Characteristics and time course of acute and chronic myocardial lesion formation after electroporation ablation in the porcine model

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Abstract

Introduction: Electroporation ablation creates deep and wide myocardial lesions. No data are available on time course and characteristics of acute lesion formation.

Methods: For the acute phase of myocardial lesion development, seven pigs were investigated. Single 200 J applications were delivered at four different epicardial right ventricular sites using a linear suction device, yielding a total of 28 lesions. Timing of applications was designed to yield lesions at seven time points: 0, 10, 20, 30, 40, 50, and 60 min, with four lesions per time point. After killing, lesion characteristics were histologically investigated. For the chronic phase of myocardial lesion development, tissue samples were used from previously conducted studies where tissue was obtained at 3 weeks and 3 months after electroporation ablation.

Results: Acute myocardial lesions induce a necrosis pattern with contraction band necrosis and interstitial edema, immediately present after electroporation ablation. No further histological changes such as hemorrhage or influx of inflammatory cells occurred in the first hour. After 3 weeks, the lesions consisted of sharply demarcated loose connective tissue that further developed to more fibrotic scar tissue after 3 months without additional changes. Within the scar tissue, arteries and nerves were unaffected.

Conclusion: Electroporation ablation immediately induces contraction band necrosis and edema without additional tissue changes in the first hour. After 3 weeks, a sharply demarked scar has been developed that remains stable during follow-up of 3 months. This is highly relevant for clinical application of electroporation ablation in terms of the electrophysiological endpoint and waiting period after ablation.

KEYWORDS
electroporation, histology, myocardial lesion, pulsed field ablation, time course
1 | INTRODUCTION

Electroporation ablation is a highly promising ablation modality for catheter ablation in patients with cardiac arrhythmias. In our previous animal studies, large myocardial lesions could be created safely and effectively.1–4 However, the time course and characteristics of lesion formation, highly relevant for the electrophysiological endpoint and waiting period after catheter ablation, has not been studied yet. The purpose of the present study is to investigate the development of myocardial lesions during the first 60 min after epicardial electroporation ablation, after 3 weeks and 3 months follow-up.

2 | METHODS

All pigs in the present study were also used in other (main) studies, performed after prior approval from the Animal Experimentation Committee of Utrecht University, and in compliance with the Guide for Care and Use of Laboratory Animals. Ablation lesions that were analyzed for the present study were either created directly before scheduled killing (acute phase) or collected as part of the treatment protocol (chronic phase).

2.1 | Anesthetic protocols

In all studies, 60–75 kg Dalland landrace pigs were premedicated with 10 mg/kg ketamine, .4 mg/kg midazolam, and .5 mg atropine. Anesthesia was induced with 4 mg/kg thiopental sodium. During the rest of the procedure, .5 mg/kg/h midazolam, 2.5 mg/kg/h sufentanil, and .1 mg/kg/h pancuronium bromide were administered.

2.2 | Creation and collection of lesion samples

Lesions obtained ≤60 min after ablation were created especially for the present study at 0, 10, 20, 30, 40, 50, and 60 min before killing. After medial sternotomy, epicardial ablation was performed with a custom-made linear suction device, which has been used in previous studies.1,2,5 In short, the custom linear suction device comprised a 35 mm-long and 6 mm-wide linear noninsulated electrode inside a 42 mm-long and 7 mm-wide insulated plastic suction cup. The suction device was sucked with a constant vacuum of 50–60 cm H₂O on the epicardium. The constant underpressure ensured a good and stable electrode-to-myocardium contact. After opening the chest, the suction device was positioned on the epicardial side of the right ventricle (RV), perpendicular to the left anterior descending artery. Coronary arteries were not targeted.

A single cathodal 200 J application was delivered at four different nonoverlapping sites between the RV base and RV apex. In each of the seven pigs, energy was delivered at four different time points in accordance with a predefined tight time schedule comprising fixed time intervals in the 60 min before killing. This schedule resulted in a set of four epicardial lesions for every predefined 10 min time interval (Table 1).

For investigation of myocardial lesion development in the chronic phase, lesion samples were selected from our collection of samples taken 3 weeks and 3 months after electroporation ablation.1–4 These lesions had been created on the epicardial side of the left ventricle, either with a circular multi-electrode catheter under at least 1 cm of blood, inside the pericardial space using a 12 mm diameter circular catheter, or using a linear suction device.

