

Original Article

Pathological observation of acute myocardial infarction in Chinese miniswine

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Abstract: The acute myocardial infarction (AMI) model in Chinese miniswine was built by percutaneous coronary artery occlusion. Pathological observation of AMI was performed, and the expression of tumor necrosis factor alpha (TNF- α) in the infarct sites was detected at different days after modeling in Chinese miniswine. The experimental findings may be used as the basis for blood flow reconstruction and intervention after AMI. Seven experimental Chinese miniswine were subjected to general anesthesia and Seldinger right femoral artery puncture. After coronary angiography, the gelfoam was injected via the microtube to occlude the obtuse marginal branch (OM branch). At 1 d, 3 d, 5 d, 7 d, 10 d, 14 d and 17 d after modeling, hematoxylin-eosin (HE) staining was performed to observe the pathological changes and to detect the expression of TNF- α in the myocardial tissues. Cytoplasmic acidophilia of the necrotic myocardial tissues at 1 d after modeling was enhanced, and cytoplasmic granules were formed; at 3 d, the margins of the necrotic myocardial tissues were infiltrated by a large number of inflammatory cells; at 5 d, the nuclei of the necrotic myocardial cells were fragmented; at 7 d, extensive granulation tissues were formed at the margin of the necrotic myocardial tissues; at 10 d, part of the granulation tissues were replaced by fibrous scar tissues; at 14-17 d, all granulation tissues were replaced by fibrous scar tissues. Immunohistochemical detection indicated that no TNF- α expression in normal myocardial tissues. The TNF- α expression was first detected at 3 d in the necrotic myocardial tissues and then increased at 5 d and 7 d. After reaching the peak at 10 d, the expression began to decrease at 14 d and the decrease continued at 17 d. Coronary angiography showed the disappearance of blood flow at the distal end of OM branch occluded by gelfoam, indicating that AMI model was constructed successfully. The repair of the infarcted myocardium began at 10-17 d after modeling with safe blood flow reconstruction. TNF- α expression in the infarcted myocardium was the highest at 10 d, which can be explained by inflammation and repair of the infarcted myocardium.

Keywords: Acute myocardial infarction (AMI), tumor necrosis factor-alpha, Chinese miniswine, animal model

Introduction

Acute myocardial infarction (AMI) is a heart disease with rapid onset, high mortality and high disability rate. Percutaneous coronary intervention (PCI) is the most effective salvage for the ischemic myocardium. However, the hospitals at the grassroots-level are not fully qualified for the performance of emergency PCI, and the best timing for the surgery (12 h after AMI) is missed. Major concerns for the physicians lie in blood flow reconstruction after ischemia and alleviation of ischemia-perfusion injury, which are also hot topics debated throughout the world. Through animal models of AMI, the path-

ological observation of AMI at different stages can be performed, and the TNF- α expression be monitored dynamically so as to find new clues. Chinese miniswine share many similarities in heart anatomy and cardiac blood vessel distribution with human. By coronary occlusion, the ischemic heart disease having similar pathogenesis as in human can be simulated. Therefore, Chinese miniswine are highly preferred for the modeling of cardiovascular diseases. Myocardial inflammation usually ensues after AMI, which affects the subsequent myocardial repair and prognosis. Tumor necrosis factor alpha (TNF- α) is a multi-directional inflammatory factor that is extremely sensitive to myocar-

dial ischemia and strongly regulates the survival and apoptosis of myocardial cells, triggering other inflammatory response. Blood sampling is the conventional method for observing the dynamic changes of TNF- α expression, but the use in the detection of TNF- α expression in AMI is rarely reported. The AMI model in Chinese miniswine was built by occlusion of OM branch with gelfoam. Then pathological observation and detection of TNF- α expression in the necrotic myocardial tissues were performed dynamically and continuously. We attempted to clarify the relationship between the timing of blood flow reconstruction after AMI, TNF- α expression in infarcted myocardium and myocardial repair.

Subjects and methods

Subjects

Eight Chinese miniswine weighted 13-18 kg were provided by Taizhou Taihe Biotechnology Co., Ltd and passed the quarantine inspection (quarantine certificate No. 3205197447, license No. SCXK (Jiangsu) 2011-0002. The Chinese miniswine were all males in the same litter, and those having poor health, bad appetite and diseased recently were excluded.

