Histological findings induced by different energy sources in experimental atrial ablation in sheep

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Abstract

The aim of this study was a detailed comparative investigation of acute cardiac alterations induced by different energy sources and approaches in a sheep model. Experiments were performed on 39 sheep. Circular lesions were created endo- or epicardially in the left atrium and at the pulmonary veins using different energy sources: cryo, microwave, laser and unipolar or bipolar radiofrequency (RF). Electrophysiological examinations were performed immediately post treatment and 2 h after ablation to prove conduction block. Altered areas of the atria and pulmonary veins were investigated histopathologically. Endocardial ablation resulted in transmural lesions, confirmed by electrophysiological examinations. However, endocardial microwave and laser induced intensive thrombus formation, whereas radiofrequency and cryoaablation induced more circumscribed necrosis and led to little endocardial thrombi. Epicardial cryoaablation and microwave energy were not successful in acute phase in 8 of 9 animals. In contrast, epicardial bipolar RF was efficient and resulted in well demarcated slim lesion lines but induced marked thrombus formation. It can be summarized that surgical ablation techniques using different energy sources and approaches in this acute animal model resulted in different electrophysiological effectiveness and histomorphological lesions. Further mid and long term studies are necessary to confirm these results.

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Keywords: Heart; Ablation; Arrhythmia; Experimental surgery; Histology

1. Introduction

Atrial fibrillation is the most common cause of arrhythmia in humans. Different therapeutic concepts have been developed to establish successful but minimal invasive techniques. The creation of transmural lesions by different energy sources to induce electrically isolating lesions and to interrupt the circuits required to propagate atrial fibrillation is the actual way of treatment [1].

Tissue heating can be produced by dielectric (microwave), ohmic (radiofrequency) or optical (laser) devices. Radiofrequency (RF) is the main procedure, typically applied in a unipolar fashion, occasionally bipolar RF is used also [2]. Cooling of the ablation RF electrode with saline has been proposed to increase the lesion size [3].

Cryoaablation results in ice crystals, which do not penetrate cell membranes, but cause irreversible alterations of cytoplasmic components and nuclei [4].

Successes of different treatments of atrial fibrillation differ in the numerous reports published until now. Furthermore, post operative complications, including e.g. esophageal perforation [5,6], thrombo-embolic events [1], and pulmonary vein stenosis appear in variable incidence [1,7].

The aim of this study was a comparative investigation of acute cardiac alterations induced by different energy sources and of morphological approaches in a sheep model. The results may give some further information about the effectiveness and risks of different techniques, leading to a clinical improvement of the methods used in practice.

2. Materials and methods

In this study sheep are used as animal model, because they are relatively easy to handle and tolerate off-pump as well as on-pump surgical interventions.

Thirty-nine female Merino sheep (7.3±1.6 months old, body weight 59.2±6.7 kg) were used. All animals received humane care in compliance with standard guidelines. The study was approved by local governmental offices.

Routine anesthesia and clinical monitoring were performed. After standard median sternotomy, the heart was exposed by opening the pericardium, and pulmonary veins were dissected.

2.1. Procedure for electrophysiological measurements

For pacing and threshold measurements bipolar electrodes were placed on the left atrial appendage (LAA) and pul-
monary veins (PV). Pacing thresholds were determined from the PVs and LAA prior to ablation, immediately after, and 2 h after ablation procedure to confirm electrical isolation. Insulation was defined as the inability to capture the heart with a frequency of 140 and maximum stimulus strength of 25 mA at 0.5 ms.

The energy sources and technical equipment used for endocardial or epicardial ablation are listed in Table 1.

### 2.2. Endocardial ablation procedure

For endocardial ablations, extracorporeal circulation was always used. The procedures were executed in mild hypothermia (32 °C). Twenty-five thousand units of heparin were administered to prevent thrombus formation. Arterial cannulation (18 French) of the supraaortic truncus and venous cannulation (28 French) of the right atrium was performed. The aorta was cross clamped. Myocardial protection was achieved by using antegrade cold blood cardioplegia (16 mmol/l potassium) delivered over 3 min. A small left atriotomy was performed to enable direct access to the ostia of pulmonary veins and mitral valve annulus. Continuous lesion lines were created around the common pulmonary vein and ostium of the left atrial appendage (Fig. 1). After closing the heart, protaminsulfat was administered to facilitate possible thrombus formation on the ablated endocardial surface.

