Myocardial Lesion Depth With Circular Electroporation Ablation

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Background—Recently, we demonstrated the feasibility and safety of circular electroporation ablation in porcine pulmonary vein ostia, but the relationship between the magnitude of the application and lesion dimensions is still unknown.

Methods and Results—An in vivo porcine study was performed on left ventricular epicardium submerged under 10 mm of blood, using devices that mimic a 20-mm-diameter 7F circular ablation catheter. Model D contained 10 separate electrodes, whereas model M consisted of 1 circular electrode. Ablations were performed at 50, 100, and 200 J with model D and at 100 J with model M. Lesion dimensions were measured after 3-week survival. All applications resulted in smooth voltage waveforms demonstrating the absence of vapor globe formation, arcing, and a pressure wave. Applications up to 100 J with model D resulted in separate lesions under the electrodes. At 200 J, continuous deep circular lesions were created despite the use of separate electrodes. There was a significant relationship between applied current and median lesion depth, with a slope of 0.17 mm/A. At 100 J, there was no difference in lesion depth or width between models D and M. The electrodes and ablation site directly after ablation showed no signs of thermal damage.

Conclusions—In an epicardial porcine model with blood around the application site, continuous circular lesions, deep enough for electric pulmonary vein isolation, were created with a single circular 200-J application. Lesions were continuous despite the use of separate electrodes. Lesion depth increased with the magnitude of the application. (Circ Arrhythm Electrophysiol. 2012;5:581-586.)

Key Words: atrial fibrillation • catheter ablation • ventricular tachycardia • electroporation
The pericardial space is filled with heparinized blood and the cine basal left ventricular epicardium with 3 sutures. Thereafter, 20-mm-diameter circular devices (Figure 1). Decapolar device D is temporarily fixated to the porcine basal left ventricular epicardium with 3 sutures (Figure 2). No additional contact on the tissue, as is common with tissue electrocoagulation. There was never a white imprint of the electrode directly after the application, never revealed any blood clots or charring. There was never a white imprint of the electrode contact on the tissue, as is common with tissue electrocoagulation. After 200-J applications, a light purplish circular epicardial colorization was visible.

**Histological Evaluation**

After fixation, multiple (range, 3–10) 3- to 4-mm-thick segments were dissected from each circular lesion. All sections were taken perpendicular to the epicardial surface. As many radial segments as possible were taken to facilitate measurement of lesion width and depth. Of each circular lesion, we also collected at least one tangential section to study lesion continuity. Paraffin-embedded segments were sectioned and stained with hematoxylin-eosin and with elastic–van Gieson. Slides were digitized, and lesion depth and width were measured.

**Measurement of Lesion Size**

Large lesions often showed tissue thinning, as is also common after myocardial infarction. When sufficient undamaged myocardium was present in the histological section, the estimated original epicardial contour was used to measure lesion depth. Lesion depth and width were measured in each histological section. Some sections included multiple lesions; in those sections, lesion depth and width were measured for each lesion separately. At a few sites, 2 neighboring tangential sections were available; from those sections, maximal lesion depth was taken. Along each circular ablation line, median lesion depth and width were calculated.

**Statistical Analysis**

Differences in applied peak currents and median lesion depths and widths between the 100-J applications via devices D and M were investigated with a paired t test. Continuous variables were expressed as mean ± SD. The relation between delivered peak current and median lesion depths obtained with device D was investigated by randomized block regression analysis. Statistical significance was defined as P ≤ 0.05.

**Results**

The applications ranged between 950 and 2150 V, with a peak current between 15 and 37 A and a pulse duration of <10 ms. All applications resulted in smooth voltage waveforms, demonstrating the absence of electrically isolating gas bubbles around electrodes and arcing. The only noticeable response to the applications was cardiac and peripheral muscle contraction. Visual inspection of the ablation area and electrodes, directly after the application, never revealed any blood clots or charring. There was never a white imprint of the electrode contact on the tissue, as is common with tissue electrocoagulation. After 200-J applications, a light purplish circular epicardial colorization was visible.
The 3-week survival period was uneventful, except in 1 animal that experienced a period of sickness and fever, presumably due to pericarditis. At removal of the hearts, the pericardium was adhered to the epicardium in most animals. Visual inspection of tissue surrounding the heart did not reveal any lesions. The 3 sutures that had held the ablation devices and whitish colorization of the lesion(s) allowed identification of the 4 circular application areas, even when superficial epicardial damage due to pericardial adhesion obscured the epicardial aspect of the lesions. Except for some 50-J ablation sites, the original position of the 10 electrodes of device D could not be identified, because those ablations had resulted in 1 continuous whitish ablation ring on the epicardial surface.

Lesion Characteristics

Tangential sections of lesions obtained with device D at 50 J always showed separate lesions under the presumed electrode contact sites (Figure 3). Tangential sections of decapolar 100-J applications showed separate lesions at 2 of 5 ablation sites, a mixed pattern with deeper lesions under the electrodes and shallower lesions between electrodes at 2 ablation sites, and 1 continuous lesion at 1 ablation site. Tangential sections of lesions obtained at 100 J with device M always showed continuous lesions. Tangential sections of lesions obtained after a single 200-J application via decapolar device D were also continuous. In 3 of 5 lesions obtained at 200 J, a surviving central island was present in at least 1 of the histological sections (Figure 4).

