Relationship between sympathetic nerve sprouting and repolarization dispersion at peri-infarct zone after myocardial infarction

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Abstract

Sympathetic nerve sprouting is thought to contribute to sudden cardiac death (SCD) in chronic myocardial infarction (MI). However, the mechanisms remain unclear. This study investigated the relationship between sympathetic nerve sprouting and repolarization dispersion at peri-infarct zones after MI. Thirty adult New Zealand White rabbits underwent coronary artery ligation (MI group: n = 20) or sham operation (SO group: n = 10). Eight weeks after surgery, transmural dispersion of repolarization (TDR) was examined at the peri-infarct zones in MI group and corresponding zones in the SO group at baseline and during sympathetic nerve stimulation. Sympathetic nerve sprouting was detected by immunocytochemical staining using anti-growth associated protein 43 (GAP43) and anti-tyrosine hydroxylase (TH) antibodies. The results demonstrated that TDR was significantly larger at peri-infarct zones in MI group than the corresponding zone in SO group at baseline or during sympathetic nerve stimulation. The densities of both GAP43- and TH-positive nerves were significantly higher at peri-infarct zones in infracted hearts than the corresponding zones in control hearts (both p < 0.01). In the MI group, the density of GAP43- or TH-positive nerves at peri-infarct zones had a significantly positive correlation with the TDR or ΔTDR (change in TDR) at baseline as well as with sympathetic nerve stimulation (p < 0.05 for all). These results suggested that sympathetic nerve sprouting is more pronounced and heterogeneous at peri-infarct zones at 8 weeks after MI. The excessive sprouting of sympathetic nerves increases local ventricular TDR, which may be a potential mechanism for SCD in chronic MI.

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1. Introduction

Half of the patients who survived acute myocardial infarction (MI) ultimately die of sudden cardiac death (SCD). Malignant ventricular arrhythmias account for most of the SCDs. Previous studies have shown electrophysiological changes termed as electrical remodeling in infarcted ventricles, which contribute to the occurrence of ventricular arrhythmias (Pinto and Boyden, 1999; Qin et al., 1996). There may also be a neural mechanism for SCD after MI, because most commonly, fatal arrhythmias occurring after MI are the result of a rapid increase in cardiac sympathetic nerve activity (Vanoli and Schwartz, 1990). Recently, a SCD model based on the hypothesis of sympathetic nerve sprouting was proposed (Cao et al., 2000a,b; Chen et al., 2001; Dae et al.; 1997; Lai et al., 2000). A series of studies demonstrated that abnormal increase in sympathetic nerve sprouting was responsible for the ventricular arrhythmogenesis after MI. Infusion of nerve growth factor (NGF) to the left stellate ganglion in MI dogs accelerated and intensified the magnitude of nerve sprouting, resulting in a high incidence of SCD. The magnitude of sympathetic nerve sprouting was an important determinant of SCD in chronic MI (Cao et al., 2000a,b; Chen et al., 2001; Lai et al., 2000). It is evident that sympathetic neural remodeling in the form of sympathetic nerve sprouting and hyperinnervation contributes to ventricular arrhythmogenesis and SCD after MI.
Although these studies have demonstrated the association between sympathetic nerve sprouting and arrhythmic risk after MI, mechanisms underlying the arrhythmogenic effect of sympathetic nerve sprouting remain unclear. Since electrophysiological changes can be the key factor leading to arrhythmogenesis, we hypothesized that there might be some link between sympathetic nerve sprouting and electrophysiological properties. In the present study, we examined the relationship between sympathetic nerve sprouting and transmural dispersion of depolarization (TDR) after MI in the rabbit heart.

It is thought that the peri-infarct zone can be an important component of the reentrant circuit, and thus critical for ventricular arrhythmogenesis in chronic MI (Cabo and Boyden, 2003; Verma et al., 2005; Wong et al., 1982). The peri-infarct zone was also the area with most pronounced sympathetic nerve sprouting found in the studies by Oh et al. (2006) and Xi et al. (2004). Therefore, we elected to investigate the peri-infarct zone to clarify the relationship between sympathetic nerve sprouting and repolarization dispersion in this MI model. Moreover, sympathetic nerve stimulation was performed to observe the change of local repolarization dispersion at peri-infarct zone.

