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Effects of local radiofrequency denervation on ventricular electrophysiological properties in normal and acute myocardial ischemia heart

Y. HUANG[1], D.-N. WANG[1], P. LIU[1], Y. SONG[2], H.-M. CUI[1], J.-Y. ZHANG[1], J. BLACKWELL[3], D.-N. LIAO[1]

[1]Department of Cardiology, and [2]Department of Endocrinology; Shanghai Changzheng Hospital, Second Military Medical University, Shanghai, China
[3]Wake Forest School of Medicine, Winston-Salem, NC, USA

Ying Huang and *Dan-Ning Wang* contributed equally to this manuscript

Abstract. – OBJECTIVE: To observe the effects of local radiofrequency denervation on ventricular effective refractory periods, electrical alternans and ventricular arrhythmia susceptibility post myocardial infarction.

MATERIALS AND METHODS: Thirty-four mongrel dogs were randomly divided into the normal heart group (n = 16, 8 in sham and 8 in local sympathetic denervation – LSD) and the acute myocardial ischemia (AMI) group (n = 18, 9 in control and 9 in LSD). The left cardiac sympathetic nerve was denervated with irrigated catheter radiofrequency ablation. Left ventricular effective refractory periods (ERP), monophasic action potential duration at 90% (APD_{90}) and APD alternans were measured at baseline and 2 hours after LSD in the normal heart group. AMI was induced by ligating the left anterior descending coronary artery 2 hours after LSD was performed. Then APD_{90}, the occurrence of ventricular arrhythmias (VAs) were measured.

RESULTS: Compared with baseline, LSD significantly prolonged ventricular ERP and APD_{90} at all sites ($p < 0.05$ for all) in the LSD group, whereas no significant change was shown in the sham group. But their spatial dispersions did not change in both groups. APD alternans occurred at shorter pacing cycle length at each site after LAD→LSD when compared to the sham group ($p < 0.05$ for all). After AMI, the occurrence of VAs was significantly lower in the LSD group than in the control group ($p < 0.05$).

CONCLUSIONS: LSD may have a beneficial impact on ventricular arrhythmias induced by AMI through modulation of autonomic tone.

Key Words:
Radiofrequency ablation, Sympathetic nerve, Myocardial infarction, Arrhythmia.

Introduction

There is a high prevalence of ventricular arrhythmia and sudden cardiac death (SCD) in patients with myocardial ischemia. Evidence from histological studies and direct nerve activity recordings have suggested that increased sympathetic nerve density and activity contribute to the generation of ventricular arrhythmia and SCD[1].

For the prevention of ventricular arrhythmia and SCD, pharmacological therapy is still the first line intervention. Multiple randomized control trials have proven that beta-blocker therapy could significantly reduce the incidence of SCD after myocardial infarction (MI)[2,3]. Some studies suggest left cardiac sympathetic denervation (LSCD) might be a treatment for primary inherited arrhythmia syndromes, such as long-QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT)[4,5]. Renal sympathetic denervation (RSDN) has provided a protection against ventricular arrhythmias and SCD in both animal models and human patients[6]. Therefore, the inhibition of general and localized sympathetic nerves is likely to have a positive effect on the cardiac electrophysiological stability and the recovery of cardiac function in myocardial infarction patients.

It is known that all mammals' hearts are richly innervated. Cardiac autonomic nerves include sympathetic and parasympathetic systems. Sympathetic nerve fibers originate from the hypothalamus and project out of the spinal cord at the level of T1-T5 segments, where they synapse with neurons in the cardiothoracic ganglion and the stellate ganglion, producing sympathetic post-

Corresponding Author: De-Ning Liao, MD; e-mail: : liao6007@163.com

ganglionic fibers. These fibers then travel below the epicardium to control cardiac function[7]. The parasympathetic innervation originates predominantly in the nucleus ambiguous of the medulla oblongata. The parasympathetic preganglionic fibers are carried almost entirely within the vagus nerve and are divided into superior, middle, and inferior branches. Most of the vagal nerve fibers converge at a distinct fat pad between the superior vena cava and the aorta (known as the third fat pad) en route to the sinus and atrioventricular nodes[8]. In the present study, we explored the effects on ventricular electrophysiological characteristics and induced ventricular arrhythmias by the local ablation of nerve fibers associated with the coronary arteries following myocardial infarction.

