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· Original article ·

Effect of local radiofrequency denervation on ventricular effective refractory period and ventricular arrhythmia susceptibility after acute myocardial infarction

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[**Abstract**] **Objective** To introduce a new method of local denervation by ablation and to observe its effect on ventricular effective refractory periods and ventricular arrhythmia susceptibility after acute myocardial infarction. **Methods** Eighteen mongrel dogs were used to make myocardial infarction model by ligation of the 1st diagonal artery. Then local denervation was achieved by using six radiofrequency ablations (each at 8 W for 2 min) along the left anterior descending artery. Effective refractory periods were tested at six sites on the left ventricular epicardium (above, in and below the infarction areas, 2 sites each area) before and after local denervation by S1S2 stimulation. Ventricular arrhythmia was also induced after myocardial infarction and after denervation by burst pacing, and the number of arrhythmia episodes was recorded. **Results** The effective refractory periods were significantly prolonged after ablation ([191.3±24.9] ms vs [209.0±27.2] ms, $P<0.05$). And the prolongation of effective refractory periods in infarcted area ([11.3±8.8] ms) was significantly shorter than those in the other 2 areas ([23.2±10.2] ms and [18.7±11.5] ms). After ablation, 8 of the 11 susceptible dogs were free of induced ventricular arrhythmia, while 3 of them were still inducible by burst pacing. The induced ventricular arrhythmia rate was reduced from 61.1% (11/18) to 16.7 %(3/18) ($P=0.007$). **Conclusion** The method in this study is promising for preventing ventricular arrhythmia in patients with acute myocardial infarction receiving percutaneous coronary intervention.

[**Key words**] sympathetic nerve; denervation; radiofrequency ablation; effective refractory period; ventricular arrhythmia

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Autonomic nervous system plays an important role in the genesis of life-threatening arrhythmias. Cellular mediators of this autonomic imbalance probably contribute significantly to ventricular fibrillation (VF). Over the last several years, a number of studies have focused on the role of ion channel in inducing VF during myocardial ischemia. Data have suggested that alterations in ion channel activity are responsible for VF induced by myocardial ischemia[1-2]. Several anti-adrenergic interventions have been shown to protect against VF in canine model of sudden cardiac death[3]. Removal of the left stellate ganglion has been proven to be the most effective intervention. As Billman mentioned previously, β-adrenoceptor blockade has been proven less effective than destruction of the cardiac sympathetic nerves, which protect less sustained canine[4].

Sympathetic nerve system increases the calcium current by releasing sympathetic transmitter

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and activating β_2-adrenoceptor, without altering calcium reuptake by the sarcoplasmic reticulum. The resultant elevation in intracellular calcium could provoke oscillations in membrane potential, which could trigger arrhythmias. Thus, in the diseased heart, β_2-adrenoceptor activation tends to reduce the cardiac electrical stability and increase the propensity for the genesis of malignant arrhythmias. Activation of potassium ATP (K_{ATP}) channels during acute myocardial ischemia has also been found to be arrhythmogenic by shortening ventricular repolarization and refractoriness[5-6].

Sympathetic nerves go along the left anterior descending artery (LAD), and transmural left ventricular myocardial infarction in patients and dogs produces sympathetic and vagal denervation in viable myocardium apical to the infracted area[7]. Sympathetic nerves mainly spread in the epicardium while vagal nerves mainly in the endocardium. We conjectured that direct modulation of local sympathetic nerves have impact on the electrical characters and contribute to the treatment of ventricular arrhythmia after acute myocardial infarction.

1 Materials and methods

1.1 Animals and equipments Eighteen mongrel dogs weighing 12-15 kg were used in this study. The animal procedures were approved by the Animal Care and Use Committee of Second Military Medical University. All the experiments were performed under general anesthesia with 3% pentobarbital sodium (30 mg/kg iv, and the animals were mechanically ventilated. Adequacy of anesthesia was assessed by toe pinch and palpebral reflex. Additional anesthetics were given when necessary to maintain anesthesia throughout the experiment. Dogs were ventilated with room air via a cuffed endotracheal tube using a constant volume-cycled respirator. The right femoral vein and artery were cannulated to infuse normal saline (100-200 mL/h) for replenishing body fluid loss. Electrocardiogram (ECG) was monitored through a multichannel electrophysiological amplifier system TOP2001 (Hongtong Biology Technology Company, Shanghai, China). The chest of animals was then opened through the 4th intercostal and the pericardium was opened and sewn to chest wall to expose and cradle the heart. Monophasic action potentials were recorded by multichannel electrophysiological amplifier system BL-420 (Taimeng Biology Technology Company, Chengdu, China) with a detector sewn to the apex.