In all sections along each circular ablation line, a lesion was found, except at 4 sites (1 at 50 J, D; 2 at 100 J, M; and 1 at 200 J, D) where epicardial fat, thicker than the median lesion depth of those particular lesions, was present.

The relationship between delivered peak current and median lesion depth along each circular lesion is shown in Figure 5 and Table. This relationship is significant, with a slope of 0.17 mm/A ($P=0.001$; 95% CI, 0.09–0.25). When all sections of 200-J (D) lesions are combined, the 5th and 95th percentiles of all observed lesion depths are 2.9 and 8.7 mm, respectively (Figure 6). The corresponding values for 50- and 100-J applications via device D are 0.8 to 4.6 and 1.2 to 5.5 mm, respectively.

Ablations at 100 J via devices D and M resulted in average peak currents of 24.3±1.3 and 25.7±1.6 A, respectively ($P=0.14$). Median lesion depths and widths were not significantly different between these 2 types of ablation ($P=0.49$ and $P=0.27$, respectively). The 5th and 95th percentile range of all lesion depths observed in sections of 100-J applications via device M was 0.5 to 5.3 mm.

Of 102 histological sections, 3 showed an exceptionally narrow and deep lesion, as if the ablation current had followed a specific path through the myocardium (Figure 7). This path was always perpendicular through the myocardial
wall and not directed toward the indifferent skin electrode. Such lesions were observed at each energy level. The depth/width ratio of these 3 lesions was 11±1, whereas it was 1.0±0.6 for all other lesions. Rootlike extensions that were usually observed at the border of the lesions (Figures 3 and 4) were not included in lesion depth and width.

Lesion Histological Features
All lesions showed complete replacement of cardiomyocytes by granulation tissue consisting of fibroblasts with loose collagen fibers and capillaries. Lesions have irregular outer margins, with tiny extensions at their borders (Figure 4).

Discussion
This study demonstrates a significant relationship between the magnitude of the application and median lesion depth. In addition, it demonstrates the possibility of creating continuous 20-mm circular lesions with a single ultrashort 200-J DC application via 10 electrodes (7F and 2-mm diameter). At that energy level, voltage waveforms were smooth, demonstrating the absence of vapor globe development and of hazardous adverse effects associated with DC ablation.

Lesion Depth
In this study, 200-J applications delivered via device D resulted in continuous lesions with a median depth of 5.2±1.2 mm. The 0.05 to 0.95 percentile range of lesion depths is 2.9 to 8.7 mm, suggesting that electric PV isolation is a realistic goal for this development.

Myocardial cells do not sense total applied energy, only local current density and impulse duration. Local current density is directly proportional to delivered current at any moment, independent of electrode interface impedance, polarization, and position of the indifferent electrode. Therefore, delivered current (and duration) is the most direct measure of the magnitude of the application (Figure 5). Total peak current delivered with a 200-J application is only twice that of a 50-J application, which was the standard energy level for endocardial cardioversion before the arrival of biphasic defibrillators (Table).

Unanticipated Lesion Formation
Three histological sections showed an exceptionally deep and narrow lesion with an =10 times larger depth/width ratio than of the other lesions (Figure 7). Further studies might reveal why the ablative current sometimes finds such an abnormal path through myocardial tissue.

Thermal Effects
Blood clots and/or carbonization were never observed on the electrodes or application site. Energetically, 20 J per electrode is <0.7 seconds of radiofrequency (RF) delivery at 30W. Thermally, this energy level cannot explain a 7-mm lesion depth. At the application sites, a whitish tissue colorization, as is common with thermal RF ablation, was never observed acutely. In addition, RF lesions have a fairly sharp demarcation between damaged and healthy myocardium, whereas lesions in the present study have a more irregular border with tiny fibrous extensions. The exact local temperature increase

Table. Data of the 4 Types of Circular Epicardial Applications and Resulting Lesions

<table>
<thead>
<tr>
<th>Device</th>
<th>Energy, J</th>
<th>Peak Current, A</th>
<th>Median Depth, mm</th>
<th>Median Width, mm</th>
<th>Lesion Continuity</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>50</td>
<td>16.1±1.3</td>
<td>2.1±0.6</td>
<td>2.6±0.7</td>
<td>0/5</td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td>24.3±1.3</td>
<td>2.9±1.2</td>
<td>4.5±1.2</td>
<td>1/5</td>
</tr>
<tr>
<td>M</td>
<td>100</td>
<td>25.7±1.6</td>
<td>2.8±1.1</td>
<td>3.7±1.2</td>
<td>5/5</td>
</tr>
<tr>
<td>D</td>
<td>200</td>
<td>34.9±2.1</td>
<td>5.2±1.2</td>
<td>5.3±3.0</td>
<td>5/5</td>
</tr>
</tbody>
</table>

Ablations were performed with a 7F 20-mm-diameter decapolar device (D) at 50, 100, and 200 J and with a 20-mm-diameter 7F ring electrode (M) at 100 J. Lesion depth and width were measured in all histological sections, and their median values were calculated per circular lesion. Data are given as the average±SD of the 5 peak ablation currents and of the 5 median lesion depths and widths obtained per type of application. Lesion continuity, determined in tangential sections, is scored in the last column. Values obtained at 100 J with devices D and M were compared using paired t-tests.