Materials and Methods

Animal handling was performed in accordance with the Shanghai Directive for Animal Research and the current Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85-23, revised 1996). The Ethics Committee at Second Military Medical University approved the study protocol.

Experimental Models

Thirty-four mongrel dogs were randomly divided into the normal heart group (n=16, 8 in sham and 8 in LSD) and the acute myocardial ischemia (AMI) groups (n = 18, 9 in control and 9 in LSD). All of the dogs were anesthetized with sodium pentobarbital (30 mg/kg, IV), intubated, and ventilated with room air supplemented with oxygen from a respirator (LTV-1000, Pulmonetic Systems, USA). Additional maintenance doses of 2 mg/kg sodium pentobarbital were administrated at the end of each hour during the procedure. Standard surface electrocardiogram (ECG) was continuously monitored using a computer-based Lab System (TOP 2001, Hongtong Biology Technology Company, Shanghai, China).

Local Sympathetic Denervation by Radiofrequency

Radiofrequency was applied along the left anterior descending (LAD) artery in order to ablate sympathetic nerves which travel along the LAD and then spread through the epicardium to the myocardium. The 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 8W for 2 min) were performed. Current was delivered from the LAD artery bifurcation to the apex. Catheter tip impedance and temperature were constantly monitored. ECG was also monitored to evaluate the potential for myocardial ischemia caused by inadvertent LAD injury.

Electrophysiological Measurements

Effective Refractory Periods (ERP)

Two multi-electrode catheters with 1 cm interelectrode distance were sutured to evaluate effective refractory periods (ERP) at six epicardial sites from the apex to the base of the left ventricular free walls. The ventricular ERP in each site was determined by programmed pacing that consisted of eight drive stimuli (S1) followed by an extra-stimulus (S2) at twice threshold pacing current with a 2 ms pulse duration. The S1S2 interval was progressively decreased until refractoriness was achieved. The ERP was defined as the longest S1S2 interval that failed to capture the ventricles as described previously[9]. ERPs were measured at the baseline and after local denervation. ERP dispersion was defined as the coefficient of variation (CV) of the ERP at all six sites.

Monophasic Action Potential

Monophasic action potentials (MAP) were recorded using a custom-made Ag-AgCl catheter from the epicardial surface at the infarcted area and infarcted remote area in all animals. MAP signals were amplified and filtered at 1-1200 Hz and were analyzed using the BL-420 (Taimeng Biology Technology Company, Chengdu, China). The $MAPD_{90}$, was defined as monophasic action potential duration (MAPD) at 90% repolarization. A dynamic steady state pacing protocol (S1S1) was performed to determine APD alternans. The pulse train was delivered at an initial pacing CL slightly shorter than the sinus CL and maintained for 30 s to achieve a steady state. The pacing cycle length was progressively decreased in an initial stepwise fashion by 20 or 30 ms until APD alternans occurred, the pacing was interrupted for 2 min before the next pacing train. The cycle length at which APD alternans occurred was recorded.

Acute Ischemia Protocol

After two hours local denervation or sham treatment, the first diagonal artery was isolated in the AMI group and the AMI+ LSD group, then occluded by ligature (3-0 silk) for one hour 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 would not change any further before proceeding.

Measurement of Ventricular Arrhythmias Occurrences

Electrocardiogram was continuously monitored for 1 hour to record the occurrences and duration of ventricular arrhythmias including ventricular premature contraction (VPC, identifiable premature QRS complexes), ventricular tachycardia (VT, three or more consecutive VPCs at a rate faster than the resting sinus rate) and ventricular fibrillation (VF, unidentifiable and low voltage QRS complexes). Especially, if VT progresses within a few beats to VF (there are no sinus beats between VT and VF), we classified these VTs as VF.