### 2.3. Epicardial ablation procedure

Epicardial ablation was performed off-pump as described previously [8]. Circular atrial lesions (Fig. 2) were created around the pacing electrodes preventing direct contact.

### 2.4. Post ablation procedure

Sheep were killed 2 h after ablation procedure. The heart was fixed in 4% formalin for 48–72 h, and lesions were investigated macroscopically. The thickness of atrial wall, diameter (width) and depth (transmurality) of lesions at ablated sites were measured.

Representative specimens of the grossly identified lesions in the left atrium, and the pulmonary vein were cross sectioned, embedded in paraplast and stained with hematoxylin-eosin and Luxol fast blue. For semiquantitative histopathological investigations specimens were blinded.

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**Table 1** Technical equipment, energy sources and approaches used in atrial ablation in this sheep model

<table>
<thead>
<tr>
<th>Energy source</th>
<th>Approach</th>
<th>Technical equipment</th>
<th>Technical data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwave</td>
<td>epicardial (n=3)</td>
<td>Flex 4®, AFX, Fremont, California, USA</td>
<td>90 s, 65 Watt</td>
</tr>
<tr>
<td></td>
<td>endocardial (n=6)</td>
<td>Surgifrost®, CryoCath, Endocare Inc. Irvine, California, USA</td>
<td>Argon, max – 160 °C, endocardial 60 s, epicardial 120 s</td>
</tr>
<tr>
<td>Cryoablation</td>
<td>epicardial (n=6)</td>
<td>Cobra bipolar® Boston Scientific Inc., Minneapolis, USA</td>
<td>30 s, 50 Watt, max. 80 °C</td>
</tr>
<tr>
<td></td>
<td>endocardial (n=6)</td>
<td>Endocare Inc. Irvine, endocardial 60 s, epicardial 120 s</td>
<td></td>
</tr>
<tr>
<td>Bipolar radiofrequency</td>
<td>epiaclial (n=6)</td>
<td>Cardioblate®, Medtronic Inc., Minneapolis, USA</td>
<td>10–20 s, 25 Watt</td>
</tr>
<tr>
<td></td>
<td>endocardial (n=6)</td>
<td>Edwards Optimize®, Surgical Ablation System 980 nm wave length</td>
<td>36 s, 60 Watt</td>
</tr>
<tr>
<td>Laser</td>
<td>endocardial (n=6)</td>
<td></td>
<td>980 nm wave length</td>
</tr>
</tbody>
</table>

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Fig. 1. Endocardial ablation lines: RAA: right atrial appendage, RV: right ventricle, LAA: left atrial appendage; LV: left ventricle, SVC: superior vena cava, PV: pulmonary vein; pacing leads (arrows), endocardial ablation lines (continuous lines). (Schematic illustration modified from R. Nickel, A. Schumacher, E. Seiferle; Lehrbuch der Anatomie der Haustiere, Vol. III, 2. Ed., page 63. Paul Parey; Berlin und Hamburg 1984).

Fig. 2. Epicardial ablation lines: RAA: right atrial appendage, RV: right ventricle, LAA: left atrial appendage; LV: left ventricle, SVC: superior vena cava, PV: pulmonary vein; pacing leads (arrows), epicardial ablation lines (continuous lines). (Schematic illustration modified from R. Nickel, A. Schumacher, E. Seiferle; Lehrbuch der Anatomie der Haustiere, Vol. III, 2. Ed., page 63. Paul Parey; Berlin und Hamburg 1984).
Table 2
Electrophysiologically proved success of ablation and macroscopically visible diameters of lesions (two hours after application of different energy sources and approaches in this sheep model)