Figure 6. Histogram of all lesion depths observed after 200-J applications via decapolar device D. The 5th and 95th percentiles (dashed lines) are 2.9 and 8.7 mm, respectively.

Figure 7. Histological elastic–van Gieson–stained tangential section of a 50-J application via decapolar device D. Three separate lesions, of which the lesion at the right is exceptionally deep and narrow, can be appreciated. Lesion borders are marked with a dashed line. In total, 3 of such lesions were found. The cause of this phenomenon is unknown, but apparently the ablative current has followed a narrow path through the myocardium.
near the electrodes still needs to be investigated, but our data suggest that tissue heating is not the ablative mechanism.10

**Tissue Contact**
Given the better conductivity of blood than of tissue, electrode-tissue contact will definitely affect lesion depth. Final catheter design and perhaps electric means to measure tissue contact may determine the success of this technique for PV antrum isolation.

**Lesion Width**
The main purpose of our study was to investigate lesion depth and continuity at various energy levels. Lesion width is important too, because we expect that circular IRE ablations must be placed just inside PV ostia to achieve sufficient electrode-tissue contact. Lesion width will then determine how much of the antrum will be ablated, and this may affect clinical success (Table).10–12

**Catheter Configuration**
For 3D localization, mapping, and PV isolation, a circular catheter with multiple electrodes may be more attractive than one with a single circular electrode. The ultimate catheter design will also depend on whether mapping of PV potentials remains important for PV isolation with IRE, but the results of our study suggest that electrode design is not a critical issue as long as electrode size is large enough to prevent arcing.

**Limitations**
The design of the decapolar device did not allow for measurement of individual electrode impedances. Theoretically, impedance differences may cause differences in delivered current between electrodes. However, except for a wedged situation, measurement of electrode impedance with a roving endocardial catheter usually shows little variation. We presumed that the same would be true for the electrodes of device D.

Measurement of lesion size from radial segments could have missed the center and presumably greatest lesion depth and width of separate 50- or 100-J lesions. Tangential sections may also have missed greatest lesion depth. This could have led to an underestimation of lesion depth. Despite this, lesions were always found in all sections along each circular ablation line, except at 4 locations with relatively thick epicardial fat.

Lesion size with IRE will depend on electrode-tissue contact and, thus, on the ultimate catheter design and measures to ensure electrode-tissue contact. Given the major differences between the present model and circular catheter ablation of PV ostia, extrapolation of the results of this study to PV isolation should be performed with great caution. Data of this study, however, suggest that electroporation technology definitely has the potential to facilitate extremely fast PV isolation.13,14 Further studies must address potential complications, such as PV and coronary stenosis, nerve damage, esophageal fistula, and other unwanted adverse effects.15,16

**Side Contact**
The plane of the circular ablation device was positioned parallel to the tissue (Figure 2). The plane of a circular ablation catheter positioned against a PV antrum may also be parallel to the tissue. With a circular arrangement of electrodes, the ablation current will preferentially be directed outward. With the catheter hoop positioned inside a PV ostium, an even deeper lesion than what was obtained in the present study may be expected.

**Clinical Implications**
IRE ablation might not be the optimal approach for all other cardiac arrhythmias. RF and cryoablation are much more controllable because they allow monitoring the electrophysiologic response during energy application. However, for electric PV isolation and in cases in which deep transmural lesions are desired, a fast technique that does not require tissue heating may be preferable.

The design used in the present study had a 7F 20-mm-diameter hoop. In clinical use, a different hoop diameter may be required. With identical electrode size and spacing, total applied current should be scaled proportional to the diameter of the ablation hoop to maintain the same lesion depth and safety margin below arcing threshold.

**Conclusions**
The data of this study demonstrate a significant relationship between the magnitude of the application and myocardial lesion depth. In a blood-myocardial tissue environment, continuous 20-mm circular lesions, deep enough for electric PV isolation, can be created with a single 200-J application of a few milliseconds in duration. Tissue heating does not appear to play a role in lesion formation.

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**Disclosures**
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**References**


CLINICAL PERSPECTIVE

This article presents data of lesions created with a novel energy source that induces irreversible myocyte membrane electroporation, leading to apoptosis. The study demonstrates that a single 6-ms high current application, delivered via a circular arrangement of electrodes in a blood-tissue environment, can create a continuous circular lesion sufficiently deep for pulmonary vein antrum isolation. The data of this study suggest that the application does not cause enough temperature elevation to induce blood or tissue coagulation. The technology would allow for ultrafast nonthermal electric pulmonary vein isolation as an alternative for multiple sequential radiofrequency applications. The ablation technology still relies on electrode-tissue contact. Clinical application will, therefore, require the means to determine electrode-tissue contact to ensure sufficient lesion depth.
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