2. Methods

All experiments were approved by the Institutional Animal Care and Use Committee of Wuhan University, and were conducted in accordance with the Guideline for the Care and Use of Laboratory Animals.

2.1. Animal preparation

Thirty New Zealand White rabbits, including both male and female, weighing 2.0–2.5 kg, were randomized into...
either MI group \((n=20)\) or a sham operation (SO) group \((n=10)\). All rabbits were anesthetized with sodium pentobarbital \((30 \text{ mg/kg i.v.)}\). Under controlled ventilation, a thoracotomy through a left parasternal incision was performed, the pericardium was incised and the anterior wall of the left ventricle was exposed. The left anterior descending (LAD) coronary artery of the animals in the MI group was carefully ligated 2 mm distal to the origin of the first diagonal artery. A successful MI model was confirmed by elevation of the ST segment by more than 0.2 mV in leads I, II and aVL. The animals in the SO group underwent thoracotomy without ligation of the LAD. Postoperatively, each rabbit received 400,000 IU penicillin intramuscularly twice daily for 2 days and was fed with standard diet for 8 weeks.

2.2. Monophasic action potential recording

Eight weeks after surgery, all surviving rabbits including the MI group and SO group underwent a second thoracotomy. In order to pace and stimulate the heart, a slower intrinsic heart rate was needed. Thus the sinoatrial node was destroyed by injection of 0.3 ml of formaldehyde \((100 \text{ ml/l})\) into the region between the right atrial appendage and superior vena cava. Continuous epicardial bipolar pacing was delivered from the high right atrium at a cycle length \((CL)\) of 300 ms.

The technique of recording monophasic action potential \((MAP)\) has been described in detail in our previous studies \((Huang et al., 2004, 2006; Jiang et al., 2006)\). The MAP recordings consist of electrograms from epicardial, midmyocardial and endocardial sites. The MAP electrodes were penetrated into the peri-infarct zone in the MI group or corresponding zone in the SO group. The reference electrode was placed on the thorax. The peri-infarct zone was defined as the zone within 3 mm of the marginal zone between the infarct zone and non-infarct zone. The boundary between the infarct zone and non-infarct zone was visually identified by differences in color, and confirmed by HE staining (see Results and Fig. 1). All signals were recorded with a polygraph \((LEAD2000B, \text{ Jinjiang Ltd, China})\) and were filtered between 0.05 Hz and 300 Hz. The \(\text{MAPD}_{90\text{Epi}}, \text{MAPD}_{90\text{Mid}}, \text{MAPD}_{90\text{Endo}}\) and transmural dispersion of depolarization \((TDR)\) at baseline were acquired. \(\text{MAPD}_{90\text{Epi}}, \text{MAPD}_{90\text{Mid}}\) and \(\text{MAPD}_{90\text{Endo}}\) were defined as monophasic action potential duration \((\text{MAPD})\) at 90% repolarization in epicardium \((\text{Epi})\), midmyocardium \((\text{Mid})\) and endocardium \((\text{Endo})\), respectively. \(TDR\) was defined as the difference between the longest and the shortest \(\text{MAPD}_{90}\) among \(\text{Epi}, \text{Mid}\) and \(\text{Endo}\).

