Alcohol ablation at the posterior papillary muscle prevents ventricular fibrillation in swine without affecting mitral valve function

Lei-Sheng Guo1,4†, Xu Zhou1†, Yan-Hui Li1, Jun Cai1, Dong-Mei Wei2, Liang Shi1, Gang Yang1, Antonis A. Armoundas3, and Xin-Chun Yang1*

1Department of Cardiology, Chaoyang Hospital, Capital Medical University, Beijing 100020, People’s Republic of China; 2Department of Echography, Chaoyang Hospital, Capital Medical University, Beijing 100020, People’s Republic of China; 3Cardiovascular Research Center, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA; and 4Department of Cardiology, Henan Provincial People’s Hospital, Zhengzhou 450003, People’s Republic of China

Received 25 May 2010; accepted after revision 16 August 2010; online publish-ahead-of-print 21 September 2010

Aims

Radiofrequency ablation at the posterior papillary muscle (PM) significantly reduced ventricular fibrillation (VF) inducibility in rabbits and dogs, suggesting that PM may be involved in the generation of VF. However, the effect of ablation at the PM on VF inducibility remains unknown in normal intact swine hearts because in this species radiofrequency energy delivered at PM provoked incessant VF.

Methods and results

Twelve anesthetized swine underwent median sternotomy. Under the ultrasonographic guidance, chemical ablation was performed via injection of dehydrated alcohol into the base of the posterior PM (group PM, n = 6) or anterior wall (control group, n = 6) in the left ventricle. Ventricular fibrillation inducibility and mitral valve function were measured pre- and post-ablation. Hearts were explanted and the ablated myocardium was stained with haematoxylin and eosin. Ventricular fibrillation inducibility was significantly decreased from 100 ± 0% pre-ablation to 11.9 ± 7.8% post-ablation in group PM (P = 0.001), whereas it was not statistically different in the control group (100 ± 0 vs. 92.9 ± 7.1%, pre-ablation vs. post-ablation). Haemorrhage and cellular necrosis was observed in the centre of ablated myocardium and no significant mitral regurgitation was observed following ablation at the posterior PM.

Conclusion

Alcohol ablation of the left posterior PM reduced VF inducibility in normal intact swine hearts, with no significant mitral regurgitation. This suggests that the posterior PM may be involved in the generation of VF, and the recurrence of VF may be prevented by chemical ablation at the posterior PM.

Keywords

Chemical ablation • Ventricular fibrillation • Posterior papillary muscle • Swine

Introduction

Despite substantial advances in the prevention and treatment of cardiovascular diseases, sudden cardiac death (SCD) remains a major concern in the world. Nearly 50% of all deaths are attributed to cardiovascular disease that is sudden in nature. In the USA, SCD is responsible for 400 000–450 000 deaths per year. Ventricular tachycardia/ventricular fibrillation (VT/VF) accounts for 75–80% of patients who experience SCD.

Radiofrequency catheter ablation has been proposed as an effective approach in preventing recurrence of idiopathic VF. In those cases, the Purkinje system has played an important role in initiation and maintenance of VF and has been the main target of radiofrequency ablation to cure VF.

The papillary muscle (PM) of the left ventricle is rich in Purkinje fibres and has complex structural heterogeneity at the PM-ventricular junction of the right ventricle. Emerging evidence has indicated that the posterior PM may play an important role in sustaining VF. For example, Wu et al. has reported a mother rotor anchored on the PM of the left ventricle during VF in Langendorff-perfused rabbit hearts. Furthermore, ablation of the PM significantly reduced the inducibility of VF in rabbits.
and ischaemic dogs. The effect of PM ablation on VF inducibility is not clear in swine, because application of radiofrequency energy near the posterior PM frequently initiated VF, preventing subsequent testing of VF inducibility. In contrast, operation along the left ventricular posterior wall beside the posterior PM in swine, demonstrated that VF inducibility was significantly decreased. Therefore, it is unclear whether PM is responsible for maintaining VF in swine. The present study aims to investigate the hypothesis that chemical ablation (through dehydrated alcohol) of the posterior PM reduces VF inducibility without affecting the mitral valve function.

**Methods**

**Surgical preparation**

Twelve male mature mini-swine (mean weight ± standard deviation, 22 ± 2.4 kg) were used for this study. The animals were handled in accordance with guidelines established by the American Heart Association on research animal use. The protocol was approved by the Animal Care and Use Committee at the Chaoyang Hospital of Capital Medical University.