Immunohistochemistry

At the end of the experiment, the hearts were quickly collected from 2 dogs in each group. Samples from the ablation area were harvested for immunostaining. Sections (5 μm thick) were mounted on charged slides. A modified immunohistochemical ABC method was used for immunostaining for TH (tyrosine hydroxylase, a marker of sympathetic nerves). Integrated optical density (IOD) was used to assess TH+ nerve fibers[10]. In each section, 4 fields evenly distributed throughout the areas of interest were analyzed. The IOD of TH+ fibers were calculated by Image-Pro Plus (IPP) 6.0 image analysis software.

Statistical Analysis

Data is expressed as mean ± SD, the mean ERP, $MAPD_{90}$ and sympathetic density acquired before and after local denervation were compared using student's paired *t*-test. The independent sample *t*-test was used to compare the number of PVC and VT/VF between the two groups. Data was analyzed using SPSS21.0 software (IBM Corp, Armonk, NY, USA). Statistical significance was defined as $p < 0.05$.

Results

Effect of LSD on Hemodynamics

As shown in Table I, no significant changes were seen in heart rate or SBP at 2 hours after LSD or sham operation. Systolic BP decreased significantly during AMI, but there were no significant differences between the LSD and the control group. Heart rate, however, did not change during AMI and was not affected by LSD.

Effect of LSD on Ventricular ERP in the Normal Heart

Figure 1 summarizes ventricular ERP at 6 epicardial sites in the LSD group and Sham group. As a result of LSD, left ventricular ERP were significantly prolonged when compared to baseline. (LVA1, 163.6 ± 12.7 ms *vs.* 175 ± 13 ms; LVA2, 164.1 ± 11.8 ms *vs.* 171.6 ± 12 ms; LVA3, 163.3 ± 7 ms *vs*. 171.7 ± 9.1 ms; LVB1, 162.8 ± 11.7 ms *vs.* 173.8 ± 12.3 ms; LVB2, 161.6 ± 11.2 ms *vs.* 171.2 ± 12.1 ms; LVB3, 162.7 ± 11.7 ms *vs.* 171.7 ± 13 ms, $p < 0.05$ for all). However, left ventricular ERP in the sham group did not change significantly. Furthermore, LSD did not increase the ERP dispersion as measured by CV-ERP (Figure 2).

Effect of LSD on $MAPD_{90}$

In comparison with baseline, $MAPD_{90}$ from the anticipated infarcted area and non-infarcted

Table I. Heart rate and systolic BP at baseline and after ablation.

	Heart rate (bpm)		Systolic BP (mmHg)	
	Baseline	Ablation	Baseline	Ablation
LSD group	150 ± 11	148 ± 9	148 ± 18	146 ± 14
Sham group	153 ± 14	149 ± 11	143 ± 14	141 ± 13

BP, blood pressure.