2.3. Sympathetic nerve stimulation

Anatomically, most sympathetic efferent nerve fibers innervating the left ventricle are contained in the left inferior cardiac sympathetic nerve \((\text{LICSN, arising from the left stellate ganglion})\) \((Tomokazu, 2005)\), which accordingly should contain the main fibers of the sprouting sympathetic nerves which was injured due to MI in left ventricle. Thus stimulation of LICSN could excite sprouting sympathetic nerves especially in the peri-infarct area, which has the most pronounced sprouting and hyperinnervation. The method of LICSN stimulation was previously described by other investigators \((Yoshioka et al., 2000; Miyamoto et al., 2004)\). The left ventricular end systolic pressure \((\text{LVESP})\) was monitored via a catheter placed in the right common carotid artery. An increase in LVESP was used as an indicator to assess the efficacy of LICSN stimulation. Bilateral cervical vagus nerves were isolated and disconnected to eliminate any vagal effect on the heart during LICSN stimulation. The LICSN was exposed, carefully isolated from the surrounding tissues, and placed on a pair of silver electrodes for stimulation. To prevent drying and provide insulation from the surrounding tissues, the stimulation electrodes and the nerves were immersed in a mixture of Vaseline and liquid paraffin. Continuous nerve stimulation was obtained via a stimulator \((\text{Grass Instrument, USA})\) at a frequency of 5 Hz and a pulse width of 2 ms. The stimulation amplitude was adjusted to induce a 20 mmHg rise in LVESP and sustained for 30 s before recording. The amplitude of sympathetic nerve stimulation ranged from 2 to 4 V.

\(\text{MAPD}_{90}, TDR\) and \(\Delta TDR\) at the peri-infarct zone were measured during sympathetic nerve stimulation. \(\Delta TDR\), defined as the change in \(TDR\) from prior to \((i.e. \text{after bilateral cervical vagus nerves disconnected})\) and then during sympathetic nerve stimulation, was used to reflect the net effect of sympathetic nerve stimulation on \(TDR\).

2.4. Immunocytochemical staining

Antibodies including anti-growth associated protein 43 \((\text{GAP43})\) antibody and anti-tyrosine hydroxylase \((\text{TH})\) antibody \((\text{monoclonal mouse anti-GAP43 and anti-TH, Zymed Inc, USA})\) were used for immunocytochemical staining. GAP43, a protein expressed in the growth cones of sprouting axons, has been shown to be a marker of nerve sprouting \((Meiri et al., 1986)\). TH is the rate-limiting enzyme of norepinephrine \((\text{NE})\) synthesis, which serves as not only a marker for sympathetic nerve location \((Wharton et al., 1990)\), but also an indirect indicator of sympathetic activity \((Li et al., 2004)\). The combination of GAP43 and TH can therefore precisely reflect the sprouting of sympathetic nerves. Tissues were vertically sectioned from epicardium to endocardium at the peri-infarct zone in the MI group or the corresponding zone in the SO group where \(TDR\) was measured. Nerve densities were determined by a computer-assisted image analysis system \((\text{Image-Pro Plus 3.0, Media Cybernetics, USA})\). Each slide was examined under a microscope to select 3 fields with the highest density of nerves. The computer then automatically calculated the area occupied by the nerves in the field. The nerve density was the area occupied by the nerves divided by the total area.
examined (μm²/mm²). The mean density of nerves in these 3 selected fields was used to represent the nerve density of that slide.

2.5. Statistical analysis

All values were expressed as mean ± SD. Non-paired t test was used for comparison between the MI group and the SO group. Paired t test was used for comparison between before and during sympathetic nerve stimulation. Linear correlation analysis was used to determine the correlation of the indices between sympathetic nerve sprouting and repolarization dispersion. Statistical significance was defined as \( p \leq 0.05 \).

3. Results

3.1. Animal preparation

In the MI group, after LAD ligation, all animals showed ST-segment elevation in leads I, II and aVL. There was no change in the ST segment in the SO group. One rabbit in the MI group died of ventricular fibrillation immediately after LAD ligation. Another one died with diarrhea on the 35th day after LAD ligation. The study was completed in the 28 surviving animals (18 in the MI group and 10 in the SO group).

Eight weeks after surgery, all surviving animals in the MI group showed a deep Q wave in leads I, II and aVL. The peri-infarct zone was distinctly located between the pale infarct zone and the dark red non-infarct zone. HE staining (Fig. 1) showed typical histopathological changes of the myocytes, such as coagulation necrosis at the infarct zone, a near normal histomorphology at the adjacent non-infarct zone and a mixed appearance at the peri-infarct zone.