The animals were anesthetized with intravenous bolus of pentobarbital (30–35 mg/kg) and succinylcholine (1 mg/kg). Each animal was intubated with a cuffed endotracheal tube and ventilated with 30–60% oxygen via a Dräger SA2 respirator (Dräger Medical, Germany) (16 beats/min and 15 mL/kg tidal volume). Pentobarbital was infused at a rate of ~0.05 mg/kg/min via a left ear vein to achieve adequate anaesthesia. The dose of pentobarbital was adjusted according to the anaesthetic depth, which was assessed by signs of shivering, eyelid reflexes, and pain reaction. Systemic blood pressure was continuously monitored on an oscilloscope via the right femoral artery. Blood samples were withdrawn every 30–60 min to perform blood gas and electrolytes analysis. Ringer’s lactate was continuously infused and supplemented with potassium chloride, sodium bicarbonate, and calcium chloride when necessary, to maintain normal status of pH value and electrolytes.

After adequate anaesthesia, the chest was opened through a median sternotomy, and the heart was suspended in a pericardial cradle. A multi-electrode catheter was percutaneously introduced into the right ventricular apex via the femoral vein to record right ventricular electrograms and to induce VF. Surface electrocardiograms of all six limb leads (I, II, III, aVR, aVL, and aVF) and intracardiac electrograms from the right ventricular apex were continuously monitored and stored by EP-WorkMate™ MapMate Platform (EPMedSystems Inc., USA).

Ventricular fibrillation was induced by the modified protocol of programmed ventricular stimulations, as previously reported. Briefly, electrical stimuli (20 mA amplitude and 5 ms duration) were delivered from the right ventricular apex via EP-WorkMate™. The S1–S1 cycle length was 350 or 400 ms. An extra stimulus (S2) was delivered in diastole after eight S1 stimuli. The coupling interval of S1–S2 was decreased at 10 ms step until VF was induced or an effective refractory period was reached. There was a 3 s interval between each pace sequence. If VF was not induced, S2 was set to 10 ms above the ventricular effective refractory period, and the extra stimuli could then be delivered to S3 or S4 until VF was induced.

Ventricular fibrillation was induced when the electrocardiogram was a disorganized rhythm with no clearly defined QRS complexes, and the configuration of arterial blood pressure was turned into a straight line. Ventricular fibrillation lasted for at least 10 s and the sinus rhythm was then restored by extrathoracic cardioversion with a 150 J biphasic shock (Zoll Mseries, Zoll Medical Corporation, USA) using cutaneous pads placed in the conventional sternal–apical position. Ventricular fibrillation was not induced within 10 min after electrical defibrillation to restore physiological homeostasis.

**Chemical ablation**

A long thin needle was used to inject dehydrated alcohol into the base of the posterior PM (group PM, n = 6). In order to accurately inject the alcohol, the posterior PM was sited by Terason t3000 ultrasonograph (Terason t3000, Teratech, USA) from the long-axis view and short-axis view of the left ventricle. During injection, the needle was under the real-time guidance of an ultrasonograph. One millilitre dehydrated alcohol was injected into the base of the posterior PM.

In order to differentiate the effect of ablation on the inducibility of VF, the anterior free wall of the LV was also injected with dehydrated alcohol 2 cm to the left of the anterior descending coronary artery (control group, n = 6). After the needle was inserted ~4–5 mm into the anterior wall, 1 mL dehydrated alcohol was injected.

**Experimental protocol**

The function of the mitral valve and the electrophysiologic protocol in inducing VF were recorded pre- and post-ablation. The function of mitral valve and the inducibility of the VF were not examined until 20 min after ablation, to allow sufficient time for the alcohol to effectively ablate the myocardium.

The function of the mitral valve was determined based on whether or not chemical ablation induced or made mitral valve regurgitation more severe. Parameters that were used to assess mitral valve regurgitation included regurgitation volume, regurgitation area, regurgitation velocity, and regurgitation pressure gradient, which were measured by two-dimensional and Doppler echocardiography.

Ventricular fibrillation induction was attempted twice to determine whether it could effectively induce VF pre-ablation. The same VF protocol was used to induce VF post-ablation. Ventricular fibrillation was induced seven times after ablation and VF inducibility was defined as the quotient of the times of VF induction to the total number of attempts.

At the completion of each experiment, the animal was sacrificed by induction of VF. The hearts were excised and fixed in formalin. Sections of posterior PM and anterior wall underwent haematoxylin-eosin staining.

