Mechanisms of renal sympathetic denervation on improving ventricular arrhythmias after acute myocardial infarction in rats

Yong-Quan Lu1,2, Li-Yuan Zhu3, Yin-Fen Zhang2, Zi-Guan Zhang2,3, Hong-Lang Huang1, Lin Lin4, Jun Li3, Wu-Yang Zheng1, Xin Jin3, Qiang Xie1

1Department of Cardiology and Xiamen Institute of Cardiovascular Diseases, The First Affiliated Hospital of Xiamen University, Xiamen 361003, China; 2Fujian University of Traditional Chinese Medicine, Fuzhou 350122, China; 3Medical College, Xiamen University, Xiamen 361102, China; 4The First Clinical Medical College, Fujian Medical University, Fuzhou 350108, China

Received April 1, 2019; Accepted April 10, 2019; Epub July 15, 2019; Published July 30, 2019

Abstract: Background: More than 50% of acute myocardial infarction (MI) survivors died from malignant ventricular arrhythmias (VA). Renal sympathetic denervation (RSD) has been demonstrated to exert remarkable effects on VA, but the mechanism remains unclear. Methods: Thirty Sprague Dawley rats were divided into three groups randomly, that is Sham, MI (ligation of left anterior descending artery) and MI+RSD (ethanol ablation). Six hours after modeling, electrocardiogram was recorded. Four weeks later, the left ventricular function indexes were obtained through echocardiography, and cardiac tissues were stained by Masson trichrome for fibrotic analysis. Whole-cell patch-clamp recordings were performed to record the transient outward K+ current (Ito) and the protein expression of Kv4.2 and Kv4.3 in the left ventricle were detected using Western blot. Results: Compared to that in MI group, RSD group showed reduced incidence of premature ventricular contractions and ventricular tachycardia, increased left ventricular ejection fraction and fractional shortening, and decreased left ventricular end diastolic diameter and left ventricular end systolic diameter. RSD attenuated collagen deposition in the cardiac tissue. RSD group alleviated prolonged action potential duration (p < 0.05) especially APD20. The Ito current density was significantly decreased in the MI group compared to the sham group, and was reversed by RSD. MI-induced a decreased cardiac protein expression of Kv4.2 and Kv4.3 in the left ventricle were detected using Western blot. Conclusions: RSD reduced the incidence of VA after MI in rats. This may be due to the improvement of left ventricular function, the recovery of cardiac Ito density and Kv4.2 protein expression.

Keywords: Renal sympathetic denervation, ventricular arrhythmias, myocardial infarction, left ventricular function, Ito current, Kv4.2

Introduction

Ventricular arrhythmia (VA) is the most common complication of acute myocardial infarction (AMI), and it is also an important factor affecting prognosis [1]. Malignant ventricular arrhythmias, such as persistent ventricular tachycardia or ventricular fibrillation, are the main causes of sudden death in AMI patients [2]. The mechanism of ventricular arrhythmias mainly involves reentry and triggering [3, 4], but the VA treatment including antiarrhythmic drugs and catheter ablation are both unsatisfactory at present [5]. More than 50% of AMI survivors died from malignant ventricular arrhythmias [6]. In recent years, the role of the plant nerve system in the occurrence of arrhythmia is becoming more and more important. The relationship between the autonomic nervous system and ventricular arrhythmia is one of the emphases in the research field of cardiac electrophysiology [7]. Substantial evidence has shown that excessive activation of the sympathetic nervous system could increase the susceptibility to ventricular arrhythmias [8]. The renal sympathetic afferent nerve is the origin of excessive activation of the central sympathetic nerve, and the central nervous system trans-
mits activation signals to the kidney, meanwhile taking signals to the heart or other highly sympathetic dominating organs [9-11]. Tsai provided the direct evidence that RSD could reduce the cardiac sympathetic nerve activity, which prompts that RSD has a potential role in reducing the incidence rate of ventricular arrhythmias. Krum [12] first proposed RSD as a new therapeutic measure for refractory hypertension, and follow-up studies found that RSD also had a therapeutic effect on ventricular arrhythmia. Ukena [13] first reported that two patients with chronic heart failure complicated with refractory ventricular arrhythmias were successfully treated by RSD. Boris [14] also reported that a patient with ventricular arrhythmias after acute ST-segment elevation myocardial infarction was effectively controlled by RSD. Armaganian [15] took 10 patients with refractory arrhythmia and implanted them with ICD, and the results showed that the average times of the VT/VF, antitachycardia and electrical cardioversion before RSD were 28.5 (1~106), 20.5 (0~52), 8 (0~88), but they were all reduced after RSD operation 6 months later, respectively times were 0 (0~9), 0 (0~7), 0 (0~3). Evranos [16] made a similar clinical trial to Armaganian and the results also showed that RSD could reduce the average times of the VT/VF, antitachycardia and electrical cardioversion, which suggested that RSD was an effective adjuvant therapy in the treatment of refractory arrhythmia. Linz [17] found that RSD was capable of inhibiting the occurrence of ventricular arrhythmias after acute myocardial ischemia in pigs. Conclusively, a growing body of evidence [18, 19] has confirmed that RSD could reduce the incidence of ventricular arrhythmias, but the mechanism is still unclear. In the research work of this paper, we work to reveal the mechanism of RSD on improving VA after acute myocardial infarction, by studying the relationship between RSD and multiple physiological features of the rat heart, based on the rat model of AMI-induced arrhythmias.

