Effects of angiotensin converting enzyme inhibition on cardiac innervation and ventricular arrhythmias after myocardial infarction

Su-hua Yan¹
He-sheng Hu¹
Le-xin Wang²
Qi-chong Xing¹
Wen-juan Cheng¹
Mei Xue¹

¹Department of Cardiology, Qianfoshan Hospital of Shandong Province, Jinan, 250014, China
²School of Biomedical Sciences Charles Sturt University Wagga Wagga, NSW 2678, Australia

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Abstract

Purpose: To investigate the influence of angiotensin-converting enzyme inhibitor (ACEI) on cardiac innervation and inducible ventricular arrhythmias (VAs) in healed myocardial infarction (MI).

Methods: Left anterior descending coronary artery was ligated to induce MI in 30 rabbits. After oral captopril (10mg/kg/d) for 8 weeks, electrophysiological study was performed to evaluate the incidence of inducible VAs. RT-PCR and immunohistochemistry were used to measure the cardiac innervation.

Results: Eight weeks after the operation, the incidence of inducible VAs in the MI-placebo group was higher (58.3%, 7/12) than in the sham operation group (16.7%, 2/12, P<0.05). However, the incidence of inducible VAs in the MI-captopril group was lower (27.2%, 3/11) than in the MI-placebo group (P<0.05). Proliferation and growth of nerve fibres in the MI-placebo group were mainly distributed at the periphery of the infarcted and perivascular regions of the myocardium. The density of nerve fibres increased in the MI-placebo group (3889±521 μm²/mm²) compared with the sham group (1727±304 μm²/mm², P<0.01) at the infarct border. In the MI-captopril group, the density of nerve fibres (3507±433 μm²/mm²) at the infarct border did not differ from that in the MI-placebo group (P=0.07). MI-induced abnormal nerve fibre distribution was partly restored by captopril treatment.

Conclusion: In this study, prolonged captopril treatment was effective in preventing VAs in healed MI, partly by attenuating the spatial heterogeneity of cardiac innervation.

Nerve sprouting and heterogeneous cardiac reinnervation may be induced by myocardial infarction (MI), rapid pacing, radiofrequency catheter ablation, hypercholesterolemia and stem cell transplantation.¹ Excessive reinnervation leads to hyperinnervation and spatial heterogeneity of cardiac nerves and contributes to arrhythmogenesis and sudden cardiac death post MI.²-⁴ Spatial variation of regional innervation results in heterogeneity in cardiac repolarization after sympathetic stimulation, which may cause a high incidence of ventricular arrhythmias (VAs).⁵ These responses suggest that restoration of abnormal cardiac reinnervation may become a novel anti-arrhythmic strategy after MI.

Multiple randomized trials have demonstrated that angiotensin converting enzyme inhibitors (ACEI), such as captopril, reduce mortality and morbidity after MI.⁶-⁷ Long-term ACEI treatment has proven benefi-
cial in improving left ventricular remodeling and reducing the QT dispersion (QTd) following MI. Increased QTd is a marker of electrical instability predisposing to VAs and sudden cardiac death. The reduction of ventricular repolarization inhomogeneity indicates an antiarrhythmic effect but the exact mechanisms are still unknown.

Long-term ACEI treatment attenuated the abnormalities of cardiac innervation in chronic heart failure dogs and prevented reduction of periarterial innervation in spontaneous hypertensive rats. However, whether prolonged treatment with ACEI after MI could influence cardiac innervation and reduce the incidence of inducible VAs is still unknown. The present study was undertaken to explore the effects of long-term ACEI treatment on cardiac innervation and inducible VAs in a rabbit model.

Materials and Methods

New Zealand white rabbits were obtained from the animal house of Shandong University, China. The study was conducted according to the institutional guidelines for animal care and research, which are in line with guidelines for the care and use of animal by National Institute of Health (NIH, 1996, No.85-23).

Animal model

Forty-two rabbits, 2.5-3 kg, were used for the study. Animals were anesthetized with intraperitoneal injection of pentobarbital sodium (40 mg/kg). Electrodes were attached to the four limbs to record the electrocardiogram during the experiment. In 30 animals, the left anterior descending coronary artery (LAD) was ligated to create MI as described previously. A sham group (n=12) also underwent thoracotomy and pericardiotomy but without coronary artery ligation. Successful creation of MI was verified by the occurrence of ST-segment elevation on the ECG and by colour changes in the epicardium from the affected areas. After the operation, all rabbits were individually housed in cages in a room with air conditioning and were fed with standard feed and water.

MI animals were assigned randomly to MI-ACEI (captopril 10mg/kg/d, Squibb, China, n=15) group or MI-placebo group (n=15) for 8 weeks of treatment after the operation.