Materials

Anesthetics: Diazepam injection (Harbin Pharmaceutical Group Holding Co., Ltd), ketamine (Jiangsu Hengrun Pharmaceutical Co., Ltd), lidocaine (Jiangsu Pharmaceutical Group Xinzheng Holding Co., Ltd); contrast agent: iopromide (Bayer Healthcare Company Limited Guangzhou Branch); Reagents for HE staining: dimethylbenzene (Tianjin Yongda Chemical Reagent Co., Ltd), anhydrous alcohol (Tianjin Yongda Chemical Reagent Co., Ltd), formaldehyde (Shanghai Ruji Biotechnology Development Co., Ltd), eosin (Tianjin Kermel Chemical Reagent Development Center), hematoxylin (Shanghai Shanpu Chemical Co., Ltd), TNF- α ELISA kit (ABCAM, USA, item number AB1793); Materials for blood vessel occlusion: gelfoam (Guangzhou Yanda Trade Co., Ltd); defibrillator (TEC-5521C, NIHON KOHDEN CORP., Japan); digital subtraction angiograph (DSA) (PHILIPS-MML19); ECG monitor (Philips Medizin Systeme BoebliGen GmbH German M8001A); suction machine (TE-A, Yuyue Medical Equipment Co., Ltd); electro-thermostatic drying oven (202-1,

Tianjin Taisite Instrument Co., Ltd), glass slide (Helen, 25 mm \times 7.5 mm, thickness 1-1.2 mm), coverslip (0.13-0.17 mm, Yancheng Xintai Medical Instrument Factory); embedding machine (PPDB/21, Hubei Xiangfan Leike Electrical Instrument Factory); paraffin refrigerator (PPDB/21, Hubei Xiangfan Leike Electrical Instrument Factory), microtome (LEICA RM2235); digital electronic microscope (NIKON ECLIPSE 80i); interventional materials: 6 F arterial sheath, 6FJR, microtube, guide wire, J-shaped tip guide wire (all medical wastes that had been disinfected by epoxyethane); Alternate drugs: heparin (Shandong LuKang cisen Pharmaceutical Co., Ltd), adrenalin (Tianjin Pharmaceutical Group), dopamine (Shanghai Hefeng Pharmaceutical Co., Ltd), nikethamide (Tianjin Pharmaceutical Group), atropine (Furen Pharmaceutical Group Xinyang Co., Ltd), potassium chloride (Beijing Yimin Pharmaceutical Co., Ltd), trinitroglycerol (Beijing Yimin Pharmaceutical Co., Ltd), dexamethasone pins (Kaifeng Pharmaceutical Group Co., Ltd).

AMI modeling

Anesthesia: Chinese miniswine were fasted from food for 12 h and fasted from water for 8 h. Anesthesia was induced by intramuscular injection of ketamine at 10 mg/Kg. After cleaning, the four limbs of the Chinese miniswine were fixed to the wood plank and the miniswine were placed on the catheter bed. Oxygen was supplied at the rate of 3 L/min, and skin preparation was performed on the chest and the four limbs. Under continuous ECG monitoring, venous access was made at the marginal ear vein using a cannula needle, and maintained throughout the operation, and defibrillator and suction machine were used d 1 ml diazepam and 10 mg ketamine were injected. Anesthesia was when necessary.

Seldinger right femoral artery puncture was performed to build AMI model: Disinfection with iodophor and draping were performed conventionally; at the site of strongest pulse in the right femoral artery, lidocaine was injected for local anesthesia. After puncture, bright fresh arterial blood squirted out from the tail of the needle. Then the guide wire was inserted, and 6 F arterial sheath was delivered along the guide wire. Pellet injection of 1000 U heparin was performed, and 2000 U heparin was given additionally every half hour during the opera-