Materials and methods

Animals and model preparation

The Male Sprague-Dawley rats (200-250 g) were purchased from Animal Center (Wushi, Fuzhou, China) and were housed in a 12 h dark/light cycle. The temperature was 22~25°C, and the relative humidity was 55~60%. Thirty experimental rats were divided into three groups randomly, that is sham group (n=10), MI group (n=10) and MI+RSD group (n=10).

In the MI and MI+RSD groups, the rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (3.5 mL/kg), and were ventilated at 75 breaths/min, 5.5 mL tidal volume (Rodent Ventilator, ALC-V8S, Shanghai, China). The heart was exposed and pericardium was incised. The chest was closed and lungs were reinflated using positive end-expiratory pressure. In the MI+RSD group, the bilateral renal artery and vein was exposed immediately after MI operation and the sympathetic innervation was surgically denervated by cutting all visible nerves, and then the vascular wall was daubed with absolute ethanol for 10 minutes. The rats in the sham group, the surgical procedure was performed without coronary artery ligation and RSD was performed.

TTC staining

Infarct size was assessed using TTC staining as described previously [20]. Briefly, the rat hearts were harvested and frozen at -80°C for 5 min, and then rapidly cut into 6 slices approximately 1 mm thick each. The slices were incubated with 1% triphenyl-tetrazolium chloride (TTC, Solarbio, Beijing, China) for 20 min at 37°C and then fixed in 4% formaldehyde for another 30 min. The viable perfused myocardium was colored in red and the infarcted area in white. The ratio of infarcted area to total area was calculated by computerized planimetry with ImageJ software (NIH, USA). The results were expressed in average of percentage of infarcted area on total area in the 6 slices.

H&E staining

To evaluate the effectiveness of the RSD, the renal artery sections were stained with hematoxylin and eosin (HE, Jiancheng, Nanjing, China) to label sympathetic nerves.
Mechanisms of RSD on improving VA

ECG assessment

A standard II lead of electrocardiogram (ECG) was recorded after modeling for 6 hours by using Multichannel physiological signal acquisition system (RM6240CD type, Chengdu, China). Ventricular arrhythmias observed in the ECG were classified as premature ventricular contractions (PVCs), ventricular tachycardia (VT) and ventricular fibrillation (VF) according to the Lambeth standard.

Echocardiographic assessment of left ventricular function

The echocardiogram was performed with high-resolution ultrasound (20 MHz) imaging system (Vevo 2100, Visual Sonics, Ontario, Canada). The left ventricular ejection fraction (LVEF), fractional shortening (FS), left ventricular end diastolic dimension (LVEDD) and left ventricular end systolic dimension (LVESD) were measured before the surgery and after 28 days of left anterior descending coronary artery ligation. The LVEF were defined by the formula LVEF = (LVEDD-LVESD)/LVEDD * 100%. All echocardiograms were measured in triplicate by the same sonographer, and the averages were taken for analysis.

Masson’s staining

The rats were sacrificed by an overdose of chloral hydrate, and then the left ventricles were rapidly harvested and fixed in 4% neutral buffered formalin for 18 hours and embedded in optimal cutting temperature compound (OCTc). Longitudinal sections (6 um) of hearts were prepared using a microtome. The sections were stained with Masson’s trichrome (Maixing, Fuzhou, China) for measurement of fibrosis. The collagen volume fraction in the peri-infarcted areas of left ventricular was calculated by measuring the optical density of fibrotic area using ImageJ software (NIH, USA).