In total, the following samples were available:

1. Twenty-eight acute lesion samples obtained 0, 10, 20, 30, 40, 50, and 60 min after ablation (four lesions samples per time interval).
2. Thirty chronic lesion samples obtained 3 weeks after ablation.
3. Thirty chronic lesion samples obtained 3 months after ablation.

2.3 | Energy delivery

The ablation energy was generated using a monophasic external defibrillator (Lifepak 9, Physio-Control, Inc.). A large skin patch (7506, Valleylab, Inc.) on the lower back of the animal served as an indifferent electrode. A single, 6 ms cathodal application was delivered. A cathodal shock polarity was chosen, because that has the highest threshold for arcing in a blood environment. All lesions <60 min had been created using a single 200 J application. The other lesions had been created with single applications ranging from 30 to 200 J. Voltage and current waveforms of all applications were recorded as previously described.3

2.4 | Histological evaluation

All animals were killed by exsanguination. The heart was removed en bloc, the area with ablation lesions was excised integrally, and lesions...
were separated and fixed in formalin. After fixation, 3–4 mm-thick segments were taken. All paraffin-embedded segments were sectioned and stained using hematoxylin–eosin and/or Elastic–van Gieson (EvG).

3 | RESULTS

3.1 | Inspection of all lesions

Visual inspection of the ablation area and the electrodes directly after the energy application never revealed any perforation, blood clots, or charring. There were no differences between the ablation devices. The suction device did cause some local epicardial hematoma due to bursting of superficial tiny epicardial blood vessels (Figure 1).

After every energy application, a light purplish colorization around the bruised area was visible. In addition, in contrast to the adjacent unaffected myocardium, the ablated area did not contract during ventricular systole. A sharp zone between lesion and undamaged myocardium could be observed, as a cross-section of the lesion was obtained (Figure 2).

3.2 | Acute phase lesion histology (<60 min)

Lesions were readily identified from normal myocardium. In comparison with control myocardium, all sections with ablated myocardium showed interstitial edema, recognizable as empty space between cardiomyocytes and also contraction band necrosis (Figure 3A–D). All lesions were transmural. Other well-known characteristics of myocardial necrosis, such as nuclear condensation, cardiomyocyte swelling, inflammatory cells, and hemorrhage were not present. The histological features were present from the very beginning (t = 0 min) with no changes in the next hour. Adverse mechanical complications, such as myocardial rupture, were not noticed.

The ablated myocardium was well demarcated from the unaffected myocardium. However, extensions containing contraction bands (eosinophilic staining cross-bands reflecting cardiomyocyte hypercontraction) created an irregular border zone between lesion and unaffected myocardium (Figure 4).

FIGURE 1 Electroporation ablation of the right ventricular free wall: using a custom-made suction device, a single cathodal 200 J application was delivered at four sites of the right ventricle between the base and apex, perpendicularly and just aside from the left anterior descending coronary artery (green arrow). (A) The suction device brought into position for the second energy application; the first energy application was delivered 10 min earlier, more apically. (B) The situation preceding the fourth energy application from a slightly different angle, three energy applications were delivered 50, 40, and 20 min earlier, respectively. The ablated myocardium is greyish colored. A linear hematoma at previous ablation sites, due to the negative pressure of the suction device, can be identified. LV, left ventricle; RV, right ventricle. Green arrow, left anterior descending artery; white arrows, edges of the pericardium; 1, 2, 3, 4 (in blue), ablation application sites.

FIGURE 2 Cross-section of the lesion in the right ventricular free wall, created with a single 200 J application, using a custom-made suction device. A sharp border zone between lesion and normal myocardium can be observed. Blue arrows, lesion at the pericardial sites; dashed green lines, demarcation of the lesion.
Lesions were clearly distinguishable from normal myocardium. Lesions 3 weeks after electroporation ablation consist of loose connective tissue, in the EvG staining pale purple colored (Figure 5). As has been noted in previous electroporation studies, coronary arteries, veins, and nerves were unaffected.1,3,4,6 In the lesions 3 months after electroporation ablation, connective tissue production has increased (intensely purple colored). Again, hemorrhage and inflammatory cells were almost absent. Different energy settings did not affect lesion characteristics. Except for a more distinct connective tissue deposition, myocardial lesions 3 months after electroporation ablation are similar to lesions 3 weeks after electroporation ablation.

