Original Article
Association of TNF-α with ventricular arrhythmias during early acute myocardial infarction

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Abstract: To explore the effects of tumor necrosis factor-α (TNF-α) on ventricular arrhythmias (VAs) in rats with acute myocardial infarction (AMI). A rat model of AMI was created by ligation of the left anterior descending (LAD) coronary artery. ECG and spontaneous VAs were observed during the whole experiment. Monophasic action potentials (MAP) among ischemic zone, border zone and non-ischemia zone was observed and Monophasic action potential repolarization dispersion (MAPDd) were calculated. Linear correlation analysis was used to assess the relationship between TNF-α protein expression and MAPDd of the border zone. The incidence of VAs in AMI group was markedly increased at each time interval after ligation compared with Etanercept group (P<0.05). The MAPDd from the border zone of the ischemic myocardium was significantly increased at each time point after ligation compared with the ischemic zone and non-ischemic zone of the epicardium (P<0.05). Moreover, the MAPDd from the border zone of ischemic myocardium was significantly increased in the AMI group compared with the Etanercept group (P<0.05). The temporal changes of the MAPDd in the border zone coincided with the incidence of VAs in vivo during early AMI. The levels of TNF-α protein expression in the AMI group began to increase at 10 min and reached a peak at 20 min after ligation; then, they decreased. The correlation coefficient of TNF-α protein expression and MAPDd of the border zone was 0.96 (P<0.01) and showed a positive linear correlation. TNF-α could increase the MAPDd in the border zone, and promote the onset of VAs, while Etanercept could decrease the MAPDd in the border zone and lessen the incidence of VAs in rats with AMI.

Keywords: Acute myocardial infarction, ventricular arrhythmias, monophasic action potential

Introduction

Coronary heart disease (CHD) is a common cardiovascular disease. Seventy five to eighty percent of the world of sudden cardiac death (SCD) is the direct cause of coronary heart disease, especially the occurrence of ventricular arrhythmia after myocardial infarction [1]. Acute Myocardial Infarction (AMI) is one of the most common fatal cardiovascular diseases. However, ventricular arrhythmia is the most serious complications of AMI [2]. More than one million people died from myocardial infarction disease every year [3]. Ventricular arrhythmia after myocardial infarction is one of the most common causes of SCD [4]. Therefore, it is of great scientific significance to study the mechanism of ventricular arrhythmia after acute myocardial infarction.

Tumor necrosis factor-α (TNF-α) is an inflammatory cytokine with pleiotropic biological effects and plays an important role in inflammatory reactions. In the circulatory system, TNF-α is responsible for many myocardial diseases such as acute myocardial infarction (AMI) [5, 6], myocarditis [7], and congestive heart failure (CHF) [8]. Recent reports have documented that TNF-α is involved in the onset of atrial and ventricular arrhythmias (VAs). Over expression of TNF-α contributes to the initiation of rapid re-entrant atrial arrhythmias in mouse model of heart failure [9]. Asynchronous ventricular activation (AVA) caused by right apical ventricular pacing (RAVP) in patients with sick sinus syndrome leads to an undesirable increase in TNF-α. The effect of APD prolongation by TNF-α may be associated with susceptibility to lethal ventricular arrhythmias and sudden death in CHF.
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[10]. In transgenic mice, overexpression of the inflammatory cytokine TNF-α (TNF-α mice) in the heart causes mice to develop a progressive heart failure syndrome characterized by ventricular dilatation, decreased ejection fraction, and atrial and ventricular arrhythmias on ambulatory telemetry monitoring [11].

AMI is currently thought to be an inflammation process [12]. TNF-α may be found augmented in the myocardium of AMI [13] and serves as one of the cytokine-mediated attractants for neutrophil migration into the ischemic region and enhances the inflammatory reaction related to progressive tissue destruction [14]. Inflammatory reaction caused by TNF-α stimulates paraplasm and hypertrophy of the myocardium and plays important roles in recovery and ventricular remodeling after AMI [15-17]. When AMI occurs, in accordance with different degrees of ischemia, myocardium could be divided into the ischemic zone, the nonischemic zone, and the border zone [18]. However, whether TNF-α plays an important role in the occurrence of VAs remains unknown.