**Data analysis**

Data are expressed as mean values ± standard error of mean (SEM). Student’s t-test was used for comparison of mitral valve function between pre- and post-ablation. Fisher’s exact test was used to compare inducibility of VF between pre- and post-ablation. P-values of <0.05 were considered statistically significant.

**Results**

**Comparison of ventricular fibrillation inducibility between pre-ablation and post-ablation**

The electrophysiologic parameters and inducibility of VF are summarized in Table 1. Before ablation, the inducibility of VF
was 100 ± 0% in the group PM as well as in the control groups. Moreover, while the VF induction parameter of programmed ventricular stimulation was not uniform in all animals, it was identical for the two pre-ablation inductions in each of the animals.

After ablation with dehydrated alcohol, the VF inducibility in group PM was significantly decreased from 100 ± 0% pre-ablation to 11.9 ± 7.8% post-ablation ($P = 0.001$, Figure 1). After alcohol ablation, VF could not be induced by the same VF parameter in four swine, which could induce VF pre-ablation. Ventricular fibrillation was induced twice and three times, respectively, in the other two animals in the seven post-ablation inductions. Overall, it required more aggressive ventricular stimulus to induce VF than pre-ablation.

In six animals, the anterior wall of the left ventricle also underwent alcohol ablation. Ventricular fibrillation inducibility was not significantly different between pre- and post-ablation, which were 100 ± 0 and 92.9 ± 7.1% ($P = 1.0$, Figure 1), respectively. Specifically, VF inducibility after ablation was 100% in five animals, while in the remaining animal it was 57.1% (4/7).

**Evaluation of mitral valve function**

The posterior PM could be accurately sited by ultrasonograph from views of the long-axis and short-axis of the left ventricle. Local echo immediately increased after injection of dehydrated alcohol into ventricular myocardium (Figure 2).

Colour Doppler was used to determine whether ablation resulted in increased mitral regurgitation or not (Figure 2). Three swine had no regurgitation pre-ablation, although the other three had mild regurgitation. After ablation at the base of the posterior PM, two swine still had no regurgitation, while the other had mild regurgitation.

Overall, there were no significant differences in the regurgitation volume, regurgitation area, regurgitation velocity, and regurgitation

---

**Table 1 Characteristics of ventricular fibrillation induction at pre- and post-ablation**

<table>
<thead>
<tr>
<th>Animal #</th>
<th>Induction parameter (ms)</th>
<th>Inducibility of VF</th>
<th>Induction parameter (ms)</th>
<th>Inducibility of VF</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM-1</td>
<td>400/280/160</td>
<td>100% (2/2)</td>
<td>400/280/110 (ERP)</td>
<td>0% (0/7)</td>
</tr>
<tr>
<td>PM-2</td>
<td>400/280/180</td>
<td>100% (2/2)</td>
<td>400/280/150</td>
<td>43% (3/7)</td>
</tr>
<tr>
<td>PM-3</td>
<td>350/200/130/110</td>
<td>100% (2/2)</td>
<td>350/200/130/50 (ERP)</td>
<td>0% (0/7)</td>
</tr>
<tr>
<td>PM-4</td>
<td>400/260/210</td>
<td>100% (2/2)</td>
<td>400/260/170 (ERP)</td>
<td>0% (0/7)</td>
</tr>
<tr>
<td>PM-5</td>
<td>400/260/180</td>
<td>100% (2/2)</td>
<td>400/260/60 (ERP)</td>
<td>0% (0/7)</td>
</tr>
<tr>
<td>PM-6</td>
<td>400/190</td>
<td>100% (2/2)</td>
<td>400/130</td>
<td>29% (2/7)</td>
</tr>
<tr>
<td>Mean ± STE</td>
<td>400/280/180/110</td>
<td>100 ± 0%</td>
<td>400/280/180/140</td>
<td>11.9 ± 7.8%*</td>
</tr>
<tr>
<td>AW-1</td>
<td>350/200/90</td>
<td>100% (2/2)</td>
<td>350/200/100</td>
<td>100% (7/7)</td>
</tr>
<tr>
<td>AW-2</td>
<td>400/140</td>
<td>100% (2/2)</td>
<td>400/200</td>
<td>100% (7/7)</td>
</tr>
<tr>
<td>AW-3</td>
<td>400/260/130</td>
<td>100% (2/2)</td>
<td>400/260/150</td>
<td>100% (7/7)</td>
</tr>
<tr>
<td>AW-4</td>
<td>400/260/140</td>
<td>100% (2/2)</td>
<td>400/260/210</td>
<td>100% (7/7)</td>
</tr>
<tr>
<td>AW-5</td>
<td>400/140</td>
<td>100% (2/2)</td>
<td>400/200</td>
<td>57% (4/7)</td>
</tr>
<tr>
<td>AW-6</td>
<td>400/190</td>
<td>100% (2/2)</td>
<td>400/280/180/140</td>
<td>92.9 ± 7.1%* #</td>
</tr>
</tbody>
</table>