1.2 Animal model of myocardial infarction The first diagonal artery was dissected longitudinally with glass needle and then ligated by 4-0 prolene suture at the origin from LAD; then waited until the ischemic part turned dark red so as to make sure that the ligation was successful. ECG was recorded and analyzed continuously before and after myocardial infarction. To achieve a stable status, we gave the animals a 90 min-pause to make sure the ECG was unchangeable before moving to the next step.

1.3 Local denervation by radiofrequency To achieve left ventricular denervation, radiofrequency was applied along the LAD artery because sympathetic nerves go along the LAD and then spread through the epicardium to myocardium. Irrigated radiofrequency catheter tip was placed on the left ventricular side, which was only 2 mm away from LAD artery. Up to six ablations (each at 8 W for 2 min) were performed. Interventions were delivered from the LAD artery bifurcation to the apex and placed longitudinally. Catheter tip impedance and temperature were constantly monitored. ECG was also monitored to evaluate the potential myocardial ischemic caused by LAD injury.

1.4 Measurement site selection and measuring effective refractory periods (ERPs) Estimation of ischemic area was made before the first diagonal artery occlusion (described previously). Six sites were chosen for ventricular ERP measurements. As a result, two sites corresponded to the locations

of plunge electrodes in the circumflex distribution above the ischemic area (1 cm away from LAD and circumflex artery, 1 cm away from each other), two corresponded to the 1st diagonal branch artery in ischemic area (1 cm away from LAD and 1 cm away from each other), and two corresponded to the apex region below the ischemic area (1 cm away from each other). ERP measurements were performed at 90 min after the 1st diagonal branch artery myocardial infarction and 60 min after local denervation by radiofrequency ablation. S1S2 pacing was performed at each site by using eight drive stimuli (S1) followed by an extra-stimulus (S2) at twice threshold pacing current with a 2 ms pulse duration. Pacing threshold was determined by gradually decreasing the stimulus current intensity during continuous ventricular pacing until loss of consistent ventricular capture occurred and ranged from 0.1 mA to 1.4 mA. Based on the findings of our preliminary experiment, we chose 300 ms as the drive-cycle length (S1), which could maintain consistent capture without competition from the basal heart rate for each animal. S2 was delivered 10 ms decrements starting from 300 ms until the capture was lost, then it was delivered in 1 ms decrements from 10 ms above the last one until ERP was obtained (defined as the longest S1S2 interval failing to yield a ventricular response). ERPs were measured at the base line, after myocardial infarction and local denervation.

1.5　VT/VF induction　Ventricular burst pacing (50 beats) with progressively shorter coupling intervals was used to elicit ventricular tachycardia (VT)/VF. Sustained VT was defined as a stable tachycardia with uniform QRS and MAP configuration patterns that lasted more than 30 seconds and required termination by overdrive pacing or by direct current (DC) cardioversion.

1.6　Statistical analysis　Data was expressed as $\bar{x}\pm s$. ERP change was analyzed by paired student t test. Arrhythmia incidences before and after local denervation were analyzed by Fisher's exact test. $P<0.05$ was considered statistically significant.

2　Results

2.1　ERP change　We compared ERPs before and after radiofrequency ablation in all the six sites and found that ERPs were significantly prolonged after ablation ([191.3±24.9] ms vs [209.0±27.2] ms, $P<0.05$)(Fig 1). The average prolongation was (17.7±13.8) ms. As reported by previous researchers, sympathetic nerve is affected in infarcted area[8]. To make sure whether there was any difference in ERP prolongation among the sites which were above, in and below the infarcted areas, we calculated the average ERPs of the two sites above the infarcted area (REGION 1), two sites in the area (REGION 2) and two sites below the area (REGION 3). Then we calculated the difference between these 3 areas (REGION 1, 2 and 3). Finally, we found that ERP prolongation ([11.3±8.8] ms) in infarcted area (REGION 2) was significantly smaller than those in the other 2 areas (REGION 1&3) ($P<0.05$), but there was no significant difference between REGION 1 and REGION 3 ([23.2±10.2] ms vs [18.7±11.5] ms, $P>0.05$)(Fig 2).