Cardiomyocyte isolation and whole-cell patch-clamp recording

Ventricular cardiomyocytes were isolated by enzymatic dissociation using a method previously reported [21]. Briefly, the rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (3.5 mL/kg). Hearts were rapidly excised and the infarct border zones were harvested. Myocardial tissue was first decomposed by Ca²⁺-free Tyrode’s solution containing II type collagenase (0.5 g/L), bovine serum albumin (1 g/L) and XIV type protease (0.1 g/L) for 50 min, followed by decomposition with the similar solution containing II type collagenase (0.5 g/L) and bovine serum albumin (1 g/L) for 40 min. The whole process was carried out in constant oxygen perfusion at 37°C. The freshly isolated myocytes were centrifuged at 1000 rpm for 3 min and resuspended in KB solution, followed by resting for 3 hours and then preserved at 4°C before electrophysiological recording. The rod-shaped cells with clear cross-striation and without spontaneous contraction were selected for patch recording.

The Whole-cell patch-clamp techniques were performed by an Axopatch 200B amplifier (Axon instrument, USA). Recording pipettes with a tip resistance of 3-5 MΩ when filled with the internal pipette solution were used. For I_to recordings, the pipette solution contained in mM: 20 KCl, 110 K-Asparate, 1 MgCl₂·6H₂O, 5 Na₂-Phosphocreatine, 10 HEPES, 5 K₂-EGTA, 0.1 GTP and 5 Mg₂-ATP (pH adjusted to 7.2 with KOH). 0.2 µM BaCl₂ was used to block I_to and I_K1 respectively. I_to was elicited by a 300 ms depolarizing current stepped from -30 mV to 60 mV with 10 mV increments from a holding potential of -80 mV. The data were acquired with pCLAMP system and analyzed by Origin 7.5 software.

Western blot

The ventricle samples of infarct border zone were lysed, and total protein was extracted using RIPA Lysis Buffer. Before the experiment, the BCA method was used to determine the protein concentration. The 2 mg/mL BSA standard sample was diluted into 8 groups of 0-2000 µg/mL gradient concentrations using deionized water, and the absorbance value corresponding to 562 nm was used as the ordinate to draw the standard concentration curve of the protein sample, and then the protein concentration of the sample to be tested was calculated according to the standard curve. The protein samples were separated on 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinyl-
Mechanisms of RSD on improving VA


Auto-radiography images were analyzed by Carestream Molecular Imaging analysis software.

Statistical analysis

Experimental data were obtained from the statistical average of multiple samples in the same group of subjects and all values were presented as the mean ± standard deviation. Statistical analysis was performed with SPSS 20.0 software. First, the experimental values were statistically analyzed, and the experimental values collected from each sample of sham, MI and MI+RSD group were consistent with normal distribution and odd variance. And then one-way analysis of variance was applied to compare mean differences among the sham, MI and MI+RSD groups. Furthermore, LSD method was used for multiple comparisons, and sham, MI, MI+RSD groups were compared in pairs to analyze whether there were significant differences between them. The statistical method of t test was used for significance test and the p-value of less than 0.05 was considered to be statistically significant.

Results

MI induced by ligating left anterior descending coronary artery

TTC staining was performed to confirm whether the rat model of MI was successfully established. The result showed that the myocardium infarction region was stained white, while the non-infarct area remained red (Figure 1A).

Figure 1. TTC staining was performed to measure the infarct size in rat hearts. A. Photos showed the myocardium infarction region was stained white, whereas the non-infarct area remained red. B. Infarct sizes were presented as a percentage of the infarct area to the total left ventricular area. Values are presented as the mean ± SD. *p < 0.05 versus the sham group; *p > 0.05 versus the MI group.

Figure 2. H&E (hematoxylin and eosin) staining of the renal artery and surrounding nerves with or without RSD (renal sympathetic denervation). Aa. Renal artery and ganglions without RSD (×200). Ab. Magnified view showing a healthy ganglion from image A (×400). Ac. Renal artery and ganglions with RSD (×200). Ad. Magnified view of a damaged ganglion from image C (×400). The ganglionic cells have lost histological details after RSD. B. Sympathetic nerves in RSD group were significantly decreased in comparison with sham group. *p < 0.05.
Mechanisms of RSD on improving VA

Histology of the renal artery and nerves

The renal artery and surrounding nerves were stained with H&E (Figure 2A). The results showed that sympathetic nerves in RSD group were significantly decreased in comparison with sham group (0.25±0.50 vs 2.75±0.50, \( p < 0.05 \) (Figure 2B)). Some nerves lost histological details after RSD. In contrast, these alterations were not observed in rats without ablation.