* $P = 0.001$ inducibility of VF at post-ablation vs. pre-ablation in group PM.
* $P = 1.0$ inducibility of VF at post-ablation vs. pre-ablation in group AW.
* $P = 0.002$ inducibility of VF in group PM vs. group AW at post-ablation.
VF, ventricular fibrillation; ERP, effective refractory period; PM, ablation was performed at the base of posterior PM of left ventricle; AW, ablation was performed in the anterior wall of left ventricle.

**Figure 1** Ventricular fibrillation inducibility of group PM and control group at pre- and post-ablation. Ventricular fibrillation inducibility significantly decreased after alcohol ablation at posterior PM in group PM. It did not markedly change after ablation at anterior wall of left ventricle in the control group. It was lower at post-ablation in group PM than in group anterior wall. VF, ventricular fibrillation; PM, papillary muscle.
pressure gradient between pre- and post-ablation at the base of posterior PM (Table 2 and Figure 2D).

**Microscopic examination of the ablated tissue**

Overall, there was no change of the gross appearance of the tissue except for local bleeding in the injected site. We then sought to examine the effect of ablation on the histology of the PM. Compared with the anterior wall, the orientation of the myocardial fibres in the posterior PM was disorganized (Figure 3A and B). Moreover, haemorrhage and cellular necrosis was observed in the centre of the ablated myocardium. Furthermore, in the centre of the injecting site, the nuclei disappeared and the cytoplasm was markedly dissolved (Figure 3D).

![Figure 2](https://academic.oup.com/europace/article-abstract/12/12/1781/432781)

**Table 2** Mitral regurgitation parameters at pre- and post-ablation in group papillary muscle

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-ablation</th>
<th>Post-ablation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regurgitation area (cm²)</td>
<td>0.18 ± 0.11</td>
<td>0.29 ± 0.12</td>
<td>0.375</td>
</tr>
<tr>
<td>Regurgitation volume (mL)</td>
<td>0.16 ± 0.12</td>
<td>0.10 ± 0.05</td>
<td>0.440</td>
</tr>
<tr>
<td>Regurgitation velocity (cm/s)</td>
<td>110.33 ± 53.18</td>
<td>128.17 ± 43.53</td>
<td>0.721</td>
</tr>
<tr>
<td>Regurgitation pressure gradient (mmHg)</td>
<td>7.73 ± 3.85</td>
<td>11.72 ± 3.86</td>
<td>0.335</td>
</tr>
</tbody>
</table>

Mitral regurgitation parameters were not significantly different between pre- and post-ablation at posterior PM of left ventricle in group PM.
Discussion

Idiopathic VF has been a lethal cardiac rhythm disorder which is challenging to treat, with only a limited number of treatment options available. The present study provides a promising therapeutic approach by exploring the effect of chemical ablation on VF inducibility. Specifically, we have shown that, first, chemical ablation with dehydrated alcohol at the base of left ventricular posterior PM significantly reduced the inducibility of VF; second, this effect relates specifically to the posterior PM while ablation at the anterior wall of left ventricle failed to affect VF inducibility; third, chemical ablation of the posterior PM did not affect the function of the mitral valve.

Papillary muscle, especially its root, has distinct structural heterogeneities such as abrupt discontinuities in cardiac fibre orientation and complex coupling between Purkinje fibres and ventricular myocardium. Recent studies have suggested that anatomic heterogeneities might be important causes of wavefront block and might be involved in the VF onset; in particular, the posterior PM might be an important structure contributing to the maintenance of VF. Nielsen et al. found that during VF the breakthrough clustered over the posterior PM of the left ventricle. This finding supports the theory that the PM may affect VF activation.

Recently, a new syndrome of ventricular arrhythmia involving the posterior PM was proposed. Doppalapudi et al. reported a syndrome of ventricular tachycardia originating from the base of the left posterior PM in seven patients. In these patients, the earliest site of activation localized at the base of posterior PM. Recently, Crawford et al. also reported that ventricular arrhythmias may originate in the right ventricular PMs, and radiofrequency ablation could effectively reduce the incidence of premature ventricular complexes and ventricular arrhythmias.