<table>
<thead>
<tr>
<th>Energy Source</th>
<th>Successful electrical isolation LAA</th>
<th>Diameter of lesions LAA</th>
<th>Successful electrical isolation PV</th>
<th>Diameter of lesions PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwave epicardial</td>
<td>0/3</td>
<td>8.3±0.6 mm</td>
<td>0/3</td>
<td>8.0±1.7 mm</td>
</tr>
<tr>
<td>Cryoablation epicardial</td>
<td>1/6</td>
<td>6.6±2.0 mm</td>
<td>4/6</td>
<td>5.4±1.2 mm</td>
</tr>
<tr>
<td>Bipolar RF epicardial</td>
<td>6/6</td>
<td>3.1±1.4 mm</td>
<td>6/6</td>
<td>4.1±1.0 mm</td>
</tr>
<tr>
<td>Cryoablation endocardial</td>
<td>4/6</td>
<td>8.2±2.2 mm</td>
<td>6/6</td>
<td>7.7±3.1 mm</td>
</tr>
<tr>
<td>Microwave endocardial</td>
<td>6/6</td>
<td>9.7±3.7 mm</td>
<td>6/6</td>
<td>8.3±2.3 mm</td>
</tr>
<tr>
<td>Unipolar RF endocardial</td>
<td>6/6</td>
<td>8.5±0.6 mm</td>
<td>6/6</td>
<td>7.2±1.9 mm</td>
</tr>
<tr>
<td>Laser endocardial</td>
<td>6/6</td>
<td>6.8±1.2 mm</td>
<td>6/6</td>
<td>5.6±1.2 mm</td>
</tr>
</tbody>
</table>

3. Results

3.1. Left atrial appendage

3.1.1. Electrophysiological examinations

In most cases successful electrical isolation was induced, but there were no reliable effects in sheep treated with cryoablation or epicardial microwave energy immediately and 2 h after ablation (Table 2).

3.1.2. Macroscopical findings

The left atrial wall was 3.2±0.8 mm thick. In all cases continuous circular ablation lines appeared grayish-white, surrounded by more or less extended hemorrhages. Microwave induced widest lesion lines (9.7±3.7 mm), whereas bipolar RF resulted in slim lines (3.1±1.4 mm) (Table 2).

Transmural necroses were quite obvious in most cases. However, transmurality of necrosis after endo- and epicardial cryoablation and epicardial microwave application could not be identified macroscopically (Fig. 3) in all cases (Table 2).

Endocardial necroses and thrombi were grossly most prominent after microwave or laser and bipolar RF, whereas cryoablation did not induce macroscopically obvious endocardial thrombi.

3.1.3. Histopathological findings in general

In hematoxylin-eosin stained slides, the lesions showed similar findings, independent of the energy sources applied. Diameter of altered tissue corresponded to the macroscopic findings.

In general, the affected endocardium was characterized by necrotic endothelial cells, destructed collagen fibers, edema, hemorrhages, and more or less thrombus formation (Fig. 2).

Myocardial necrosis and hemorrhages of different degrees were obvious in all cases. Three types of myocardial necrosis could be identified (Fig. 4a–c), especially after using the Luxol fast blue stain: (1) ‘Myocardial coagulation necrosis with lost cross striation’. (2) ‘Myocardial coagulation necrosis with preserved cross striation’. (3) ‘Myofibrillar contraction band necrosis’ (Table 3).

In intact tissue LFB stain resulted in pink nuclei, brown erythrocytes, light reddish cardiomyocytes, and unstained collagen fibers. Contracture band necrosis showed irregular deep blue colored cross striations. Areas of coagulation necrosis with preserved cross striation appeared light bluish, whereas areas with lost cross striation stained deep blue.

Necroses of intramural vessels were characterized by necrotic and detaching endothelia, vacuolated pycnotic mediaymocytes, and adventitia with fragmented collagen fibers.

Fig. 4. (a) Myocardial coagulation necrosis with a loss of cross striation induced by cryoablation. Cardiomyocytes are shrunken and irregularly formed with disrupted sarcolemma (arrows), pycnotic nuclei and interstitial edema (E), necrotic fibroblasts (F), (Hematoxylin-eosin stain, bar = 100 μm). (b) Myocardial coagulation necrosis with preserved cross striation induced by unipolar radiofrequency. Cardiomyocytes show pycnotic nuclei but cross striation (c) is still obvious, sarcolemma is focally disrupted (arrows). (Hematoxylin-eosin stain, bar = 100 μm). (c) Myocardial contraction band necrosis induced by microwave energy. Cardiomyocytes show irregularly alternating hypercontracted (hc) and hypotensioned (ht) segments and multifocally disrupted sarcolemma (arrows). The interstitium was mildly edematous (E). (Luxol fast blue stain, bar = 100 μm).
Necroses and infiltration of neutrophilic granulocytes to the epicardium occurred predominantly after epicardial ablation (Table 3).

