI was explored. It was found that the genes and proteins in myocardial tissues of rats were changed after MI, and the expressions of collagen I and collagen III were also significantly increased. The main reason is that the proliferation of cardiac fibroblasts and the increased secretion of collagen further lead to changes in target molecules. In the present study, ATV could indeed improve the ventricular remodeling in SD rats after AMI through the ERK1/2 pathway. However, whether the proliferation of cardiac fibroblasts is related to the regulation of ERK1/2 pathway has not been confirmed yet. There is a lack of effective proof, so researchers should spare no effort in a further in-depth study, hoping to explain such a correlation.

Several studies have demonstrated that inflammatory cytokines are involved and play considerable roles in the left ventricular remodeling after AMI. Therefore, anti-inflammatory therapy can improve the ventricular remodeling and cardiac function of MI rats[21]. In the MI model, simvastatin, through regulating the expressions of inflammatory cytokines, significantly improves the left ventricular remodeling, and lowers the left ventricular end-diastolic pressure and left ventricular end-systolic pressure[22], fully confirming the above effect. Moreover, simvastatin also reduces the deposition of collagen I, thereby preventing myocardial fibrosis and cardiac dysfunction[23]. Statins have very potent effects, which can promote the survival of cardiac stem cells after transplantation into the heart after MI, thus improving the cardiac function[24]. However, the mechanism of the protective effect of statins remains to be fully revealed, and there is a lack of important breakthrough research to guide or lead the study on the protective effect of statins. It was pointed out in previous works that statins improve the microenvironment after infarction through inhibiting the RHoA/ROCK pathway[25], thereby promoting survival and therapeutic effect after transplantation. After many years, Liu

et al[26] have confirmed that ERK1/2 is involved in myocardial fibrosis during the RHoA/ROCK downstream cascade, which not only detects that ERK1/2 is the downstream substrate of RHoA/ROCK, but also demonstrates that the inhibitory effect on ERK1/2 is the reason for the protective effect of ATV on the heart[8,27]. It can be seen that ATV can improve the cardiac function through inhibiting the ERK1/2 pathway. Despite this, there are still some shortcomings in this experiment. In the subsequent research, therefore, more cell lines can be introduced and more molecular biological techniques can be adopted, so as to explore the specific protective effect of statins.

Conclusions

We demonstrated that ATV may regulate the heart failure symptoms in AMI rats through the ERK1/2 signaling pathway. Further, collagen I, collagen III, and ERK1/2 pathway-related proteins can serve as detection indexes for the therapeutic effect of AMI patients. This study provides an important experimental basis for the treatment of MI and offers theoretical and experimental references for the subsequent research on the treatment of MI with statins.

Conflict of Interests

The authors declare that they have no conflict of interest.

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