Electrophysiological study protocol

Rabbits were anesthetized at the end of the study. After thoracotomy, bipolar pacing was performed using a paired unipolar electrode configuration, which was implanted in the infarct border zone. Programmed electrical stimulation was performed to measure the effective refractory period (ERP) and to induce VAs. Standard pacing protocols were used to determine basic electrophysiological parameters. Briefly, a train of eight beats (S1) was delivered at a basic cycle length of 180 ms followed by the delivery of a single premature beat (S2), which was decremented by 5 ms in successive runs to determine ERP. In an attempt to induce ventricular arrhythmias, single (S1S2) and double extra stimuli (S1S2S3) during ventricular drive at a cycle length of 180, 160 and 140 ms and rapid ventricular pacing at a rate of 180 ms for 8 minutes were delivered.

A 12-lead ECG was recorded in all animals. QT dispersion (QTd) was defined as the difference between the longest and shortest QT interval among the 12-lead ECG. To avoid the impact of heart rate on QTd, Bazett’s formula (QTc = QT/RR1/2) was used to calculate the corrected QT interval dispersion.

RT-PCR

After electrophysiological study, the left ventricles were cut into two parts. One was the infarct border and the other, located more than 2 cm away from the infarct area, was selected as non-infarct left ventricle free wall (LVFW). Half of these samples were frozen in liquid N2 immediately for RT-PCR. Total RNA was extracted using Trizol (Invitrogen, USA) according to the manufacturer’s instructions. Tyrosine hydroxylase
(TH, a marker of sympathetic nerves) and neurofilament (NF, a marker of nerve axons) were selected as target genes. The primer sequences of TH (AF493546) were as follows: forward primer, AAT TCG ATT CCG ACC TGG AT; reverse primer, GAT GTA CTG GGT GCA CTG GA; length, 398 bp. The primer sequences of NF (Z47378) were as follows: forward primer, CAG CAC ATT TTC AGG AAG CA; reverse primer, 5'- CTG CTG GGC TCA GGT CTA AC-3'; length, 1115 bp. The mRNA levels of these candidate genes were measured by the semi-quantitative RT-PCR. Reverse transcription (RT) was carried out with AMV reverse transcriptase (TaKaRa). In each assay, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH, housekeeping gene, AB231852, forward primer: GCG CCT GGT CAC CAG GGC TGC TT; reverse primer: TGC CGA AGT GGT CGT GGA TGA CCT) was used for normalization, length: 465 bp. The primers for each selective gene were designed using the primer express software (Primer3) according to the sequences by the GenBank. Digital images of the stained gels were taken using UVIpro gel documentation systems (England) with an integrated UV transilluminator and a CCD camera; densitometry of PCR products was performed using basic analysis software package. Expression of the different target genes studied was normalized to the expression of GAPDH in the same cDNA sample.

**Immunocytochemistry**

Ventricular muscle samples were directly immersed in 10% formalin for 24 hr, followed by paraffin embedding, and 4 μm section for immunohistochemistry. We selected S100 antibody (DAKO, Denmark) and GAP 43 antibody (Chemicon, USA) for immuno-histochemical staining. Sections were deparaffinized and rehydrated, followed by 3% hydrogen peroxide solution to inactivate endogenous peroxidase. The slides were then treated with 0.1M citric acid buffer for 15 min at 92-98°C in a microwave oven and cooled at room temperature. After incubation with serum-free protein blocking buffer (BOSTER, China), sections were incubated with primary antibodies for 2 hr. The sections were rinsed and incubated in the biotinylated secondary antibody (BOSTER), followed by incubation in SABC (BOSTER). The studying area of myocardium was selected using methods as previously described.

Nerve fibres, distributed in the border of necrotic myocardium and blood vessels, were selected for analysis. However, if the border of the injured myocardium was not identified easily or if no myocardium injury existed, the density of nerve fibres was measured in the myocardium containing most nerve structures. The shape and distribution of nerve fibres were detected under microscope (20×); the density of stained nerve fibres was measured by a computer-assisted image analysis system (Image-Pro Plus 4.0, USA). The program automatically detected the stained nerve fibers by their color and the area occupied by the nerve fibres was calculated. The nerve fibre density was calculated as the nerve fibre area divided by the total area examined (μm²/mm²).

**Statistical analysis**

All statistical analysis was performed with SPSS 13.0. Quantitative variables were presented as mean ± standard deviation. We used independent Student’s t-test to compare the differences between the means. For analysis of categorical variables, Chi-square test was used. P < 0.05 was regarded as statistically significant.

**Results**

**General results**

In all animals there was ST segment elevation on the electrocardiogram (ECG) immediately after coronary artery ligation. The post-procedural mortality in the MI-placebo and MI-ACEI groups was 20% (3/15) and 26.7% (4/15), respectively. None of the sham group animals died. The ECG showed abnormal Q-waves in
the MI surviving rabbits, but no obvious ECG change was detected in the sham group.