It is reported that sudden cardiac death (SCD) causes approximately 3 million fatalities in the U.S. Annually Ventricular tachyarrhythmia arising from myocardial ischemia and infarction is a leading cause [19, 20]. Recently, Shimoda et al [21] found that, in AMI patients with malignant ventricular arrhythmia, spontaneous and stimulated levels of TNF-α were higher than controls. However, the relationship between TNF-α and ventricular arrhythmias arising from AMI was still unknown. Therefore, this study detected expression of TNF-α in different regions of ischemic myocardium, recorded the monophasic action potentials (MAPs) and occurrence of VAs, and then explored the effect of TNF-α expression on the occurrence of VFs and the potential mechanisms.

Materials and methods

Animal care

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. 85e23, revised 1985).

AMI rat model

Myocardial infarction (MI) was induced in 8-week-old male Sprague-Dawley rats weighing 250-300 g through ligation of the left anterior descending coronary artery as previously described [22]. Briefly, All male SD rats were anesthetized with pentobarbital sodium (30~35 mL/kg) by intraperitoneal injection. Under controlled ventilation, a thoracotomy through a left parasternal 3, 4 intercostal spaces was performed, the pericardium was incised, and the anterior wall of the left ventricle was exposed. Left anterior descending coronary artery (LAD) proximal end was ligated with 6-0 line at the junction of the pulmonary cones and the left atrial appendage, which could induce extensive infarction of left ventricular anterior wall. When the ventricular anterior wall turned to be pale or cyanosed and ECG showed ST-segment elevated, myocardial infarction model succeeded.

Experimental groups

Ninety SD rats were randomized into AMI group (n = 30), sham operation group (n = 30), and Etanercept group (n = 30). Anterior wall myocardial infarction was produced in AMI group by ligating the left anterior descending coronary artery (LAD); there was no ligation but operation in sham-operation group. Etanercept group was treated with recombinant human tumor necrosis factor receptor: Fc fusion protein (rhTNFR: Fc) (10 mg/kg), a TNF-α antagonist, 24 hours before LAD ligation. ECG and spontaneous VAs were observed during the whole experiment. Monophasic action potentials (MAPs) among the ischemic zone, the non-ischemic zone, and the border zone were observed at baseline, 10 min, 20 min, 30 min, 60 min, 3 h, 6 h, and 12 h after ligation, and monophasic action potential duration disperses (MAPDs) were calculated, while VFs were induced by S1S2 programmed electrical stimulation and recorded.

Recording of MAP and calculation of monophasic action potential repolarization dispersion (MAPDd)

Electrodes were placed on the ischemic zone, the nonischemic zone, and the border zone of the epicardium, and each zone recorded five points. Signal input terminal was connected to the BL-420F biological signal acquisition and processing system (Taimeng, China). The signals were inputted into the computer by the A/D converter. Pacing with 6Hz at high right atrium, MAPs were recorded at baseline, 10 min, 20 min, 30 min, 60 min, 3 h, 6 h, and 12 h after ligation, and monophasic action potential duration disperses (MAPDs) were calculated, while VFs were induced by S1S2 programmed electrical stimulation and recorded.
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after ligation. After measuring MAPD90 of five points of each zone.

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\text{MAPDd} = \left( \sqrt{\text{MAPD}90_i - \bar{x}} \right)^2 + \ldots + \left( \frac{\text{MAPD}90_n - \bar{x}}{5} \right)^2
\]

**Western blotting of TNF-α protein**

Total cell plasma protein of myocardium of rats in AMI group, sham operation group, and Etanercept group, respectively, were extracted by 50 mmol/L Tris eHCl (pH 7.4). Samples containing 30 mg of total protein were electrophoresed on 15% polyacrylamide gels (Bio-Rad, Hercules, CA) for 1 h (4°C, constant current for 80 mA). Separated proteins were electrophoretically transferred onto a nitrocellulose membrane (Invitrogen, Carlsbad, CA). Negative (no protein added) controls were used at the same time. Transfer time was 28 min. Blocking with 5% nonfat milk was done for 2 h at 37°C. TNF-α protein expression was detected by a 1:500 dilution of goat anti-rat TNF-α polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) as the first antibody, respectively. A 1:5000 dilution of horseradish peroxidase-conjugated rabbit antigoat (Santa Cruz Biotechnology) was used as the second antibody and then developed with the enhanced chemiluminescence Western blotting detection system (Pierce Biotechnology, Rockford, IL).