3.2. Sympathetic nerve sprouting

Significant sympathetic nerve hyperinnervation was observed in infarcted hearts. Immunohistochemical staining (Fig. 2) showed that both GAP43- and TH-positive nerves were more abundant and heterogeneously located in the peri-infarct zone in the MI group than in the corresponding zone in the SO group at 8 weeks after MI (Fig. 3). The sprouting nerves appeared to be excessively aggregated and reticulated at the peri-infarct zone in all MI animals (as shown in Fig. 2).

The ratio of TH/GAP43 was significantly higher at the peri-infarct zone in the MI group than at the corresponding zone in the SO group (80±5 vs. 70±6%, \( p < 0.01 \)), indicating that the sprouting nerves were mainly sympathetic.

3.3. TDR at baseline

As shown in Fig. 4 and Table 1, at baseline during pacing with a CL of 300 ms from the high right atrium, the MAPD₉₀

![Graph showing nerve density comparison between MI and SO groups](image-url)
was the longest at the Mid and shortest at the Epi in both MI and SO groups; however, the dispersion of MAPD90 (TDR) was much larger in infarcted hearts (MI group: 35±7 ms; SO group: 20±9 ms, \( p < 0.01 \)) than in the normal hearts.

### 3.4. TDR during sympathetic nerve stimulation

Fig. 4 shows MAPs recorded at baseline, before and during sympathetic nerve stimulation. After bilateral cervical vagus nerves were disconnected, MAPD90 and TDR did not change significantly compared to the baseline state in both groups, however, while the LICSN was effectively stimulated, the MAPD90 and TDR changed markedly: (1) In the SO group, MAPD90 shortened similarly in all 3 layers of myocardium, concomitantly, TDR decreased from 17±8 ms (before stimulation) to 10±7 ms (\( p < 0.01 \)) with a \( \Delta \)TDR = −8±6 ms; (2) In the MI group, MAPD90 of the 3 layers shortened to a widely different extent, MAPD90Epi had the greatest amount of shortening while MAPD90Mid had the least shortening, therefore, TDR increased significantly from 33±7 ms (before stimulation) to 59±11 ms (during stimulation, \( p < 0.01 \) vs. before stimulation). \( \Delta \)TDR markedly increased to 26±7 ms (\( p < 0.01 \) vs. SO group) (Fig. 4, Table 1).

### 3.5. Correlation of sympathetic nerve sprouting and repolarization dispersion

We further analyzed the correlation between the indices of sympathetic nerve sprouting and repolarization dispersion in

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**Table 1**

<table>
<thead>
<tr>
<th>Corresponding zone in SO group</th>
<th>Peri-infarct zone in MI group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Before stimulation</strong></td>
</tr>
<tr>
<td>MAPD90Epi (ms)</td>
<td>228±22</td>
</tr>
<tr>
<td>MAPD90Mid (ms)</td>
<td>247±17</td>
</tr>
<tr>
<td>MAPD90Endo (ms)</td>
<td>236±20</td>
</tr>
<tr>
<td>TDR (ms)</td>
<td>20±9</td>
</tr>
<tr>
<td>( \Delta )TDR (ms)</td>
<td>–</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Before stimulation</strong></td>
</tr>
<tr>
<td>MAPD90Epi (ms)</td>
<td>223±13</td>
</tr>
<tr>
<td>MAPD90Mid (ms)</td>
<td>258±15</td>
</tr>
<tr>
<td>MAPD90Endo (ms)</td>
<td>231±15</td>
</tr>
<tr>
<td>TDR (ms)</td>
<td>35±7</td>
</tr>
<tr>
<td>( \Delta )TDR (ms)</td>
<td>–</td>
</tr>
</tbody>
</table>

Before stimulation represents the state after bilateral cervical vagus nerves were disconnected.

MI, myocardial infarction; SO, sham operation; Epi, epicardial cells; Mid, midmyocardial cells; Endo, endocardial cells; MAPD, monophasic action potential duration (ms); TDR, transmural dispersion of repolarization (ms); \( \Delta \)TDR, the change in TDR from prior to and then during sympathetic nerve stimulation (ms).

