Effect of tumor necrosis factor-α on ventricular arrhythmias in rats with acute myocardial infarction in vivo

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BACKGROUND: Acute myocardial infarction (AMI) is an acute cardiovascular emergency. This study was undertaken to assess the effect of tumor necrosis factor-α (TNF-α) on ventricular arrhythmias induced by AMI in rats in vivo.

METHODS: Two hundred and forty male Wistar rats were randomized into a sham-operation group, an AMI group, and a recombinant human tumor necrosis factor receptor:Fc fusion protein(rhTNFR:Fc) group. Acute anterior wall myocardial infarction was produced in the AMI group by ligating the left anterior descending coronary artery (LAD), and there was no ligation but operation in the sham-operation group. The rhTNFR:Fc group was treated with rhTNFR:Fc(10 mg/kg), a TNF-α antagonist, 24 hours before LAD ligation. The spontaneous and induced programmed electrical stimulation ventricular arrhythmias were recorded at baseline and 10 minutes, 20 minutes, 30 minutes, 60 minutes, 3 hours, 6 hours and 12 hours after ligation. At the same time the protein and mRNA expression levels of TNF-α among different groups were detected by histochemistry and real-time fluorescent quantitative PCR.

RESULTS: Expression of TNF-α increased markedly from 10 minutes after infarction, peaked at 20-30 minutes, and returned to baseline gradually in the AMI group and rhTNFR:Fc group. The time-windows of spontaneous and induced ventricular arrhythmias were similar. Compared with the AMI group, the rhTNFR:Fc group showed a lesser expression of TNF-α protein and a lower incidence of ventricular arrhythmias (P<0.05). There was no obvious change in the sham-operation group.

CONCLUSION: The expression of TNF-α induced by AMI could contribute to the onset of ventricular arrhythmias.

KEY WORDS: Acute myocardial infarction; Tumor necrosis factor-α; Ventricular arrhythmia; Recombinant human tumor necrosis factor receptor: Fc fusion protein (rhTNFR: Fc)


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INTRODUCTION

Acute myocardial infarction (AMI) is a kind of serious cardiovascular emergency. It often induce various severe ventricular arrhythmias such as frequent ventricular premature beats, ventricular tachycardia, ventricular fibrillation, etc, which are the most common causes of death in early stage. Significant inflammatory responses exist in acute ischemic myocardium during AMI. Tumor necrosis factor-α(TNF-α), one of the most important inflammatory factors,[1] has shown widespread biological effects.[2] A recent study showed that severe ventricular arrhythmias easily happened in transgenic animals with TNF-α overexpression.[3] Clinical studies[4,5] confirmed that the plasma TNF-α of AMI patients markedly increased,
and was closely related to the occurrence of ventricular arrhythmias. In our animal experiments, we also found that the increased myocardial expression of TNF-α in AMI rats was closely related to the occurrence of ventricular arrhythmias. But whether TNF-α expressed by acute ischemic myocardium plays an important role in the occurrence of acute ischemic ventricular arrhythmias has not been elucidated. In this study we used recombinant human tumor necrosis factor receptor:Fc fusion protein (rhTNFR:Fc) to block the biological effects of TNF-α and to determine the effects of TNF-α expressed by acute ischemic myocardium on the occurrence of ventricular arrhythmias.

**METHODS**

**Animals**

Two hundred and forty male Wistar rats weighing 250-300 g were supplied by the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology. All experimental procedures were approved by the Institutional Authority for Laboratory Animal Care and conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85-23, revised 1985).

Major reagents and instruments included rhTNFR:Fc (Zhongxinguojian, China); goat anti-rat TNF-α/TNFSF1A Antibody (R&D, USA); TNF-α rabbit anti-goat antibody (Boshide, China); TRizol kit (invitrogen); a small animal respirator (Zhejiang University Medical Instrument Factory, China); a BL-420F biological signal acquisition and processing system (Chengdu Tai Meng, China); a Y-2 electric stimulator (Chengdu Instrument Factory, China); a HMIAS series color medical image analysis system (Olympus, Japan); and a real-time quantitative PCR system (ABI, USA).

**AMI rat model**

Acute myocardial infarction (AMI) was induced in male Wistar rats weighing 250-300 g. All rats were anesthetized by intraperitoneal injection of pentobarbital sodium (30-35 ml/kg). Under controlled ventilation, a thoracotomy was performed through the left parasternal 3, 4 intercostal spaces while the pericardium was incised and the anterior wall of the left ventricle was exposed. The proximal end of the left anterior descending coronary artery (LAD) was ligated with 6-0 line at the junction of the pulmonary conus and the left atrial appendage, which could induce extensive infarction of the left ventricular anterior wall. When the ventricular anterior wall turned to be pale or cyanosed and ECG showed ST-segment elevation, a myocardial infarction model was established.