3.1.4. Comparison of the histopathological alterations induced by different methods

Epidermal microwave ablation induced severe endocardial necroses but minimal thrombi. In contrast to the electrophysiological data, myocardial necroses were transmural and severe, not demarcated, and affected intramural vessels intensively, but hemorrhages were mild. Epicardium showed severe necrosis and mild inflammation.

In contrast, endocardial microwave energy induced marked endothelial thrombi, moderate transmural hemorrhages, and less epicardial inflammation. Transmural lesions in all cases corresponded to the electrophysiological data.

Epidermal cryoablation induced mild or severe endocardial necrosis but no thrombus formation. Myocardial necroses were partially demarcated and not transmural in 5 of 6 cases, which was reflected by electrophysiological measurements. Vascular necroses and hemorrhages as well as epicardial necroses were mild.

In contrast, endocardial cryoablation induced transmural lesions in all cases but insulation was confirmed only in 4 of 6 cases. Only a few thrombi occurred in this method.

Epidermal bipolar RF led to intensive endocardial necroses and thrombi, severe sharply demarcated transmural myocardial necroses but mild vascular lesions. Severe myocardial hemorrhages and moderate epicardial necroses were seen (Fig. 5).

Endocardial unipolar RF resulted in severe endocardial necroses but few thrombi. Necroses of the myocardium were intense, transmural and well demarcated. Vessels were affected only mildly but moderate hemorrhages occurred. Epicardial lesions were mild.

Endocardial laser ablation resulted in severe endocardial necroses and thrombus formation. Myocardial necroses were severe, transmural and mainly not demarcated.

![Fig. 5. Transmural well demarcated myocardial damage induced by bipolar radiofrequency: Normal myocardium (n), borderline zone of contraction band necrosis (cb) and zone of coagulation necrosis (cn) with marked edema (e) and hemorrhages (arrows). (Hematoxylin-eosin stain, bar = 200 μm).](image)

Severe vascular necroses and marked hemorrhages occurred. Epicardial necroses were moderately intense.

3.2. Pulmonary vein

3.2.1. Electrophysiological examinations

In most cases successful electrical isolation was induced, but there were no reliable effects in sheep treated with epicardial microwave energy or cryoablation immediately and 2 h after ablation (Table 2).

3.2.2. Macroscopical findings

In all cases pulmonary veins were approximately 1 mm thick. Altered areas appeared transmural grayish-white with extended hemorrhages. Microwave induced widest lesions (8.3 ± 2.3 mm), whereas bipolar RF resulted in slim lesion lines (3.1 ± 1.0 mm) (Table 2).

Table 3
Comparison of histomorphological characteristics of alterations of the LAA (two hours after application of different energy sources and approaches in this sheep model)

<table>
<thead>
<tr>
<th></th>
<th>Endocardial necrosis</th>
<th>Endocardiac thrombi</th>
<th>Transmurality and type of myocardial necrosis</th>
<th>Intramural vascular necrosis</th>
<th>Myocardial hemorrhages</th>
<th>Epicardial necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwave</td>
<td>++ + +</td>
<td>+</td>
<td>3/3</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>epicardial</td>
<td></td>
<td></td>
<td>Type 1, 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryoablation</td>
<td>++ + + +</td>
<td></td>
<td>1/6</td>
<td></td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>epicardial</td>
<td></td>
<td></td>
<td>Type 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bipolar RF</td>
<td>++</td>
<td>+++</td>
<td>6/6</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>epicardial</td>
<td></td>
<td></td>
<td>Type 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryoablation</td>
<td>++</td>
<td>+</td>
<td>6/6</td>
<td>+</td>
<td>+++</td>
<td>+/ +</td>
</tr>
<tr>
<td>endocardial</td>
<td></td>
<td></td>
<td>Type 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microwave</td>
<td>++ + + +</td>
<td>++</td>
<td>6/6</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>endocardial</td>
<td></td>
<td></td>
<td>Type 1, 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unipolar RF</td>
<td>++ + +</td>
<td>+</td>
<td>6/6</td>
<td>+</td>
<td>+++</td>
<td>+</td>
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<td></td>
<td></td>
<td>Type 2, 3</td>
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<tr>
<td>Laser</td>
<td>+++</td>
<td>++</td>
<td>6/6</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>endocardial</td>
<td></td>
<td></td>
<td>Type 1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- - none, + mild, ++ moderate, +++ severe.