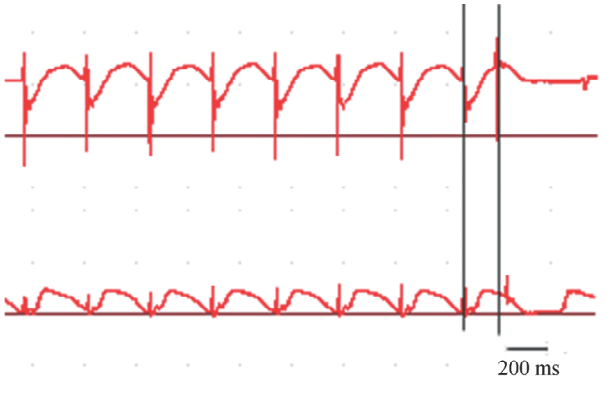

Fig 1　ERP prolongation after local denervation

The two action potential duration recordings were recorded at the same site of one dog. The upper one showed ERP=180 ms (between the two black vertical lines) after myocardial infarction and before radiofrequency ablation, while the lower one showed ERP=200 ms (between left black line and the right small red stimulation spark) after radiofrequency ablation. ERP: Effective refractory period

Eleven of the eighteen dogs were induced to VF after infarction. After ablation, 8 of the 11

susceptible dogs were free of induced ventricular arrhythmia, while 3 of them were still inducible by burst pacing. The rest 7 dogs in which arrhythmia could not be induced before ablation were still resistant to pacing induced arrhythmia. The induced arrhythmia rates were different before and after the ablation (61.1%[11/18] vs 16.7 %[3/18], $P=0.007$).

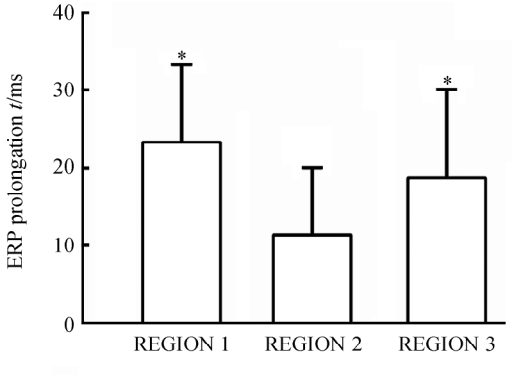

Fig 2　ERP prolongation in different regions

ERP: Effective refractory period. ERP prolongation ([11.3±8.8] ms) in the infarcted area (REGION 2) was significantly smaller than those in the other 2 areas (REGION 1 [23.2±10.2] ms, REGION 3 [18.7±11.5] ms). * $P<0.05$ vs REGION 2; $n=18$, $\bar{x}\pm s$

3　Discussion

Our experiment indicated that local denervation along LAD could prolong ERP and reduce ventricular arrhythmia induction rate after myocardial infarction. According to previous studies, high sympathetic activity in chronic heart failure patients is associated with a poor prognosis[9-10], the same is true for myocardial infarction. A target which can influence the cardiac sympathetic activity would be of great importance. So we tried to modulate sympathetic activity by directly targeting the sympathetic nerves on epicardium.