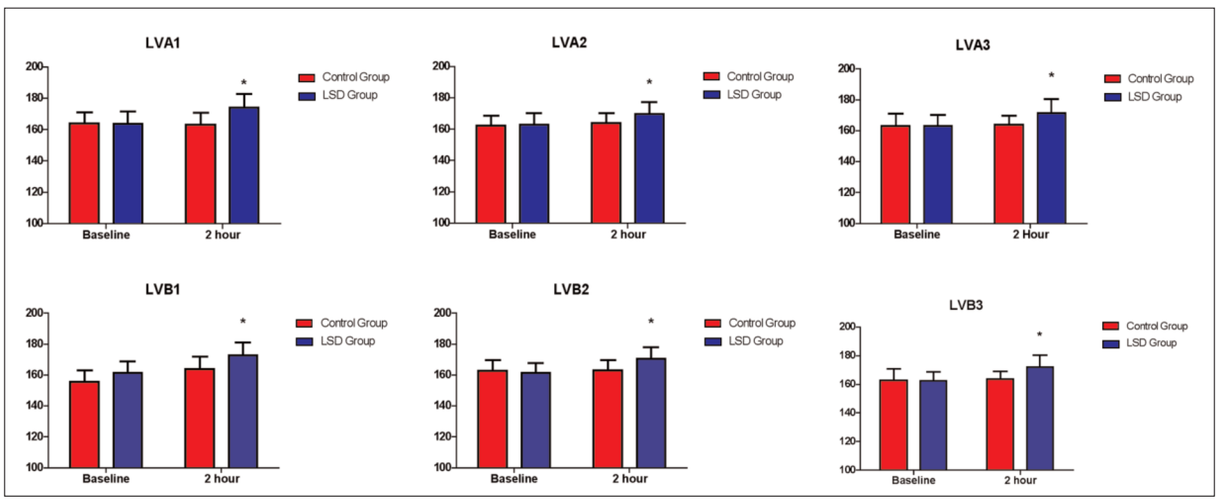

Figure 1. Effect of LSD on ERP at baseline and 2 hours after LSD or sham LSD in different sites of left ventricle. LSD significantly prolonged ventricular ERP, whereas no significant change in sham Group. *$p < 0.05$ compare to the baseline.

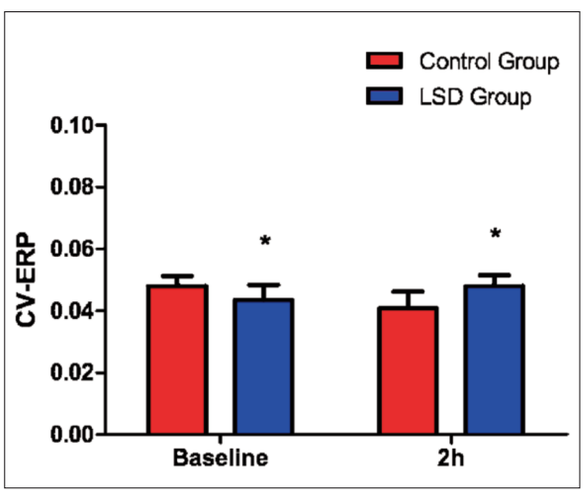

Figure 2. Effect of LSD on CV-ERP in left ventricle, *$p > 0.05$.

area were significantly prolonged after LSD in the LSD group, whereas no significant change was shown in the control group (Figure 3). After AMI, a significant decrease in $MAPD_{90}$ from the infarcted zone was shown in the AMI group but no change in the LSD+AMI group (Figure 3a). The $MAPD_{90}$ from the non-infarcted area, however, was not changed during AMI in both groups ($p > 0.05$) and kept at a level which was more or less similar to that of pre-AMI (Figure 3b).

Effect of LSD on APD Alternans in the Normal Heart

Figure 4 shows the pacing cycle length at which APD alternans occurred in the normal heart. Compared with the baseline state, APD alternans occurred at significantly shorter cycle lengths after LSD at each recording site.

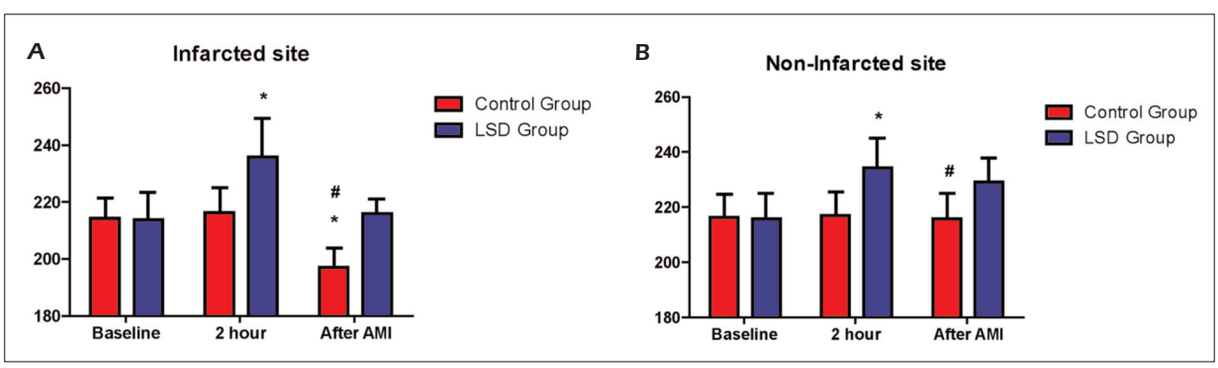

Figure 3. ***A, B,*** Effect of LSD on $MAPD_{90}$ in infarcted and non-infarcted sites on left ventricle, *$p < 0.05$ compared with baseline, $^{\#}p < 0.05$ compared with AMI group after LSD.