Eight weeks after the operation, the corrected QTd in the MI-placebo group (97.6±3.2ms) was greater than that in the sham group (87.3±2.9ms, \( P < 0.01 \)) and the MI-ACEI group (93.8±3.4ms, \( P < 0.05 \)).

**Electrophysiological study**

No rabbit experienced spontaneous VAs during the placement of the electrodes, and none died during electrophysiological study after 8 weeks of treatment. The ventricular ERP was prolonged in the MI-placebo group (120.4±8.1ms) compared with that in the sham group (92.5±5.0ms, \( P < 0.01 \)) and the MI-ACEI group (102.2±8.7ms). ERP in the MI-ACEI group (102.2±8.7ms) was shorter than in the MI-Placebo group (\( P < 0.01 \)).

Programmed ventricular stimulation induced VAs in 58.3% (7/12) of the MI-placebo group animals. Among them, 3 (25%) had monomorphic ventricular tachycardia (MVT); 1 (8.3%) had polymorphic ventricular tachycardia (PVT); the other 3 (25%) animals had inducible ventricular fibrillation (VF). In the MI-ACEI group, VT was induced in only 1 (9.1%) animal and VF was induced in 2 (18.1%). VF was induced in 2 (16.7%) of the 12 sham group animals.

**Cardiac innervation**

MI resulted in nerve sprouting and spatial heterogeneity of innervation, which were demonstrated by increased nerve density and changes in nerve fibres shown as immunopositive staining for GAP 43 and S100 (Fig 1). The densities of GAP 43 (3090±622 \( \mu \text{m}^2/\text{mm}^2 \)) and S100 (3889±521 \( \mu \text{m}^2/\text{mm}^2 \)) positive nerve fibres were higher (\( P < 0.05 \), Table 1) in the infarct border of the MI animals compared with those in sham group.

The distribution and shape of nerve fibres in the MI-placebo group were more diverse comparing with the sham group (Fig 1, B and E). In the sham group, S100 immunopositive slender nerve fibres were distributed evenly between myocardial fibres. However, the regularity of the myocardial fibres was disturbed in the MI-placebo group. It was ruptured and disordered at the infarct border. Gross nerve fibres were mainly distributed at the periphery of infarct area and perivascular regions.

**TABLE 1. Comparison of nerve fibre density between the three groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>S100 (( \mu \text{m}^2/\text{mm}^2 ))</th>
<th>GAP 43 (( \mu \text{m}^2/\text{mm}^2 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infarct border</td>
<td>Non-infarct LVFW</td>
</tr>
<tr>
<td>Sham (n=12)</td>
<td>1727±304</td>
<td>1763±272</td>
</tr>
<tr>
<td>MI-placebo (n=12)</td>
<td>3889±521 *</td>
<td>3146±459 *</td>
</tr>
<tr>
<td>MI-ACEI (n=11)</td>
<td>3507±433##</td>
<td>3214±603#</td>
</tr>
</tbody>
</table>

* \( P < 0.001 \) vs sham group
** \( P = 0.13 \) vs corresponding site in MI-placebo group
*# \( P = 0.07 \) vs corresponding site in MI-placebo group
# \( P > 0.05 \) vs corresponding site in MI-placebo group
Heterogeneous distribution of nerve fibres was easily observed in the infarct border. Some nerve fibres clustered together around the infarct border or perivascular regions. However, the irregular distribution of the myocardial fibres was partly restored in MI animals that received ACEI treatment (Fig. 1). In the MI-ACEI animals, nerve fibres became slender and were largely parallel to the orientation of myocardial fibres, which were similar to the distribution and appearance of the nerve fibres in the sham group (Fig. 1C and 1F). However, it seemed that the densities of GAP 43 (2709±552 μm²/mm²) and S100 (3507±433 μm²/mm²) positive nerve fibers may be decreased after prolonged captopril therapy in the infarct border although it was not statistically significant (P=0.13 and P=0.07 respectively).

There were no noticeable changes in the distribution and shape of nerves fibres in the non-infarct LVFW. There was no difference in the nerve fibre density between the MI-placebo and MI-ACEI groups (P>0.05). Expression of TH mRNA in the healed myocardial infarction was similar between the MI-placebo and MI-ACEI groups (Fig. 2, P>0.05 v). The expression of NF mRNA at infarct border or LVFW was also similar between the MI-ACEI and MI-placebo groups (Fig. 3, P>0.05).

Discussion

We assessed the effects of captopril treatment on cardiac reinnervation and inducible VAs in a rabbit MI model. We found that nerve sprouting and spatial heterogeneity of cardiac innervation after MI were associated with increased QTd and increased incidence of inducible VAs. More importantly, captopril treatment after MI attenuated spatial heterogeneity of cardiac innervation, reduced QTd and decreased the incidence of inducible VAs.