Effect of RSD on occurrence of AMI-induced arrhythmias

The ECG manifestations in rats were showed in Figure 3A and ST-segment elevation was

\[(41.22±2.23 \text{ vs } 40.06±2.22, \ p > 0.05) \text{ (Figure 1B)}.\]

Figure 3. A. The standard II lead of electrocardiogram (ECG) manifestations of normal, post ligation of coronary artery, PVCs and VT in rats. B. Ventricular arrhythmias could be induced by myocardial infarction. RSD (renal sympathetic denervation) treatment reduced the incidence of PVCs and VT. \(^{a} p < 0.05\) versus the sham group; \(^{b} p < 0.05\) versus the MI group.
Mechanisms of RSD on improving VA

Figure 4. Echocardiographic images of rat hearts in three groups.

clearly observed after coronary artery ligation. Ventricular arrhythmias could be significantly induced by MI. Compared with MI group, MI+RSD group has a lower incidence of PVCs (25.00±2.16 vs 42.50±10.88, p < 0.05) and VT (4.80±1.64 vs 10.00±4.08, p < 0.05) (Figure 3B).

Effect of RSD on cardiac function after MI

The echocardiographic images were showed in Figure 4. There were no significant differences in pre-operative echocardiogram parameters among the three groups. The MI group had significantly decreased LVEF (32.39±10.64 vs 75.28±8.72%, p < 0.05) and FS (16.13±5.68 vs 45.49±7.53%, p < 0.05), and increased LVEDD (8.26±0.79 vs 6.63±0.28 mm, p < 0.05) and LVESD (7.01±0.91 vs 4.85±0.73 mm, p < 0.05) at 4 weeks after coronary artery ligation compared to that of the sham group. The decline of LVEF (32.39±10.64 vs 51.49±8.91%, p < 0.05) and FS (16.13±5.68 vs 27.07±5.42%, p < 0.05) was significantly attenuated by the MI+RSD group, and the elevation of LVEDD (8.26±0.79 vs 6.63±0.28 mm, p < 0.05) and LVESD (7.01±0.91 vs 4.85±0.73 mm, p < 0.05) was also attenuated by the MI+RSD group (Table 1).

Effect of RSD on myocardial fibrosis

Masson staining showed that the cardiac myocytes were dyed red and the collagen fibers were dyed blue under light microscope (Figure 5A). The area of the collagen fraction was significantly increased in MI group compared with sham group (24.84±6.07 vs 1.23±0.15, p < 0.05). The extent of collagen deposition was attenuated in MI+RSD group compared to MI group (9.75±1.22 vs 24.84±6.07, p < 0.05) (Figure 5B).

Effect of RSD on APD in rat hearts

Action potentials were recorded in current clamp mode (Figure 6A). The result showed that MI group had prolonged action potential duration (APD, 137.5±6.81 vs 115.9±6.36, p < 0.05) and APD of 50% repolarization (APD<sub>50</sub>, 58.45±1.44 vs 43.32±2.39, p < 0.05). Compared with MI group, prolonged APD and APD<sub>50</sub> have been shortened in the MI+RSD group (Figure 6B).

Effect of RSD on I<sub>to</sub> in rat hearts

To detect the effect of RSD on I<sub>to</sub> in MI rats, we examined I<sub>to</sub> under whole-cell voltage-clamp mode. At the test potential of +60 mV, the current density of I<sub>to</sub> was significantly decreased in MI group than that in sham group (11.30±5.81 vs 22.10±10.55, p < 0.05), while RSD treatment could obviously increase the suppressed I<sub>to</sub> current density (15.82±7.80 vs 11.30±5.81, p < 0.05). The current-voltage (I-V) curves demonstrating the changes of I<sub>to</sub> in different groups were plotted in Figure 7B.

Effect of RSD on protein expression of Kv4.2 and Kv4.3

Western immunoblots indicated that the protein expression levels of myocardial Kv4.2 and Kv4.3 were significantly decreased in the MI group compared with sham group (0.79±0.04 vs 1.00±0.00, p < 0.05 and 0.86±0.06 vs 1.00±0.00, p < 0.05) (Figure 7B).
Mechanisms of RSD on improving VA

Table 1. Changes of echocardiographic parameters in the three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>LVEF (%)</th>
<th>FS (%)</th>
<th>LVEDD (mm)</th>
<th>LVESD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>10</td>
<td>75.28±8.72</td>
<td>45.49±7.53</td>
<td>5.70±0.33</td>
<td>3.37±0.71</td>
</tr>
<tr>
<td>MI</td>
<td>8</td>
<td>32.39±10.64</td>
<td>16.13±5.68</td>
<td>8.26±0.79</td>
<td>7.01±0.91</td>
</tr>
<tr>
<td>MI+RSD</td>
<td>6</td>
<td>51.49±8.91</td>
<td>27.07±5.42</td>
<td>6.63±0.28</td>
<td>4.85±0.73</td>
</tr>
</tbody>
</table>

Note: LVEF (left ventricular ejection fraction), FS (fractional shortening), LVEDD (left ventricular end diastolic dimension), LVESD (left ventricular end systolic dimension), MI (myocardial infarction), RSD (renal sympathetic denervation). Values are presented as the mean ± SD. *p < 0.05 versus the sham group; †p < 0.05 versus the MI group.