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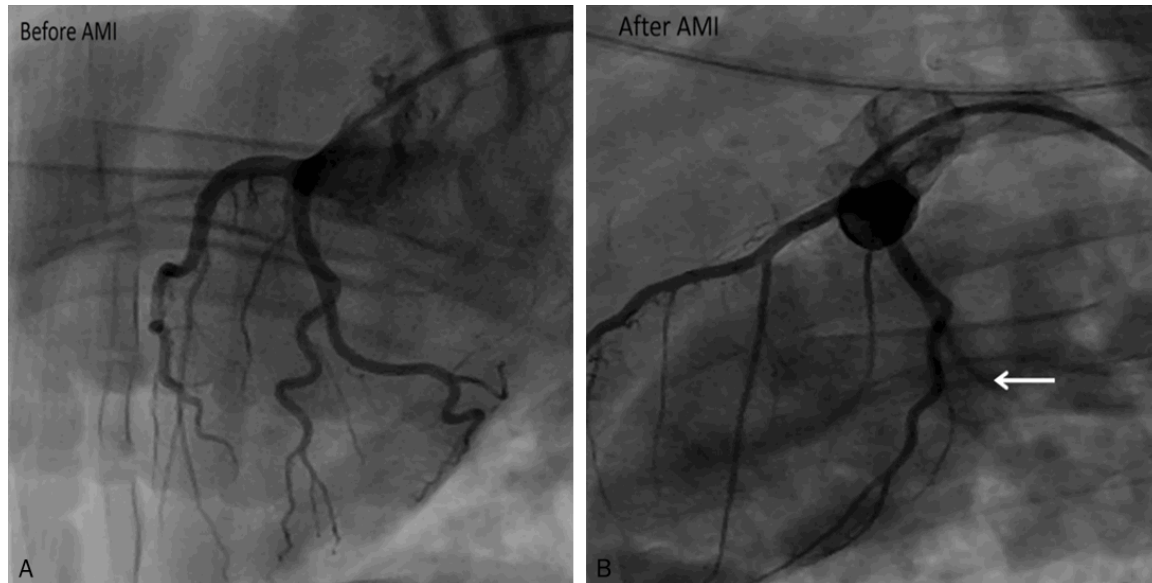


Figure 1. Coronary angiography of Chinese small porcine before and after AMI.

tion; While using the J-shaped tip guide wire, Guide wire was inserted to the root of the ascending aorta. Left and right coronary angiography was carried out in multiple positions. ECG showed sinus rhythm, 160-200 beats/min, blood pressure 160/130 mmHg. After the distal end of the Runthrough wire reached the proximal end of OM branch, the guide wire was removed. Gelfoam suspension (dissolved in iopromide) was injected via the microtube. Angiography revealed that the blood flow in the distal end of the OM branch disappeared and the microtube was removed. Vital signs and oxygen saturation were monitored; After operation, 6 F arterial sheath was removed, and subclavian vein catheter was indwelled. The incision of the femoral artery was closed; the needles were sealed with heparin, fixed at the operated site and covered with film. Chinese miniswine were sent back to the breeding center and bred separately. Measures were taken to avoid infection.

Preparation of pathological sections of necrotic myocardial tissues

Whole hearts were harvested at 1 d, 3 d, 5 d, 7 d, 10 d, 14 d and 17 d after modeling, respectively. After fixation in 4% formaldehyde, 4 blocks of infarcted myocardium and 1 block of normal myocardium were collected from the lateral wall of the left ventricle, respectively. The tissues were subjected to dehydration, par-

affin embedding, sectioning and dewaxing. Half of the tissues were examined by HE staining. The slices were sealed, observed under the 40 × digital electronic microscope, and photos were taken. The remaining half was subjected to immunohistochemical detection of TNF- α expression. Mouse anti-swine monoclonal antibodies were added as primary antibodies, and universal IHC detection kit with rabbit anti-mouse antibodies as secondary antibodies was used. The cells were cultured at room temperature with 3% H₂O₂ for 10 min to deactivate endogenous peroxidase. After washing with distilled water, the cells were soaked in PBS for three times, 5 min per time, and antigen retrieval was done by heating in citric acid. The cells were sealed with 10% normal goat serum (diluted with PBS) and cultured at room temperature for 10 min. The serum was decanted and working solution of primary antibodies (diluted 1:200) was added dropwise to culture the cells at 37°C and at 4°C overnight. Later, the cells were washed with PBS for three times, 5 min per time. A proper amount of working solution of secondary antibodies labeled with biotin was added and cultured at 37°C for 15 min. This was followed by washing with PBS for 5 min, three times. Working solution of streptavidin labeled with alkaline phosphatase was added to culture the cells at 37°C for 20 min. After washing with PBS for three times, 5 min per time, DAB reagent was added for color development for 6 min. Finally, the cells were