**Immunohistochemistry for TNF-α**

Fresh myocardial tissues of rats were fixed with formalin, dehydrated, transparent, embedded in paraffin, and then cut into 5-8 μm thick slices. After peroxidase was inactivated by hydrogen peroxide at room temperature for 10 min, the slices were repaired by microwave for 10 min, incubated with BSA at 37°C for 40 min, with a 1:200 dilution of goat anti-rat TNF-α/TNFSF1A antibody (R&D Systems, USA) TNF-α antibody at 4°C overnight, with a 1:100 dilution of biotinylated rabbit anti-goat secondary antibody (Boshide, China) at 37°C for 40 min, with avidin-biotin-horseradish peroxidase complexes at 37°C for 60 min, then colored with diaminobenzidine, stained with

![Figure 1. Ventricular Arrhythmias in AMI Rat Models. A. Recording of PVCs in ECG in AMI Rat Models. B. Recording of VT/VF in ECG in AMI Rat Models. C. The incidence of PVCs at different time intervals of post-ligation. D. The incidence of VT/VF at different time intervals of post-ligation. #versus sham group; *versus AMI group. *P<0.01, *P<0.01. 3.2 The MAPDds in the Border Zone before and after ligation.](image-url)
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haematoxylin, and, finally, observed. Selecting five sights at each slice, TNF-α expression in every section was measured and analyzed by HMIAS Series Color Medical Image Analyze System (Champion Image Ltd., China).

Figure 2. The MAPDd of the border zone (BZ) and the incidence of VAs at different time point. A. The MAPDd of the border zone at different time point. B. The incidence of VAs at different time point. Compared with AMI group, the MAPDd of the border zone significantly decreases at different point in early AMI in Etanercept group. The change of MAPDd of the border zone with time is in accordance with change of the incidence of VAs in early AMI. n=10, *versus AMI group, *P<0.05.

Statistical analysis

All values are expressed as mean ± SD. Results were analyzed by using Student’s t-test and analysis of variance (ANOVA) for multiple comparisons followed by a two-sided Dunnett’s test or Student Newman-Keuls test when appropriate. Linear correlation analysis was used for the relationship between TNF-α and ventricular arrhythmias. Statistical significance was assumed at P<0.05.

Results

Ventricular arrhythmias in AMI rat models

Ventricular arrhythmias happened in the early period after ligation in AMI rats, such as premature ventricular contractions (PVCs), ventricular tachycardia (VT) and even ventricular fibrillation (VF) in individual rats. PVCs was most common in these ventricular arrhythmias. To testify the occurrence of VAs in rat AMI model, ECG was recorded to monitor the spontaneous VAs in rat model (Figure 1A and 1B). In the AMI group, the spontaneous VAs appeared most frequently 10-30 min after ligation, reached a climax at 15-25 min, and recovered gradually then. Compared to the AMI group, the occurrence of VAs (PVCs and VT/VF) in Etanercept group significantly decreased, P<0.05 (Figure 1C). There were no ventricular arrhythmias in the sham group and the other two groups before ligation both in the induced and raw state. The occurrence of VAs was showed in Figure 1.

To explore the relation between MAPDd of the border zone (BZ) and the incidence of VAs at
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Figure 3. The expression of TNF-α protein in different group and the correlation between TNF-α expression and MAPDd of the border zone. A. The expression of TNF-α protein at different time point of border zone in AMI group. B. The expression of TNF-α protein at different time point of border zone in Etanercept group. C. The expression of TNF-α protein in different groups. Western blot showed different expressions of TNF-α at different time among groups. Compared with sham group, the expression of TNF-α protein significantly increases at different time point in both AMI group and Etanercept group. Compared with AMI group, the expression of TNF-α protein significantly decreases at different time point in Etanercept group. #versus sham group; *versus AMI group. P<0.05, *P<0.05. D. The correlation between TNF-α expression and MAPDds of the border zone. The positive correlation coefficient between TNF-α expression and MAPDds of the border zone is 0.9837 (P<0.01) in AMI group. Different time point, MAP was recorded in the ischemic zone, the border zone (BZ), and the non-ischemia zone with electrode. The MAPDds in the border zone significantly increased after LAD ligation, reached a peak time at 20 min, and recovered gradually then. At the same time point, MAPDds of AMI group were significantly greater than Etanercept group, P<0.05 (Figure 2A). The time window of the changes of MAPDds in the border zone was in accordance with the occurrence of VAs in acute ischemic myocardium. This shows that the MAPDd of the border zone promotes the occurrence of VAs (Figure 2).