Discussion

In our experiment, we first observed the effect of RSD on the occurrence of VA. Second, we recorded the APD and found the prolonged APD in MI group could be shortened by RSD especially APD_{50}. APD_{50} mainly reflected repolarization I phase of the action potential, so we detected I_{to} and its relevant ion channel proteins.

In the rat AMI model, we showed that the I_{to} density and protein expression level of Kv4.2 and Kv4.3 were decreased in the marginal zone of myocardial infarction. Furthermore, we first found that RSD could alleviate the decline of I_{to} density and up regulate Kv4.2 protein expression level. I_{to} is the main current of repolarization I phase of action potentials in cardiac myocytes, and it can affect the action potential morphology and duration. Kv4.2 and Kv4.3 are the major subunits of I_{to} in cardiomyocytes. Previous studies have shown that the protein expression levels of Kv4.2 and Kv4.3 in cardiomyocytes were decreased after MI, and it could decrease I_{to} density and then prolonged action potential duration (APD) [22, 23], which can cause the occurrence of early after depolarization and contribute to ventricular arrhythmias. Moreover, the decrease of I_{to} current density can increase the sensitivity of the myocardium to hypokalemia, ischemia and acidosis, which can increase the susceptibility to arrhythmias. Some studies have showed that cardiac nerve sprouting or isoproterenol could suppress I_{to} and increased the susceptibility to ventricular fibrillation. Ventricular electrical remodeling is one of the important factors of the occurrence of ventricular arrhythmia after myocardial infarction, and myocardial electrophysiological heterogeneity in different positions of the myocardium increases the risk of ventricular arrhythmias. Here we show that RSD can reduce the incidence of ventricular arrhythmias, and its mechanism is possibly related to attenuation of the decline of I_{to} current density through the up-

![Figure 5. Masson’s trichrome staining for assessment of myocardial fibrosis. A. Photos showed that the cardiac myocytes were dyed red and the collagen fibers were dyed blue under light microscope (×100). B. The area of the collagen fraction was significantly increased in MI groups compared with sham groups. RSD treatment attenuated myocardial fibrosis in MI rats. *p < 0.05, versus the sham group; †p < 0.05 versus the MI group.](image-url)
Mechanisms of RSD on improving VA

antiarrhythmic role. In addition, our results demonstrated RSD’s effect of improving cardiac function after MI by increasing EF and FS and reducing LVEDD and LVESD. Collagen fiber hyperplasia is an important factor in myocardial fibrosis and cardiac remodeling after MI, the Masson staining showed that RSD can reduce the degree of myocardial fibrosis, which may be an important pathological mechanism of its role improving cardiac function after MI.

Conclusions

Electrical remodeling and structural remodeling play a critical role in the occurrence and maintenance of ventricular arrhythmias after myocardial infarction. Our results indicated that the mechanism of RSD on inhibition of VA after MI was linked to the reverse of $I_{to}$ density through the upregulation of Kv4.2 protein expression and the improvement of cardiac function.

Acknowledgements

This study was supported by funds from Young and Middle-Aged High-Level Backbone Talent Training Project of Fujian Health System (2013-ZQN-ZD-32), Key Project of Fujian Science and Technology Program (2014-D023), Natural Science Foundation of Fujian Province (2016J01637) and Science and Technology Project of Xiamen (3502Z20154007).

Disclosure of conflict of interest

None.
Mechanisms of RSD on improving VA

Address correspondence to: Dr. Qiang Xie, Department of Cardiology and Xiamen Institute of Cardiovascular Diseases, The First Affiliated Hospital of Xiamen University, 55 Zhenhai Road, Xiamen 361003, China. Tel: +86-592-2139716; Fax: +86-592-2139550; E-mail: arthur2014@sina.com; Dr. Xin Jin, Medical College, Xiamen University, Xiang’an South Road, Xiamen 361102, China. Tel: +86-592-2188676; Fax: +86-592-2188676; E-mail: xinjin@xmu.edu.cn

References


Mechanisms of RSD on improving VA