Clinically, nerve sprouting may contribute to an increased hemodynamic performance of the surviving...
myocardium\textsuperscript{13} and is associated with considerable improvement in exercise performance.\textsuperscript{14} However, several recent studies demonstrated that nerve sprouting results in abnormal patterns of cardiac innervation, such as hyperinnervation and neural imbalance, which potentially increase cardiac arrhythmogenesis.\textsuperscript{2,4, 13} In the present study, MI resulted in heterogeneous cardiac nerve distribution, variable appearance of nerve fibres and increased nerve density in 8 weeks. These abnormalities in myocardial innervation may be responsible for the increased QTd and increased incidence of inducible VAs.

Previous studies suggested that heterogeneous distribution and function of cardiac sympathetic innervation contribute to pathogenesis of fatal arrhythmias.\textsuperscript{15,16,17,18} Moreover, abnormal heterogeneous sympathetic innervation has been identified in patients with Brugada syndrome\textsuperscript{19} and in some patients with congenital long QT syndrome.\textsuperscript{20} Abnormal cardiac innervation increases ventricular vulnerability by prolonging action potential duration and QT intervals, and increased repolarization dispersion\textsuperscript{21}, which influences local myocardial repolarization.\textsuperscript{22} Regional hyperinnervation increases local ventricular transmural dispersion of repolarization\textsuperscript{23}, which may lead to arrhythmogenesis.\textsuperscript{3,23} Moreover, sympathetic stimulation increases repolarization heterogeneity in hearts with heterogeneous sympathetic innervation\textsuperscript{10} and electrophysiological heterogeneity in the coexistence of denervated or hyperinnervated myocardium.\textsuperscript{1}

QTd has been proposed as a reflection of ventricular repolarization instability, which is a risk factor for ventricular arrhythmia and sudden cardiac death after MI.\textsuperscript{24} As a conventional biomarker of cardiac nerve fibres, S100 protein positive nerve fibres can be used to represent the autonomic innervation of the heart. S100 positive structures in ventricles and regenerative nerve fibers are mostly sympathetic nerve.\textsuperscript{3,25} The density of the S100 positive nerve fibres was associated with VAs.\textsuperscript{3} These studies suggest that enhanced spatial heterogeneity in cardiac S100 positive nerve fibres might amplify spatial heterogeneity of these electrophysiological properties, increase QTd and therefore facilitate the initiation of VAs.

Previous studies have reported that ACEI treatment attenuated the abnormalities of TH-immuno-staining nerve terminal\textsuperscript{9} and affect the density of CGRP-LI-containing nerve fibres in mesenteric arteries.\textsuperscript{10} However, ACEI treatment did not change the nerve fibre profiles in the normal heart.\textsuperscript{9} We found that captopril therapy normalizes the spatial distribution and the shape of cardiac innervation, especially at the infarct border. The exact mechanism by which captopril exerts its action on nerve sprouting and nerve uniform regeneration after MI is not known. However, ACEI has proven effects in improving left ventricular remodeling after myocardial infarction\textsuperscript{26,27} and in reducing infarct size in animal models.\textsuperscript{28}

In addition, previous studies have documented that ACEI promotes angiogenesis and increase capillary density in the infarct zone and border zone.\textsuperscript{29,30} These effects may improve blood supply in the infarct and peri-infarct zones. In all, these effects may affect the external matrical milieu of nerve sprouting or regeneration which facilitates cardiac reinnervation, and improve spatial heterogeneity and keep “balance” of cardiac innervation which lowers electrophysiological heterogeneity of myocardium, decreasing the incidence of VAs.

Furthermore, previous studies have demonstrated that ACEI increased cardiac parasympathetic nerve activity\textsuperscript{31} – the effect may contribute to the decrease in the incidence of VAs. GAP 43 is present in immature nerve fibres and is up-regulated in the cone of growing and terminals of sprouting nerve. We, therefore, used GAP 43 as a measure of overall nerve sprouting activity in the myocardium.\textsuperscript{32} In our study, the expression GAP 43 protein was similar between the MI-placebo and MI-ACEI groups, suggesting that captopril have no significant effect on nerve sprouting activity.

In conclusion, MI results in increased density of nerve fibres and heterogeneous ventricular nerve
sprouting, which predisposes to the occurrence of VAs. Captopril treatment attenuates the spatial heterogeneity of cardiac innervation and normalizes the abnormal appearance of cardiac nerve fibres. These effects on cardiac innervation may influence the function of cardiac autonomic nerves, decrease electrophysiological heterogeneity of cardiac muscle cells, and prevent the VAs in healed MI.

**References**