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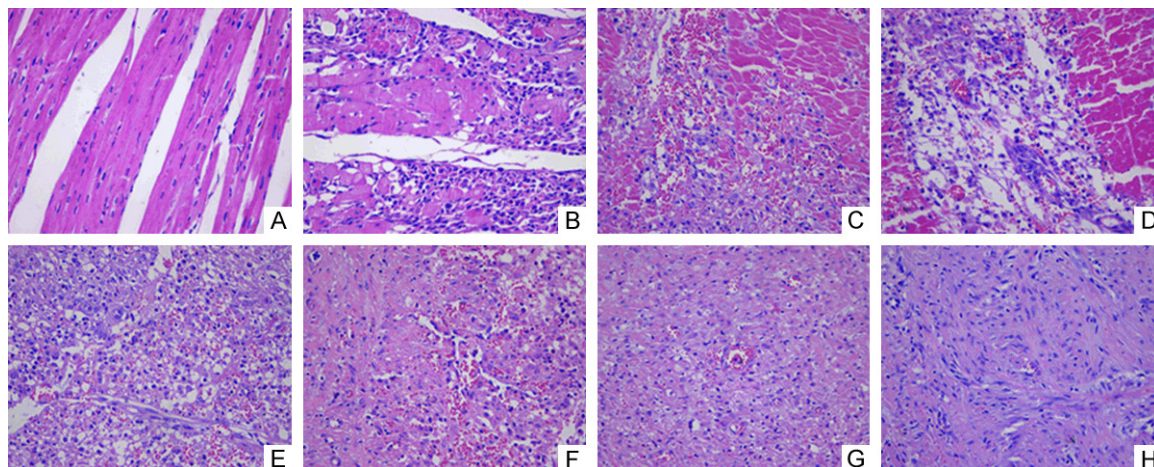


Figure 2. Pathological observation of myocardial sections post-AMI. A: Normal myocardial (400 ×); B: Infarcted myocardium at 1 d (400 ×); C: Infarcted myocardium at 3 d (400 ×); D: Infarcted myocardium at 5 d (400 ×); E: Infarcted myocardium at 7 d (400 ×); F: Infarcted myocardium at 10 d (400 ×); G: Infarcted myocardium at 14 d (400 ×); H: Infarcted myocardium at 17 d (400 ×).

washed with running water, counterstained, dehydrated, transparentized, sealed and observed under a 40 × digital electronic microscope. And the photos were taken.

Main observation indicators

Necrotic myocardial tissues at different days after AMI modeling were observed pathologically, and the dynamic changes of TNF- α expression in the necrotic myocardial tissues were detected.

Design, implementation, evaluators

The experiment was designed by the corresponding author and implemented by the first author and other co-authors. Evaluation was performed by Department of Pathology at First Affiliated Hospital of Henan University of Science and Technology, Laboratory of New District Hospital of Henan University of Science and Technology, and Biology Teaching and Research Room of Henan University of Science and Technology.

Results

Findings from coronary angiography before and after AMI (Figure 1)

Coronary angiography and blood vessel occlusion with gelfoam were performed successfully in 7 Chinese miniswine. After occlusion, blood flow in the distal end of OM branch disappeared, indicating the modeling of AMI (**Figure 1B**).

Histopathological changes

Pathological changes of infarcted myocardium were observed at 1 d, 3 d, 5 d, 7 d, 10 d and 14 d after modeling (**Figure 2**). It could be seen that the normal myocardial cells were arranged regularly with oval nuclei located near the center of the cells and the cytoplasm was evenly distributed (**Figure 2A**). At 1 d, the nuclei were long-spindle shaped in the necrotic myocardial cells, showing enhanced cytoplasmic acidophilia; cytoplasmic granules were formed in some cells (**Figure 2B**). At 3 d, the contour of the necrotic myocardial cells was obscured, and the margin of the cells was infiltrated by inflammatory cells (**Figure 2C**). At 5 d, the nuclei were fragmented, and karyolysis occurred with vacuolation (**Figure 2D**). At 7 d, the nuclei of the necrotic myocardial cells disappeared with extensive formation of granulation tissues at the margins (**Figure 2E**). At 10 d, the granulation tissues at the margins of the necrotic myocardial cells were replaced by fibrous scar tissues; there were a large number of newly formed capillary vessels, fibroblasts and lymphocytes (**Figure 2F**). At 14-17 d, all granulation tissues were replaced by fibrous scar tissues (**Figure 2G, 2H**).