\( *p < 0.01 \) vs. before stimulation; \( #p < 0.01 \) vs. corresponding zone in SO group.
MI animals. Fig. 5 shows the results of this correlation analysis. The density of GAP43- or TH-positive nerves at the peri-infarct zone had a significantly positive correlation with the TDR at baseline, i.e., TDR increased significantly as the magnitude of sympathetic nerve sprouting increased. In addition, during sympathetic nerve stimulation, there was also a positive correlation between the density of GAP43- or TH-positive nerves and TDR or ΔTDR with the same stimulus intensity ($p < 0.05$ for all), which also suggested that the magnitude of sympathetic nerve sprouting influenced the local transmural dispersion of ventricular repolarization.

4. Discussion

Previous studies by other investigators (Cao et al., 2000a, b; Chen et al., 2001; Lai et al., 2000) have demonstrated that heterogeneous cardiac sympathetic nerve sprouting and hyperinnervation play an important role in SCD in chronic MI models, however, the mechanism was unclear. The present study found that the magnitude of sympathetic nerve sprouting was associated with local transmural dispersion of ventricular repolarization, which suggested a potential mechanistic link between sympathetic nerve sprouting and ventricular arrhythmogenesis.

MI elicits nerve sprouting. The nerve fibers innervating the heart that were injured during MI triggered the reexpression of NGF (Zhou et al., 2004) or other neurotrophic factor genes (Lai et al., 2000; Habecker et al., 2005) in the nonneural cells around the injury site. These responses may lead to sympathetic nerve sprouting and regional myocardial hyperinnervation. Consistent with the reports by Xi et al. (2004) and Oh et al. (2006), sympathetic nerve sprouting was more excessive and heterogeneous at the peri-infarct zone in infarcted hearts than the corresponding zone in normal hearts at 8 weeks post infarction. We postulate that the peri-infarct zone contains necrotic cells (including myocytes, neurocytes and other cells), injured but surviving cells and cells that are minimally injured by MI. The heterogeneous groups of cells provide not only an optimal substrate for nerve regeneration but also an activity of neurotrophic factor synthesis which facilitates nerve sprouting around the injury sites. In the study by Oh et al. (2006), gene expression of neurotrophic factors, such as NGF, insulin-like growth factor, leukemia inhibitory factor, was increased up to 2 months after MI compared with normal controls. Expression of these neurotrophic factors was more pronounced and persistent in the peri-infarct area than the remote infarct area. Their findings suggest potential mechanisms for the excessive sympathetic nerve sprouting at the peri-infarct zone observed in the present study.

TH is the rate-limiting enzyme of norepinephrine (NE) synthesis. Although TH expression was temporarily decreased due to injury at the peri-infarct sites in the early stage (1 week) of MI (Li et al., 2004), it was significantly elevated at 8 weeks. The abnormal increase in TH expression may be a marker for active noradrenergic neurotransmission due to excessive sprouting of sympathetic efferent postganglionic nerves at the peri-infarct zone. That is, infarction alters both the distribution and noradrenergic properties of cardiac sympathetic nerves at the peri-infarct zone.