4 | DISCUSSION

4.1 | Major findings

This is the first study to investigate and compare the acute and chronic effects of epicardial electroporation ablation. Just as in previous studies, thermal damage was not noticed and all energy applications were delivered without arcing1–4,6. Acute lesions were mainly characterized by interstitial edema and contraction band formation, whereas tissue structural integrity was preserved, and myocardial lesions did not evolve histologically over the course of 1 h. We did not find nuclear condensation, cardiomyocyte swelling, inflammatory cells, and hemorrhage. Our findings indicate that electroporation ablation, while providing single-shot transmural lesions, does not share the adverse barotrauma and thermal effects of high-energy direct-current (DC) ablation used in the 1980s and early 1990s.

4.2 | Electrical pulses and lesion formation

Electroporation ablation provides tissue ablation without thermal damage and has been widely used for the treatment of malignant tumors.5 Cell death is established by injury of the cell membrane due
to a high voltage gradient, consequently leading to the loss of cell hemostasis and necrosis. Protein denaturation (as in necrosis with radiofrequency [RF] energy) does not occur, connective tissue is not damaged, and blood flow acting as a heat sink does not impact efficacy of lesion formation. According to current insights, cell death might also be caused by apoptosis (programmed cell death due to DNA damage), even in the absence of plasma membrane injury, with lower energies.9

4.3 | Histological examination of myocardial lesions caused by electrical pulses

Data regarding the development of myocardial lesions due to electrical pulses are mainly obtained from animal studies from the distant past, using high-energy DC pulses accompanied with arcing and barotrauma. Acute myocardial lesions were characterized by hemorrhage, interstitial edema, and cardiomyocyte alterations, such as dehiscence of intercalated disks, granulation of cytoplasm, mitochondrial swelling, pyknosis (condensation of chromatin in the nucleus), and contraction band formation.10 These characteristics resemble a necrosis pattern as in lethally injured cells undergoing reperfusion, in which a massive influx of calcium into injured cells induces hypercontraction and disruption of myocytes.11 A few days after the high-energy DC pulse, contraction bands and extensive disruption of myocardial fibers in conjunction with hemorrhage were found in lesions.12,13 A mix of granulation tissue and fibrosis was found after a few weeks, pure fibrosis with nearly inflammatory cell infiltration was found after a few months.14

Only two older studies report on lesion formation due to low-energy (nonarcing) DC ablation, however, not immediately after energy delivery but after 2–7 days.15,16 These authors found that low-energy DC and high-energy DC created equal amounts of necrosis and similar lesion characteristics, supporting the hypothesis that electroporation is the main lesion mechanism rather than thermal effects and barotrauma.

Lavee et al.17 reported the first study in which contemporary irreversible electroporation (multiple electrical pulses per energy application) was used for cardiac ablation. After 24 h, a clear demarcation between the epicardially ablated area and unaffected atrial tissue was observed. All lesions were transmural, measuring a mean depth of 0.9 cm.17 In another study, Hong et al.18 reported on histological features of myocardial lesions 1 h after epicardial electroporation ablation: again, demarcated lesions were observed, displaying contraction band formation and cardiomyocyte swelling surrounded by interstitial edema and hemorrhage.

In our study, myocardial lesions were visible immediately. However, histologically a sharp border between ablated and unaffected myocardium was not always found, neither in acute nor chronic lesions. Irregular penetrating extensions disturbing the border zone may represent preexisting strands of connective tissue, as they can also be detected in nonablated myocardium (Figures 5 and 6). On the other hand, concentration of these strands is clearly higher at the border zone and may also be caused by electroporation ablation.

FIGURE 5 Myocardial tissue of the right ventricle 3 weeks after electroporation ablation stained with Elastic–van Gieson (EvG). The lesion consists of loose connective tissue and is well demarcated (pink) from the unaffected myocardium (yellow). (A) The full section: (B–D) enlarged subsections. (B) A sharp border zone between lesion and normal myocardium. (C) The border of the lesion that also included adipose tissue (ad). (D) Two patent arteries (a) and nerve bundles (arrows) enclosed in the lesion. a, artery; ad, adipose tissue; v, vein; black arrows, surviving nerves
4.4 Considerations of efficacy for electroporation ablation

Understanding early myocardial lesion development might also be relevant to assess the necessity of multiple electroporation ablations and supplementary “touch-up” ablations in pursuing a favorable outcome. The key questions are whether the current endpoint of abolishing of local electrical conduction is enough, or do we have to wait for potential recovery, and if so, how long? Endpoints for current thermal ablation in patients include complete pulmonary vein (PV) isolation in atrial fibrillation cases and noninducibility in ventricular tachycardia cases.