**Experimental groups**

Two hundred and forty Wistar rats were randomized into an AMI group (n=80), a sham operation group (n=80) and a recombinant human tumor necrosis factor receptor:Fc fusion protein (rhTNFR:Fc) group (n=80). Anterior wall myocardial infarction was produced in the AMI group by ligating the LAD, and there was no ligation but operation in the sham-operation group. The RhTNFR:Fc group was treated with rhTNFR:Fc (10 mg/kg), a TNF-α antagonist, 24 hours before LAD ligation. ECG and spontaneous ventricular arrhythmias were observed during the whole experiment. Ventricular arrhythmias were stimulated and recorded at baseline, 10 minutes, 20 minutes, 30 minutes, 60 minutes, 3 hours, 6 hours and 12 hours after ligation. Afterwards TNF-α expression was detected by immunohistochemistry and real-time fluorescence quantitative PCR.

**ECG recording and occurrence of ventricular arrhythmias**

To observe the occurrence of spontaneous ventricular arrhythmias during the whole experiment, we recorded with a BL-420F biological signal acquisition and processing system the standard limb lead II ECG before and after AMI. Moreover to observe the induced ventricular arrhythmias, the S1S2 programmed electrical stimulus was produced by a Y-2 electric stimulator at different time points before and after LAD ligation (S1 pulse number: 8; S2 pulse number: 1; initial S1S2 interval: 100 ms; S1S2 interval decrease: 1 ms; cycle interval: 1s; cycles: 100).

**Immunohistochemistry for TNF-α**

Fresh myocardial tissues of rats were fixed with formalin, dehydrated, embedded in paraffin, and then cut into 5-8 μm thick slices. After peroxidase was inactivated by hydrogen peroxide at room temperature for 10 minutes, the slices were repaired by microwave for 10 minutes, incubated with BSA at 37°C for 40 minutes, with goat anti-rat TNF-α antibody at 4°C overnight, with biotinylated rabbit anti-goat secondary antibody at 37°C for 40 minutes, with SABC at 37°C for 60 minutes, then colored with DAB, stained with hematoxylin, and finally observed. With the true color multi-function color pathological image analysis system using five sights at
each slice, TNF-α expression was measured and analyzed by the HMIAS series color medical image analysis system.

**Real-time quantitative PCR for TNF-α mRNA**

Myocardial cell total RNA was extracted with TRIzol kit. Then reverse transcription, amplification and detecting fluorescence signals were detected by the real-time quantitative PCR instrument with an upper primer: 5′-CAGGCCGATTGCCCATTTCAT-3′, and a lower primer: 5′-ACGCCAGTCGCTTCACAGAG-3′. Thermal cycling conditions were 95 °C, 15 seconds; 56 °C, 15 seconds; 72 °C, 45 seconds; 40 cycles. TNF-α mRNA amplification multiple =2-(Ct TNF-α-Ctβ-actin).

**Statistical analysis**

All values were expressed as mean ± SD. The results of the study were analyzed by analysis of variance (ANOVA) followed by a two-sided Dunnett’s test or the Student-Newman-Keuls test when appropriate. Linear correlation analysis was made for the relationship between TNF-α and ventricular arrhythmias. P<0.05 was considered statistically significant.

**RESULTS**

**TNF-α expression detected by immunohistochemistry**

TNF-α in acute ischemic myocardium began to increase from 10 minutes after infarction, peaked at 20-30 minutes, and recovered gradually thereafter. TNF-α protein detected by immunohistochemistry was significantly less in the rhTNFR: Fc group than in the AMI group ($F=19.428$, $P=0.042$). The expression of TNF-α in the sham-operation group was extremely low (Figure 1).

**Expression of TNF-α mRNA detected by real-time fluorescence quantitative PCR**

TNF-α mRNA in acute ischemic myocardium began to increase from 10 minutes after ligation, peaked at 20-30 minutes, and recovered gradually thereafter. In the same time the expression of TNF-α mRNA in the AMI group ($F=22.189$, $P=0.002$) and rhTNFR: Fc group ($F=22.686$, $P=0.002$) was higher than that in the sham-operation group; there was no significant difference between the AMI group and rhTNFR: Fc group ($F=0.044$, $P=0.838$) (Figure 2).