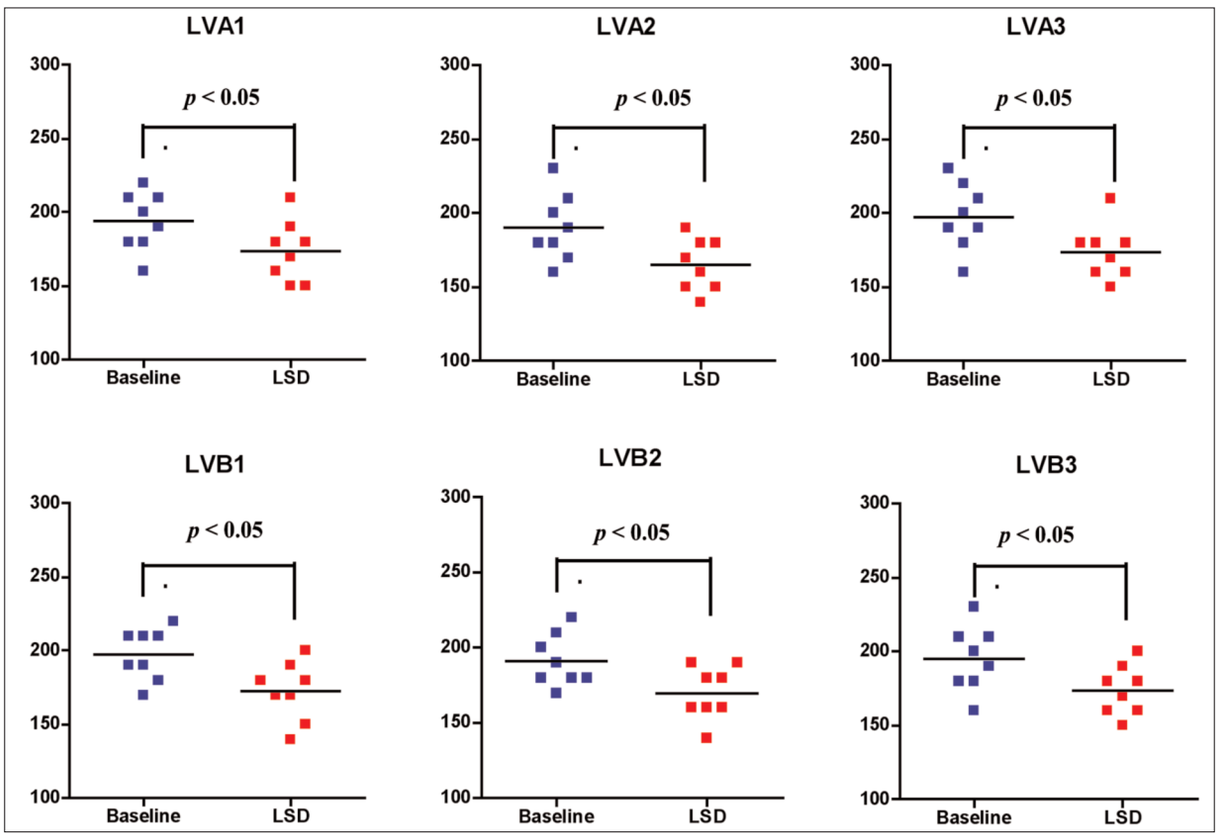

Figure 4. The APD alternans pacing cycle lengths (PCL) in different sites of left ventricle free walls at baseline and after LSD in the normal heart (n = 8). The APD alternans occurred at a significantly shorter pacing cycle length after LSD at each site (*$p < 0.05$ versus baseline).

Effect of LSD on the Sympathetic Density

We performed the TH (a marker of sympathetic nerve) immunohistochemistry staining in the ablation areas around the coronary arteries to evaluate the effects of LSD. The IOD of TH-positive nerve fibers decreased significantly in LSD group as compared to sham group (Figure 5).