Histological studies have shown abrupt changes in fibre orientation at sites of re-entry and wave splitting. As an anisotropic anatomic structure, PM plays a key role in the generation of wave splitting and in the maintenance of re-entry. Papillary muscle creates a sudden increase of muscle thickness and abruptly increases current load (sink) for the incoming wavefront (source). The source-sink mismatch created by PM reduces the safety of propagation and results in wavebreak, conduction block, and formation of re-entry in animals. According to such a scenario, PM serves as an anatomic obstacle to anchor re-entrant wavefront (spiral waves).

In prior studies, radiofrequency ablation at the posterior PM significantly reduced VF inducibility in global ischaemic dog hearts, but ablation at the anterolateral wall did not prevent VF induction; however, this approach has not been investigated in swine because radiofrequency energy delivered at the posterior PM induced incessant VF. Recently, the role of PM in swine was investigated by a cut-and-sew operation along the left ventricular wall.

Figure 3 Morphological images demonstrate representative heterogeneities of myocardial fibres of posterior (A) papillary and anterior (B) wall in left ventricle, respectively. Orientation of myocardial fibres in (A) was chaotic, however, it was parallel in (B). Gross appearance of posterior papillary muscle after alcohol ablation is shown in (C); arrowhead indicates the base of papillary muscle. Microscopic image of ablated tissue of posterior papillary muscle is shown in (D); arrowhead indicates that the nuclei have disappeared and the cytoplasm has been dissolved in the centre. Haemorrhage existed in the space of cardiac fibres. The amplification of microscopic sections was 10 x 10.
beside the posterior PM, which resulted in reduction of VF inducibility.\textsuperscript{12} The results of this study were similar to these experiments, that is, ablation at the posterior PM significantly reduces the VF inducibility, and ablation at the anterior wall fails to change VF inducibility. More importantly, the present study, which was performed in normal intact swine hearts suggests that ablation of the posterior PM of the left ventricle by injection of dehydrated alcohol results in no new or worsening mitral regurgitation.

A key finding of the present study is the use of chemical ablation with alcohol as a promising approach for preventing idiopathic VF. Given that alcohol ablation has been also used in hypertrophic obstructive cardiomyopathy,\textsuperscript{23} percutaneous alcohol ablation may be a feasible approach to treat ventricular arrhythmias resulting from anatomic structures.

In conclusion, chemical ablation at the base of posterior PM significantly reduced the inducibility of VF in the left ventricle of normal swine. This suggests that posterior PM plays an important role in the initiation of VF, and that the recurrence of VF may be prevented by alcohol ablation on the posterior PM. Moreover, chemical ablation did not affect the function of mitral valve and did not result in mitral valve regurgitation. Thus, chemical ablation may provide a promising approach for preventing VF.

**Study limitations**

There are some limitations in present study. First, sternotomy was performed in the swine to achieve alcohol ablation at the base of the left posterior PM. This is because the base of the posterior PM is broad and thick, and the lesions resulting from radiofrequency energy only account for \(\sim 30\%\) of the thickness. Alcohol may be non-invasively injected into posterior PM by retractable inject catheter such as the NOGA Myostar system.\textsuperscript{24} However, this device was not available at that time. Therefore, alcohol injections had to be guided by echocardiography on the epicardium after exposure of the heart. The second limitation is that the present study could not validate the long-term effect of alcohol ablation on VF inducibility and mitral valve function. The third one is the potential proarrhythmic effect of ablation; although the proarrhythmic potential of alcohol ablation could not be eliminated, we believe that because alcohol diffuses through the smallest capillaries, it does not create scar tissue that would make the heart proarrhythmic,\textsuperscript{25} but rather forms uniformly necrotic tissue. However, further work needs to be done in the diseased heart (e.g. in experimental models of myocardial infarction or heart failure) to verify that ablation of the posterior PM may reduce susceptibility to VF.

**Conflict of interest:** none declared.

**Funding**

This work was supported by grant number 30770875 from National Natural Science Foundation of China (X.-C.Y.), by an American Heart Association Scientist Development Grant #0635127N (A.A.A.), by NIH grants 1R21AG035128 and 1RO1HL103961 (A.A.A.) and by the Cardiovascular Research Society (A.A.A.).

**References**