TNF- α expression

TNF- α was detected by immunohistochemical staining at 1 d, 3 d, 5 d, 7 d, 10 d and 14 d after modeling (**Figure 3**). No TNF- α was detected in non-infarcted myocardium (**Figure 3A**). At 1 d,

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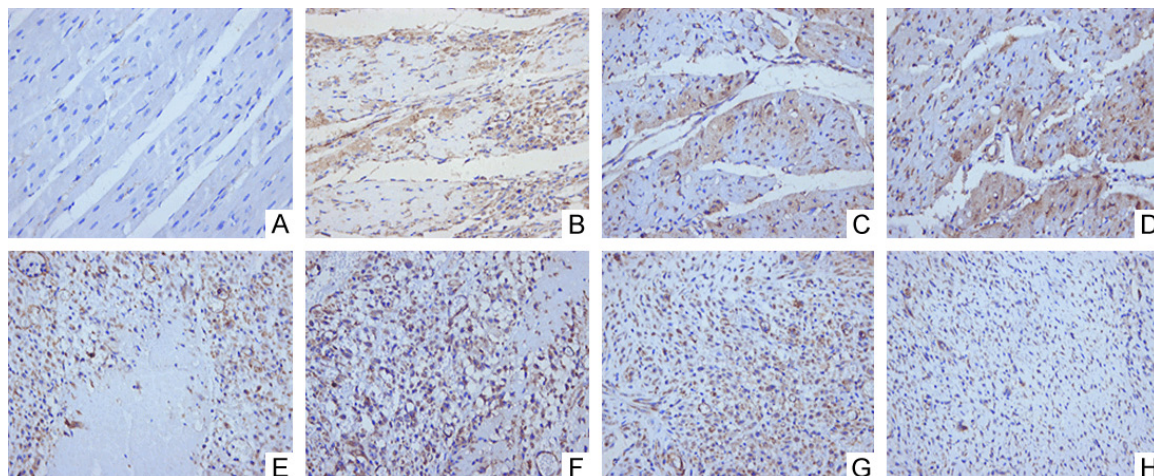


Figure 3. TNF- α expression in infarcted myocardium at different days. A: Normal myocardial (400 \times); B: Infarcted myocardium at 1 d (400 \times); C: Infarcted myocardium at 3 d (400 \times); D: Infarcted myocardium at 5 d (400 \times); E: Infarcted myocardium at 7 d (400 \times); F: Infarcted myocardium at 10 d (400 \times); G: Infarcted myocardium at 14 d (400 \times); H: Infarcted myocardium at 17 d (400 \times).

very little TNF- α was detected in the infarcted myocardium (**Figure 3B**). At 3 d, TNF- α expression was detected (**Figure 3C**). At 5 d and 7 d, TNF- α expression was increased (**Figure 3D, 3E**). The peak was reached at 10 d (**Figure 3F**), and the expression gradually decreased at 14 d and 17 d (**Figure 3G, 3H**).

Discussion

AMI is a great threat to human health, which leads to poor prognosis. PCI following AMI can rapidly restore myocardial perfusion and is the preferred therapy for ST segment elevation myocardial infarction (STEMI). However, after the reopening of the blocked blood vessels, myocardial stunning and no-reflow may result in the lowering of myocardial contractile function, arrhythmia or even severe hemodynamic disorder. A large number of clinical trials indicate that improper timing of blood flow reconstruction only causes deterioration. According to guidelines in China and western countries [1-4], reperfusion therapy is recommended at 7-10 d after onset for those whose treatment is already 12 hours after the onset of AMI. The optimal timing of blood flow reconstruction is still not determined. Most researchers believe that the timing of blood flow reconstruction should be based on the pathological changes and repair of the infarcted myocardium. Very few reports have been published concerning the pathological changes of the infarcted myocardium at different time after AMI. In the pres-