Effect of LSD on VAs Occurrence

Figure 6 shows that the episodes of PVCs, the episodes of VT as well as the mean duration of VT in LSD group was significantly lower when compared to the control group after coronary artery ligation. 1 of 9 (11.1%) animals in the LSD group had spontaneous VF compared to 3 of 9 (33.3%) in the control group ($p < 0.05$).

Discussion

In this study, we investigated the protective effects of left cardiac sympathetic denervation on ventricular electrophysiological properties using an acute myocardial infarction canine model. The results indicated that LSD pre-conditioning exhibited obvious effects on the ERP as well as $MAPD_{90}$ in animals with myocardial infarction. These results suggest that LSD may exert a pro-

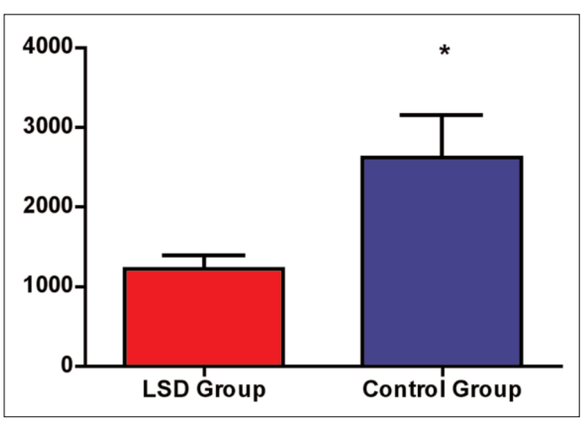

Figure 5. IOD of TH + fibres in the ablation areas around the coronary arteries *$p < 0.05$ compared with the LSD group.