RF ablation causes necrosis by heating the tissue. However, hyperthermia may also induce reversible membrane depolarization and transient loss of cellular excitability. In many centers, the ablation outcome is observed for at least 30 min to identify insufficiently treated sites.

Absence of further histological lesion development or regress in the first hour after electroporation ablation could imply that the electrophysiological evaluation can be performed immediately after ablation: there seems no need to wait and reconfirm electrophysiological endpoints after 30 min. Whether adenosine may unmask concealed PV connections after electroporation ablation remains to be determined.

Apparently, no further development of the lesion occurs after 3 weeks, except for a more intensive formation of the connective tissue. In conjunction with the absence of hemorrhage and inflammatory cell infiltration in the first hour of lesion development, the disappearance of interstitial edema after 3 weeks, and the non-thermal character with regard to clot formation, subsequent repeat ablation procedures might be safely scheduled already at 3–4 weeks after a failed initial ablation.

5 LIMITATIONS

1. We investigated only the 200 J energy setting for acute lesions. This energy setting was commonly used in our previous studies, proven to be safe and able to provide a 15–17 mm-wide transmural ventricular lesion. We have no information on acute myocardial lesion development using higher or lower energy settings.

2. In this study, the suction device was placed on the right ventricular epicardium. The right ventricular free wall is fairly regular, easily accessible, and sufficiently large, allowing for multiple electroporation applications side by side, according to a strict timetable. Lesion size was not investigated in this study. Although we might expect a similar outcome, we do not have information about the development of myocardial lesions in the thinner atrial wall.

3. We only investigated the first 60 min after electroporation ablation, considering a longer observation is not feasible in clinical practice. Development of lesion characteristics and lesion size between 60 min and 3 weeks was not addressed in the present study.

4. In early high-energy DC studies, the volume of tissue injury decreased significantly after 30 days. Thus, lesion size may decrease over time. Whether decrease of lesion size was caused by recovery of reversibly electroporated tissue or shrinkage of successfully ablated tissue remains unclear. Depending on the strength of the electrical pulse across target tissue (voltage...
gradient) and tissue characteristics, the cell membrane is either reversibly electroporated and restores in time or is irreversibly electroporated and induces cell death. In fact, areas of irreversible electroporation are always surrounded by an outer reversible electroporation zone. Other studies will be needed to explore the development of myocardial lesion size in detail.

5. We acknowledge that this study only describes the time course of macroscopic and histological lesion formation. Due to the study design, no electrophysiological study was performed. This was beyond the scope of the present study. Other studies will be needed to investigate the electrical time course after electroporation ablation in detail.

6. The monophasic waveform used in this study has been used in clinical studies, but is distinct from the waveforms used with other pulsed field ablation systems because of its pulse duration, monophasic waveform, and unipolar delivery. The observations made here cannot be applied to biphasic bipolar systems using microsecond pulse durations.

7. The suction device used in this preclinical study was chosen, because the constant underpressure ensured a good and stable electrode-to-myocardium contact. By these means, optimal lesion formation was targeted. In clinical electrophysiology, other ablation devices are used. The findings of our preclinical study cannot be transferred to other ablation devices.

6 | CONCLUSION

Immediately after electroporation ablation, myocardial lesions are visible macroscopically and histologically. Key features include interstitial edema and contraction band necrosis. Hemorrhage and inflammatory cells, as abundantly seen after high-energy DC ablation, are almost absent in the first hour of lesion development. There is no histological change in the first 60 min after electroporation ablation. Likewise, there is only minimal histological development in the period between 3 weeks and 3 months after electroporation ablation. This may have profound implications for the future clinical application of electroporation ablation, in particular with regard to the electrophysiological endpoint immediately after ablation and scheduling a repeat procedure after failed initial ablation.

CONFLICT OF INTERESTS

Harry van Wessel, Fred HM Wittkampf, and René van Es have patents regarding irreversible electroporation. Kars Neven is a consultant for Abbott, Inc. This study was funded by an unrestricted grant from Abbott, Inc.

DATA AVAILABILITY STATEMENT

Raw data were generated at the University Medical Center in Utrecht, the Netherlands. The data that support the findings of this study are available from the corresponding author on reasonable request.

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