ent study, we established AMI model in Chinese miniswine and carried out observations of the pathological changes. Many previous AMI models are built by thoractomy and coronary artery ligation, which damages the normal anatomy of the thoracic cavity and affects the cardiopulmonary function. Moreover, a series of reactions induced by surgery interfere with postoperative continuous observation. To overcome this defect, we performed percutaneous coronary artery occlusion with gelfoam. The heart of Chinese miniswine is similar to that of human in terms of anatomy and blood vessel distribution. The femoral artery is thick enough to accommodate 6 F arterial sheath, and the AMI simulated by this method shows similarity in pathogenesis as that in human. The AMI model in Chinese miniswine facilitates the pathological observations of infarcted myocardium and the determination of the timing of blood flow reconstruction.

At 1-3 d after AMI, the infarcted myocardium was yellow grey and showed obvious edema. The necrotic myocardial cells under the microscopic had an enhanced cytoplasmic acidophilia; hyperaemia occurred in the interstitial capillary vessels; the inflammatory cells infiltrated at the margin of the necrotic myocardial tissues, releasing inflammatory media that led to the dissolution of necrotic myocardial cells and heart rupture. At 3-5 d, the edema of the necrotic myocardial tissues was alleviated, and sheets of necrotic myocardial cells were

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observed. The cytoplasm of some necrotic myocardial cells was stained red uniformly, the nuclei were enlarged, and an obvious congestion zone was spotted at the margin of necrotic myocardial tissues; vacuolar degeneration occurred. At this time, the myocardial cells at the infarcted site became completely necrotic. As the myocardial repair was inconsiderable, blood flow reconstruction at this moment carried great risk. At 5-7 d, the surface of the necrotic loci was sunken, and the contour of the necrotic myocardial cells was obscured; the nuclei were fragmented, and granulation tissues appeared at the margin of the necrotic myocardial tissues. Since the tensile strength was low, the blood flow reconstruction was still risky. At 7-10 d, the necrotic site was gray white with sunken surface and hard texture. The normal structure of the myocardial cells disappeared, and the cytoplasm was stained red uniformly. The nuclei were fragmented with karyolysis and vacuolar degeneration. As the necrotic myocardial cells were dissolved, extensive granulation tissues and fibrous scar tissues appeared. Because the tensile strength of the infarcted myocardium was enhanced, the blood flow reconstruction at this moment was safe. At 10-17 d, the myocardial repair was basically complete, so reopening of the blocked coronary artery was safer 2 weeks after AMI. As shown by the above changes, blood flow reconstruction is most appropriate when the infarcted myocardium is totally replaced by granulation tissues and fibrous scar tissues which indicate complete myocardial repair. Due to social and economic factors, most patients prefer blood flow reconstruction 7-10 d after AMI. However, the effect of blood flow reconstruction at a longer interval after AMI remains unknown.

TNF- α is a cytokine that performs extensive biological functions. Belonging to tumor necrosis factor/receptor TNF/TNFR superfamily, TNF- α is related to acute inflammatory response and autoimmune diseases. Inflammatory response plays a key role in AMI, and upregulation of TNF- α in circulation and myocardial tissues is common to AMI. TNF- α can strongly trigger inflammatory response and is produced by various types of cells, such as lymphocytes, neutrophils, endotheliocytes, fibroblasts and smooth muscle cells. Ischemic myocardial cells are still capable of secreting TNF- α [5]. The difference is that after myocardial damage, TNF- α is mainly secreted by monocytes and macro-

phages [6]. According to relevant studies, TNF- α concentration in blood circulation rose significantly at 1 h after AMI [7] and reached the peak within 24 h [8]. After that, TNF- α concentration began to decrease and reached the normal level 7 days later.