We also found that prolongation in infarcted area was smaller than those in the other two areas, which might be due to the injury of local nerves and impair of ion channel in the infarcted area. After ablation, more sympathetic nerves were impaired in areas above and below the infarcted area, and sympathetic nerves were more evenly distributed in the heart. In our experiment, the induced ventricular arrhythmia rate was reduced from 61.1% (11/18) to 16.7 % (3/18) ($P=0.007$) after local denervation. The model of Moe et al.[11] showed that a random distribution of refractory periods with a sufficiently large dispersion of refractoriness could provide a substrate for reentrant arrhythmias. An increase in the dispersion following coronary artery ligation has been considered crucial to the development of reentrant rhythms in the ischemic heart[12]. Less premature beats were able to penetrate to areas of longer refractoriness before blocking than earlier premature beats were able to penetrate[13]. Many studies have focused on ion channels and found that activation of channels during acute myocardial ischemia is arrhythmogenic by shortening ventricular repolarization and refractoriness[14]. During the recovery phase of infarction, several ion channels are still functional in the healing infarct border. Sympathetic nerves participate in genesis of arrhythmia by I_{ks} channel induced cascade reaction[1-2]. Based on the extent of intracellular ATP depletion in ischemic ventricular myocytes, various K_{ATP} channel openers were found to exert different degrees of activation in the outward repolarizing current, this may result in nonuniform shortening of ventricular action potential and refractoriness in ischemic and nonischemic regions, thereby increasing spatial dispersion of repolarization and subsequently facilitating initiation of conduction block and reentry. The reduced ventricular arrhythmia rate may come from symmetrizing of ERP.

Myocardial infarction patients have pretty high incidence of ventricular arrhythmia despite of time-

ly revascularization. Our method is the first to take local sympathetic nerves as the core of treatment target. As it is known to all, beta blockers are widely used in clinical settings but are forbidden in acute phase of myocardial infarction, so our method may be promising for preventing ventricular arrhythmia in patients with acute myocardial infarction when receiving percutaneous coronary intervention (PCI) revascularization. This method may be used for preventing ventricular arrhythmia in chronic myocardial infarction patients, especially those with refractory arrhythmia after infarction; further studies should be done to evaluate the efficacy and safety of the method in chronic infarction animal models and patients. In our study, we only observed the acute effect of local denervation few hours after myocardial infarction. Further study is needed to answer what will happen in long term after local denervation.

Our studys casts new light on treatment of arrhythmia after myocardial infarction. However, the long-term safety should be taken into consideration as vascular injury is inevitable when access from coronary vein or coronary artery. Therefore, further studies are warranted to evaluate the endothelium injury and embolic risk during radiofrequency in vascular. Whether destroy of sympathetic nerves can impair pump function is not tested in our study, but according to Elvan study, a response to infused norepinephrine was still present when they measured at peri-infarct area after myocardial infarction[7], which means in the situation of myocardial infarction, myocardial is denervated but still responsible to circulative sympathetic transmitter.

4　Conflict of interest

The authors declare that there is no conflict of interest.

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射频消融局部去交感神经对心肌梗死犬心室不应期及心律失常易感性的影响

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[**摘要**] **目的**　探讨一种新的利用射频消融进行局部去交感神经的方法,并观察其对心脏电生理及程控心律失常诱发的影响。**方法**　18 只杂种犬结扎冠状动脉第一对角支建立心肌梗死模型,之后沿冠状动脉左前降支均匀地自上而下行 6 个点射频放电消融(每点 8 W,2 min)。所有实验动物均在心肌梗死后局部去神经前及去神经后通过 S1S2 程控刺激测定左心室表面 6 个部位的有效不应期(心梗上区域、心梗区域及心梗下区域各 2 个部位),并行短阵刺激诱发室性心律失常,记录诱发的心律失常数目。**结果**　沿冠状动脉消融后,左心室各部位有效不应期显著延长[(191.3±24.9) ms vs (209.0±27.2) ms,$P<0.05$]。缺血区域有效不应期延长的时间[(11.3±8.8) ms]较缺血区域以上[(23.2±10.2) ms]及缺血区域以下[(18.7±11.5) ms]短($P<0.05$)。消融前 11 只能诱发室性心律失常的犬中,消融后有 8 只未能再诱发心律失常,3 只仍能诱发心律失常;心律失常的诱发率由 61.1%(11/18)下降到 16.7%(3/18;$P=0.007$)。**结论**　本研究建立的方法可能是减少急性心肌梗死行经皮冠状动脉造影患者心律失常发生的一个可行方法。

[**关键词**] 交感神经;去神经支配;射频消融术;有效不应期;室性心律失常

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