Consistent with other reports (Cabo and Boyden, 2003; Wong et al., 1982), the present study showed abnormal electrophysiological changes at the peri-infarct zone where sympathetic nerve sprouting was extremely prominent. Widely different MAPD90 among the Epi, Mid and Endo layers made transmural heterogeneity of repolarization increase after MI. Burton et al. (2000) also demonstrated that local heterogeneity of refractoriness is more marked at the peri-infarct zone than remote zone. It is thought that these arrhythmogenic changes mainly result from the damaging effect on myocardial cells during acute MI and reparative processes (Pinto and Boyden, 1999; Qin et al., 1996). However, the present study suggests that sympathetic nerve sprouting may also be involved in this electrical remodeling process. This is supported by the fact that the density of sympathetic nerves had a significantly positive correlation with local TDR at the peri-infarct zone, i.e., the degree of repolarization dispersion increased as sympathetic nerve sprouting became more excessive. Increased transmural dispersion of ventricular repolarization predisposes the heart to reentrant ventricular arrhythmias. Another supporting fact was that a positive correlation between the density of the sprouting sympathetic nerves and TDR or ΔTDR during sympathetic nerve stimulation was also demonstrated in the present study. As stated in the Methods, LICSN, in which the main fibers of the sympathetic nerves in left ventricle are contained, was stimulated in order to examine the change of local repolarization dispersion due to sympathetic nerve excitation. With the same stimulus intensity, LICSN stimulation shortened action potential duration but increased repolarization dispersion related to the degree of sympathetic nerve sprouting, which also supports the hypothesis that the magnitude of the sympathetic nerve sprouting influences ventricular repolarization dispersion. A similar response was found in the study by Yoshioka et al. (2000), although that study focused on regional sympathetic nerve denervation induced by acute injury. In their study, the degree of repolarization dispersion increased as denervation became more severe, established at baseline or sympathetic nerve stimulation as applied in our study. Therefore, heterogeneity of sympathetic innervation, either regional hyperinnervation due to sprouting or denervation due to acute injury, contributes to increased repolarization dispersion. In another study from our laboratory (Jiang et al., 2006), we found that metoprolol, a selective β1-blocker, could inhibit sympathetic hyperinnervation and repolarization dispersion at the peri-infarct site after MI, and the association of metoprolol with improved electrical heterogeneity was related to the inhibition of sympathetic sprouting. This suggests a mechanism for the protective effect of metoprolol in prevention of SCD.

Although the present study suggests that excessive sympathetic nerve sprouting increases local transmural dispersion of ventricular repolarization, the underlying mechanisms
are yet to be clarified. One possibility is that myocardial cells among Epi, Mid and Endo layers with inherently different electrophysiological characteristics contribute different sensitivities to the neurotransmitter (NE). It also can be caused by heterogeneous distribution of sprouting sympathetic nerves in those 3 layers. The transmural staining showed markedly aggregated and reticulated sympathetic nerves, which were so heterogeneous distributed that hardly any growth pattern among the 3 layers could be identified (as shown in Fig. 2). Electrophysiological property, for example, ion channel activity, would be remodeled in the area of transmurally heterogeneous sympathetic innervations, which serve as another probable mechanism responsible for the increase of transmural dispersion of repolarization.

4.1. Study limitations

First, in this study, the method for identifying the peri-infarct zone may be not precise enough, which is the problem with such studies (Burton et al., 2000; Cabo and Boyden, 2003; Oh et al., 2006; Wong et al., 1982; Xi et al., 2004); however, peri-infarct zone was confirmed by HE staining in almost all MI animals, and we determined that the tissue for detecting sympathetic nerve sprouting was in the same place at which TDR was examined. This indicated that our results regarding the correlation between the indices of sympathetic nerve sprouting and repolarization dispersion were unaffected by spatial differences. Second, the present study showed a significant increase in the mean TDR at the peri-infarct zone, however, we did not investigate any change in ion currents, and therefore, we do not know which currents will be affected by sympathetic nerve sprouting. Third, the densities of GAP43- and TH-positive nerves among the Epi, Mid and Epi layers were not examined, because we focused on the relationship between overall transmural density of sympathetic nerve sprouting and repolarization dispersion, thus tissues were vertically (but not horizontally) sectioned to get the data of overall transmural density.

5. Conclusion

Sympathetic nerve sprouting is more pronounced and heterogeneous at the peri-infarct zone at 8 weeks after MI. The magnitude of sympathetic nerve sprouting was associated with local transmural dispersion of ventricular repolarization. The excessive sprouting of sympathetic nerves coupled with electrically remodeled myocardium may be a potential mechanism for SCD in chronic MI. Future interventions targeting sympathetic nerve sprouting may reduce the risk of SCD.

Acknowledgments

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