Type of myocardial necrosis: 1: coagulation with lost cross striation; 2: coagulation necrosis with preserved cross striation; 3. contraction band necrosis.
3.2.3. Histopathological findings

The pulmonary veins in hematoxylin-eosin stained slides showed similar lesions in all cases investigated, independent of the treatment. Severe transmural necrosis with total loss of endothelial cells and destroyed collagen and elastic fibers appeared in most cases. Thrombi and intramural hemorrhages were most striking after endocardial application of microwave energy.

However, corresponding to the electrophysiological data, epicardial microwave ablation (all sheep) and epicardial cryoablation lesions (2/6 sheep) were not transmural.

3.2.4. Correlation of electrophysiological and histopathological results

Transmural necroses were observed by light microscopy in all cases of endocardial ablation (Table 3). However, although electrophysiological data showed failure of electrical isolation after endocardial cryoablation (two of six cases) and epicardial microwave (all cases), transmural lesions were seen histologically. In contrast, epicardial cryoablation did not produce transmural changes in five of six cases, which corresponded to the electrophysiological findings.

It can be summarized that epicardial ablation techniques (cryoablation and microwave) were less successful in this acute model than endocardial approaches. However, epicardial bipolar RF was effective and resulted in well demarcated slim lesion lines but induced marked thrombus formation.

Endocardial methods were mainly effective but microwave and laser induced intensive thrombus formation. In contrast, cryoablation and unipolar RF were effective and led to little endocardial thrombi.

4. Discussion

The aim of this study was a detailed comparison of the effectiveness and morphological lesions induced by different energy sources and approaches in atrial ablation in one model.

Although only six sheep were included in each group, the results within a group were similar and only a few individual differences occurred. In most cases (36 out of 39) of our study, acute electrophysiological, macroscopical, and histological findings were closely related. However, in some cases (epicardial microwave and cryoablation), differences occurred. This phenomenon was also reported by other authors [9–11]. Santiago et al. showed that tissue temperature induced, or thickness of the atrial wall, did not correlate with transmurality of the lesions in vitro and in vivo [9,10]. In our study ovine arterial walls were approximately 3 mm thick, and variations in thickness did not correlate to failure of ablation procedure.

In our study, electrophysiological data showed a failure of insulation although histopathologically transmurality was seen. This is in contrast to the findings of van Brakel et al. who did not see transmural lesions histologically, although electrophysiological investigations confirmed successful epicardial microwave ablation [11]. The reasons for the discrepancies of histological and electrophysiological data in our study remained unclear.

In general, endocardial ablation was more reliable than epicardial approach, probably due to thermal isolation by epicardial fat tissue and blood sink phenomenon of the atrial blood flow [7,8]. Therefore, atrial tissue is clamped and delivery of energy always produces transmural lesions [2].

Histomorphological findings of acute tissue destruction and different types of myocardial necroses reported in our study correspond to the literature [8,12,13]. Luxol fast blue stain is a well-known technique to differentiate acute intravital cellular necrosis from postmortem autolysis. It has been shown in our experimental study that the deep blue color in LFB stained slides is due to severe coagulation necrosis with a loss of cross striation and not an artifact as discussed in routine post mortem material [14].

In our study, only acute lesions were investigated. In literature, chronic alterations after atrial ablation are described as more or less lesscared scars [12,13,15]. In some cases remaining intact cardiomyocytes were observed [15], probably responsible for unsatisfying results in mid and long term studies. The course and consequences of wound healing may differ between the ablation techniques performed. Epicardial inflammation may lead to adhesive epicarditis. Endocardial applications resulted in endocardial necrosis and thrombi, probably increasing the risk of thrombo-embolic diseases, valvular deformation and endocardial scars [7].

It can be summarized that this comparative acute study showed marked differences in effectiveness and morphological lesions induced by various ablation techniques and procedures. Taking into consideration that unipolar RF has induced esophageal lesions in clinical practice [5,6], this study leads to the conclusion that epicardial bipolar RF, as used in this model, is the most effective and safest method. Furthermore, endocardial cryoenergy was mainly effective and induced minimal thrombus formation. Further research experience, especially long term studies, combined with clinical data will be required to confirm the results of this study.

References