![Figure 1. Expression of TNF-α detected by immunohistochemistry. A-C: Expression of TNF-α in the sham-operation group, AMI group and rhTNFR:Fc group after ligation (original magnification ×400). D: The AMI group and rhTNFR:Fc group vs the sham-operation group, *P<0.05; the AMI group vs the rhTNFR:Fc group, #P<0.05.](www.wjem.org)
Occurrence of ventricular arrhythmias

Ventricular arrhythmias (ventricular tachycardia, ventricular flutter, ventricular fibrillation, etc) were induced by programmed electrical stimulation from 10 minutes after ligation, mostly at 30 minutes, peaked at 15-25 minutes, then recovered. Ventricular arrhythmias were markedly less in the rhTNFR:Fc group than in the AMI group (P<0.05). A little bit of ventricular arrhythmias was seen in the sham-operation group. The time window of spontaneous ventricular arrhythmias was similar to that of the induced ones. The typical ventricular arrhythmias are illustrated in Figure 3. The occurrence of ventricular arrhythmias is shown in Figure 4.

Relationship between expression of TNF-α and ventricular arrhythmias

The time window of ventricular arrhythmias was coincided with TNF-α expression in acute ischemic myocardium (Figure 5). Linear correlation analysis of the relationships between TNF-α detected by immunohistochemistry and ventricular arrhythmias was performed in both AMI group and rhTNFR:Fc group. The correlation coefficient of the AMI group was 0.908, P<0.01, and that of the rhTNFR:Fc group was 0.934, P<0.01. Both groups showed a positive linear correlation (Figure 6).
DISCUSSION

The electrophysiological mechanisms of ventricular arrhythmias associated with AMI include reentry, triggered activity and abnormal automaticity. The intimate causes, which induce the electrophysiological change, are still unknown. When AMI occurs, humoral factors and metabolites express in a wrong way, such as cAMP, catecholamine, oxyradicals, thrombin, intracellular Ca^{2+} and Na^{+} increase, all of which might participate in the occurrence of acute ischemic ventricular arrhythmias.\textsuperscript{[8-10]} TFN-α, one of the most important inflammatory factors, mainly secreted by activated macrophages, could be expressed by myocardium, especially when AMI occurs.\textsuperscript{[6]} Recent reports have documented that TFN-α plays an important role in the occurrence and development of chronic cardiac failure\textsuperscript{[11]} and viral myocarditis.\textsuperscript{[12]} However, the effects of TFN-α on acute ischemic ventricular arrhythmias are still unknown. A study\textsuperscript{[5]} indicated that TFN-α expressed by acute ischemic myocardium in rats has a close relationship with ventricular arrhythmias. RhTNFR:Fc, a dimer, consists of cell surface of TFN receptor and the Fc fragment of human IgG1, which could bind to TFN-α and block its biological effects. In the present study we used rhTNFR:Fc to establish the control group and determined the effects of TFN-α on acute ischemic ventricular arrhythmias.

We found that ventricular arrhythmias happened 10 minutes after ligation, frequently at 30 minutes, peaked at 15-25 minutes, and then recovered. The time window of ventricular arrhythmias was similar to the expression of TFN-α, showing that the occurrence of ventricular arrhythmias was closely related to the TFN-α expression. With rhTNFR:Fc, which could not interrupt the expression of TFN-α mRNA but reduce the active TFN-α protein by binding to TFN-α, the lowered occurrence of ventricular arrhythmias indicates that TFN-α secreted by acute ischemic myocardium plays a positive role in ventricular arrhythmias in AMI rats, and that rhTNFR:Fc could effectively reduce the occurrence of ventricular arrhythmias by antagonism towards TFN-α protein.

TFN-α has pleiotropic biological effects on cell proliferation, differentiation, apoptosis and inflammatory reactions. It was found to regulate Ca^{2+} inflow of cardiac myocytes by the PLA2/AA pathway\textsuperscript{[13]} and inhibit the cardiac delayed rectifier K current via the PKA pathway.\textsuperscript{[14]} TNF-α overexpression could induce electrical remodeling of myocardial cells of mice with heart failure.\textsuperscript{[15]} Therefore, TFN-α may change the electric activity of cardiac myocytes with different mechanisms, and finally causes ventricular arrhythmias. The results of the present study suggest that TFN-α as an inflammatory factor may play an important role in ventricular arrhythmias associated with AMI in rats, indicating a new way for the treatment of acute ischemic ventricular arrhythmias.

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