TNF- α is increased significantly after AMI, leading to violent myocardial inflammatory response, apoptosis and enlargement of myocardial cells, and cardiac remodeling. Finally, ejection fraction decreases, infarcted area expands, and myocardial contractile function is impaired. Some severe complications including fatal arrhythmia and heart rupture will occur. The occurrence of myocardial necrosis in AMI involves multiple inflammatory factors. TNF- α usually has a high concentration in blood during early AMI and then migrates to myocardium where it is highly expressed. Although TNF- α is crucial for the progression and repair of myocardial necrosis, the dynamic changes of TNF- α expression in the infarcted myocardium are rarely reported. We observed the TNF- α expressions at 1 d, 3 d, 5 d, 7 d, 14 d and 17 d after AMI. At 1 d, nearly no TNF- α was detected in the infarcted myocardium; TNF- α was expressed at 3 d and gradually increased at 5 d and 7 d; the expression was enhanced significantly at 10 d, after which the expression decreased. At 5-17 d, extensive granulation tissues and fibrous scar tissues appeared at the infarcted site, with the formation of new capillary vessels, fibroblasts and lymphocytes. The above changes confirmed the role of TNF- α in the occurrence and development of myocardial inflammation after AMI.

Sun et al. [9] built the AMI model in mice by ligation of left anterior descending coronary artery. They found that TNF- α level rose at the infarcted site. Acute heart rupture and chronic left ventricular dysfunction were caused by TNF- α that induced excess inflammatory response, degradation of matrix and collagen, and increased the activity of matrix metalloproteinases and cell apoptosis. Some scholars [10] studied the TNF- α -expressing offspring of transgenic mice heterozygous for TNF- α . It was discovered that over-expression of TNF- α in the myocardium of the transgenic mice caused ventricular expansion, decrease of ejection fraction, atrial or ventricular arrhythmia as well as the reduction of survival of mice. Others [11] showed that TNF- α gene and protein expressions were de-

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tected in the myocardium only half hour after the occlusion of left anterior descending coronary artery in rats. All the above experiments demonstrate the role of TNF- α in promoting the development of myocardial necrosis and slowing myocardial repair.

Whether inhibition of inflammation in AMI can facilitate myocardial repair is the major concern for many scholars. Masahiro et al. [12] built AMI model in male rats by ligation of left coronary artery and found that release of TNF- α shortly after AMI promoted myocardial injury and induced myocardial cell apoptosis. Intramuscular injection of TNF- α antagonist, i.e., plasmid DNA expressing soluble TNF- α 1 receptor (sTNFR1), can reduce the activity of TNF- α in the myocardium and the apoptosis of myocardial cells. Skyschally et al. [13] built swine coronary microthrombosis model by intracoronary injection of micro-droplets. Results showed that TNF- α not only led to myocardial contractile dysfunction, but also delayed the protective action on infarcted myocardium. Intracoronary perfusion of TNF- α antibodies can significantly reduce the infarcted area. Some scholars studied cardiac systolic function in dogs by injecting recombinant human active TNF- α (rhTNF- α) into adult hybrid dogs. After 24 h, the cardiac contractile and systolic functions decreased obviously [14]. Yokoyama et al. [15] carried out experiment using hearts from cats and the isolated myocardial cells. They found that TNF- α had negative inotropic action on the ventricles and isolated myocardial cells of adult cats. However, clearing TNF- α completely reversed this action, and treatment with TNF- α neutralizing antibody prevented the negative inotropic action exerted by TNF- α on the isolated myocardial cells.

Experiments with different animals show that clearing or neutralizing TNF- α can both reduce myocardial necrosis significantly and facilitate myocardial repair. Treatment using TNF- α monoclonal antibodies will reduce the infarcted area and lipid peroxidation in blood, mitigate harmful cardiac remodeling and inhibit immune responses caused by acute damage [16, 17]. TNF- α -converting enzyme mediates the maturity of TNF- α in monocytes and macrophages and therefore plays an important role in the poor prognosis of AMI. The inhibition of TNF- α -converting enzyme may be one method to reduce the production of TNF- α after

AMI [18]. Intramyocardial injection of TNF- α antibodies represents a key research direction due to their effect in inhibiting abnormal immune response and reversing harmful cardiac remodeling.

Blood flow reconstruction at about 10-17 d after AMI is associated with less risk of STEM and heart rupture. The inhibition of TNF- α after AMI can improve the treatment outcome and prognosis of patients with AMI. However, the sample size of the present study is limited, and the findings need to be confirmed by studies with a larger sample size.

Disclosure of conflict of interest

None.

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