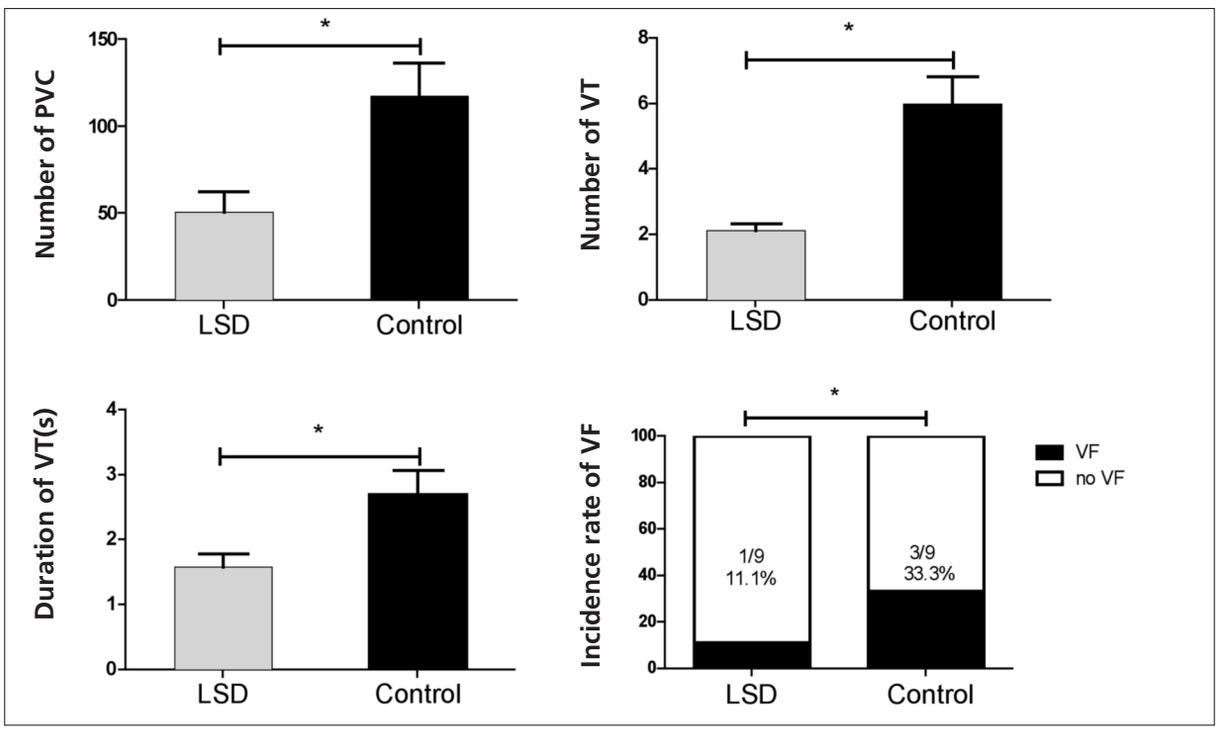

Figure 6. The occurrence of VAs episodes in both groups. *$p < 0.05$ compare to the Control group. PVC, Premature ventricular contraction; VT, ventricular tachycardia; VF, ventricular fibrillation.

tective effect against ventricular arrhythmias. Our study also demonstrated that sympathetic denervation decreases VT/VF inducibility after AMI. In the intrinsic cardiac nervous system, most ventricular ganglionated plexi (GP) are primarily located at the origins of several major cardiac blood vessels: surrounding the aortic root, the origins of the left and right coronary arteries, the origin of the posterior descending artery, and the origin of the left obtuse marginal coronary artery [11]. Meanwhile, those sympathetic fibers have little influence on the cardiac conduction system. Our study focused on the local sympathetic nerves, which can modulate the electrical characteristics of the ventricles after myocardial infarction without affecting the function of the heart. Just as seen in our study, the heart rate (HR) and blood pressure (BP) had no obvious changes before and after the LSD.

Previously studies[12,13] have demonstrated that the sympathetic system plays important roles in arrhythmogenesis and sudden cardiac death in both human patients and animal models in the setting of myocardial infarction[14]. Possible mechanisms are sympathetic nerve disorders, hyperinnervation, and heterogeneous regeneration[15,16]. In our study, we applied radiofrequency along the LAD artery to achieve local sympathetic denervation. LSD may markedly reduce the sympathetic control of the heart, indirectly increasing parasympathetic activity. We found that after LSD, the values of ERP and $MAPD_{90}$ were significantly prolonged compared to the values in the control group.

We also found that the density of the sympathetic fibers was much lower in the LSD group, which showed that our denervation method was effective on the local sympathetic nerve. Though LSD may cause a decrease in sympathetic activity of the heart, it may not be anti-arrhythmogenic in the normal heart due to the lack of an appropriate substrate for VA as shown in our study. However, AMI could provide the necessary substrate and/or trigger activity (VPCs) for a lethal VA.

At present, the common measures we take in the prevention of arrhythmias after myocardial infarction include beta-blocker therapy, ICD, and other antiarrhythmic medications. Beta-blockers have been proven to be effective in slowing heart rate, decreasing myocardial contractility and lowering blood pressure. In recent years, studies have shown renal sympathetic denervation to be effective in the treatment of electrical storm and improve autonomic nervous function of the heart[5].

Surgery to resect the stellate ganglion, however, requires thoracoscope use under general anaesthesia, which has significant risk and limitations for myocardial infarction patients. Surgical excision of the stellate ganglion also possess the risk of many complications including horner syndrome, skin paresthesia, and chronic diaphoresis[17]. Our study demonstrates a possible role for the use of radiofrequency ablation to remove the coronary blood vessels' associated cardiac sympathetic nerve fibers, which can selectively remove or weaken the left ventricular sympathetic innervation, and prolong the ERP, APD, and decrease the incidence of ventricular arrhythmia, thus improving ventricular electrophysiological properties after myocardial infarction.

Conclusions

Our work provides a novel, simple, safe and effective way to assist in the prevention of ventricular arrhythmia after myocardial infarction.

Study Limitations

There were several limitations in our study. Firstly, although we investigated the effect of LSD on ventricular electrophysiological properties in AMI hearts, further researches are required to confirm whether the current findings can be applied to chronic myocardial infarction hearts. Secondly, we investigated only the short-term effects of LSD on ventricular electrophysiology and VA occurrence; the long-term effects were not determined. Third, we did not, directly, record the cardiac sympathetic activity.

Acknowledgements

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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