TNF-α protein expression by Western blot

Western blot showed different expressions of TNF-α at different time among groups. Compared with sham group, the expression of TNF-α protein significantly increased at different time point in both AMI group and Etanercept group and reached a peak time at 20 min, and recovered gradually then. Compared with AMI group, the expression of TNF-α protein signifi-
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Significantly decreases at different time point in Etanercept group (Figure 3C). Linear correlation analysis of the relationships between detected expression of TNF-α and the MAPDds in the border zone was performed both in AMI group and Etanercept group. The correlation coefficient of AMI group was 0.9837, <0.01, and showed positive linear correlation (Figure 3D).

**TNF-α expression detected by immunohistochemistry**

TNF-α in acute ischemic myocardium began to increase at 10 min after infarction, reached a climax at 20-30 min, and recovered gradually then. At the same time point, TNF-α in the ischemic zone was higher than the others, and the second was in the border zone. Compared to the AMI group, TNF-α detected by immunohistochemistry in Etanercept group was significantly less than the AMI group (P<0.05), and the expression of TNF-α in the sham group was extremely low (Figure 4B).

**Discussion**

It is known that TNF-α is an acute phase reactive protein and a basic media of immunological...
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cal regulation. It serves as a critical role in the inflammatory reaction. AMI is thought to be an inflammation process [12]. Moreover, TNF-α participates in cardiac remodeling and in final rehabilitation after AMI [15-17, 23]. Ventricular arrhythmia followed by AMI is one of the most severe complications of AMI. However, the relationship between TNF-α and ventricular arrhythmias arising from AMI was unknown. Therefore, we performed this study. In this study, we duplicated AMI rat models. Ventricular arrhythmias happened in the early period after ligation in AMI rats, including PVC, VT. Meanwhile, protein level of TNF-α began to increase at 10 min after ligation and reached a peak time at 20-30 min, then decreased (Figures 3 and 4). The time-dependent manner coincided with the previous report [24]. Protein expression levels of TNF-α were higher in AMI group than in Etanercept group (P<0.05). In AMI group, the occurrence time of ventricular arrhythmias coincided with the secretion of TNF-α. These results indicated that the considerable secretion of TNF-α may be related to ventricular arrhythmias arising from AMI. Immunohistochemical staining showed TNF-α expression predominated in surviving cardiomyocytes of the infarct zone and cardiomyocytes in non-infarct zone. Thus, we could conclude that TNF-α may autocrine from cardiocyte in the rats of AMI.

The occurrence of VAs after ligation happened most frequently in 10 to 30 min and gradually decreased in rat AMI model, which coincided with the previous report [25]. After peak time, the decreasing occurrence of VAs may be related to coronary collateral circulation formation, water and salt electrolyte disorder, energy metabolism. But the mechanism of the occurrence of VAs after AMI is not clear yet [26]. Compared to the AMI group, the occurrence of VAs (PVCs and VT/VF) in Etanercept group significantly decreased, but the extent of increase sharply reduced. Meanwhile, the appearance of VAs significantly decreased as well, which suggested that TNF-α promotes the occurrence of acute ischemic VAs by enlarging MAPDs in the border zone, and rhTNFR: Fc decreases the occurrence of VAs by inhibiting the biological effects of TNF-α. The time window of the changes of MAPDs in the border zone was in accordance with the occurrence of VAs in acute ischemic myocardium. This shows that the MAPD of the border zone promotes the occurrence of VAs (Figure 2).

Our findings first suggested that TNF-α promotes acute ischemic VAs through enlarging MAPDs of the border zone. The underlying mechanisms need to be further studied. Besides, whether there are some other pathways in the process that TNF-α causes VAs remains unknown.

The expression of TNF-α increased greatly after acute myocardial infarction. TNF-α could enlarge the MADDs in the border zone and promote the onset of VAs while rhTNFR: Fc could diminish the MADDs in the border zone and lessen the onset of VAs in AMI rats. Our results demonstrated that TNF-α expressed by ischemic myocardium may play an important role in the occurrence of VAs in AMI rats.

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Disclosure of conflict of interest

None.

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References


