Epicardial radiofrequency applications: in vitro and in vivo studies on human atrial myocardium

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Abstract

Objectives: To obtain a better understanding of tissue damage induced in human atria by epicardial radiofrequency ablation and its correlation with intra-tissue temperatures measured sub-epicardially and sub-endocardially. Methods: Radiofrequency (RF) currents were delivered to human atrial tissues using experimental set-ups to simulate surgical RF epicardial ablation at 80, 85 and 90 °C. Sub-endocardial and sub-epicardial temperatures were measured with thermocouples during the ablations. Twelve samples from in vitro epicardial ablations were histologically assessed. Localized RF epicardial ablations at same temperatures were performed on 38 mitral patients with concomitant atrial fibrillation (AF) before full cardiopulmonary bypass and samples histologically assessed. All patients had endocardial RF ablation at 70 °C to treat AF. Results: In vitro: Sub-endocardial temperatures were lower than 50 °C except on thin atria (< 2–3 mm) in ablations at 80 and 85 °C and on thicker atria (> 5 mm) in ablations at 90 °C. Lesions measured 0.85–1.98 mm, all showed epicardial and myocardial damage but none were transmural. Mitral patients: Lesions measured 0.38–3.25 mm and 13/25 induced at 70 °C, 2/8 at 80 °C, 1/4 at 85 °C and 0/1 at 90 °C were confined to the epicardium leaving the myocardium undamaged. The remaining had damage of the epicardium and of variable portions of the myocardium, and three were transmural. Conclusions: The application temperature and the intra-tissue temperature are not the sole factors that determine lesion depth. The thickness and the composition of the epicardium and of the myocardium are major determinants in the formation of the lesion.

Keywords: Radiofrequency ablation; Tissue temperature; Lesion histology

1. Introduction

Radiofrequency catheter ablation is accepted as a safe and effective way of curing many types of cardiac arrhythmias. Our group introduced the concept of the surgical treatment of atrial fibrillation by performing the bilateral isolation of the pulmonary veins with radiofrequency ablation [1].

There are several publications on electrode/tissue temperature using in vitro and in vivo experimental [2–4] and theoretical models [5,6] simulating percutaneous approaches, as well as in vivo/in vitro experiments of epicardial radiofrequency (RF) ablation in animals [7,8]. However there is, to our knowledge, no data relating intra-tissue temperatures with histological assessment of the lesions induced by surgical epicardial RF applications in human atrial myocardium.

We studied the temperatures measured sub-endocardially and sub-epicardially under different settings of radiofrequency epicardial applications and their relation with the thickness of the atrial wall. We used fragments of human atrial myocardium in our experimental model. Similar applications were performed in selected mitral patients with concomitant atrial fibrillation submitted to valve surgery and bilateral isolation of the pulmonary veins with endocardial RF ablation. The various lesions were histologically studied to evaluate acute tissue alterations induced by the RF application, to measure the lesion depth and assess transmurality.
2. Materials and methods

2.1. RF ablations in vitro

The in vitro experiments were carried out on 28 fragments of human atrial walls from organ donors aged 17–82 years (40.0 ± 18.4 years), ten males and five females. Three points, approximately 5 mm apart, were marked on the epicardium of the atrial fragment along a straight line. The wall thickness was measured at those points, with an error of 0.3 mm, using a digitizer Threespace (Polhemus, Vermont, CA). Each atrial fragment had an area of approximately 30 cm². T-type thermocouples (0.10 mm thick) were inserted sub-endocardially and sub-epicardially at those points and connected to a signal processing unit (Boston Scientific, San Jose, CA). The unit was connected to a PC for data acquisition and real time graphics display of sub-endocardial and sub-epicardial temperatures.

The atrial tissue was mounted with its endocardial surface against the lateral opening of a custom built bath filled with circulating saline solution kept at 37 °C. In order to simulate conditions found in surgical epicardial RF ablation on a beating heart a roller pump was used to keep the saline solution circulating with a debit of 3 l/min. Radiofrequency currents were delivered to the tissue between the catheter placed over the three points marked on the epicardial surface and the dispersive electrode placed underneath the bath. We used a malleable Thermaline probe connected to a radio-frequency generator [9] (Boston Scientific, San Jose, CA) to perform epicardial ablations at set temperatures of 80 °C (n = 10), 85 °C (n = 12) and 90 °C (n = 6) for 2 min.

Twelve samples from RF applications at 80, 85 and 90 °C were histologically assessed.

2.2. RF ablations in mitral patients

RF epicardial applications at set temperatures of 70 °C (n = 25), 80 °C (n = 8), 85 °C (n = 4) and 90 °C (n = 1) were performed for 2 min on the edge of the LA incision in front of the right pulmonary veins of 38 patients (aged 33–73 years (58.3 ± 10.9) of which 27 were females) with concomitant atrial fibrillation submitted to mitral valve surgery. The epicardial ablations were always performed under normothermia on a beating heart during partial cardiopulmonary bypass (CPB). The heart was arrested after the epicardial ablation and fragments of tissue were removed from the zone of ablation of the 38 patients and were observed histologically.

Radiofrequency endocardial applications were then performed at a set temperature of 70 °C for 2 min in all patients to achieve bilateral isolation of the pulmonary veins as previously described by the authors [10,11].

2.3. Histopathological assessment

The samples were fixed in 10% buffered formalin, and fragments were serially taken from the whole line of the sample (containing a fragment of the RF induced lesion) in sections that were perpendicular to the line and included the whole thickness of the atrial wall. After paraffin embedding 2 μm cuts were stained with histochemical dyes – haematoxylin/eosin, Gomori’s trichrome and elastic van Gieson. The sections were analyzed under light microscopy by two observers. A metric eyepiece (with a precision of 0.02 mm) was used to measure the thickness of the left atrial wall, of its layers and of the lesions caused by radiofrequency.

3. Results

3.1. RF ablations in vitro

Generally the sub-endocardial temperatures were lower than 50 °C except in ablations on thin atria (<2–3 mm) at 80 and 85 °C (Fig. 1) and on thicker atria (<5 mm) in ablations at 90 °C.

Table 1 shows the values of the sub-endocardial temperatures, the thickness of the epicardium, the depth of the lesions in the myocardial layer and the lesion total

![Fig. 1. Sub-endocardial (send) and sub-epicardial (sepi) temperatures measured at three points of human atrial myocardium during epicardial RF application at 85°C. The initial temperature overshoot is related to the generator response time and the arrows show the end of the ablation.](image-url)
depth, obtained during epicardial radiofrequency applications at set temperatures of 80, 85 and 90 °C on atrial walls of various thickness. For each value of the application temperature, results are split between two values of the atrial wall thickness. Table 1 shows only the values obtained in samples that have histological information. The sub-endocardial temperatures reached during applications at 80, 85 and 90 °C in samples that were not histologically assessed are not shown as they fell in the range of the values of Table 1.

Histologically all the lesions showed damage of the whole epicardium with coagulation or liquefaction necrosis, ‘shrinkage’ of adipose tissue and damage of the muscle fibers. The latter showed cytoplasm homogenization with total loss of cross striations, nuclear hyperchromasia or picnosis and ill-defined cell membrane (histological features of coagulation necrosis). The larger vessels and the nerves did not show significant damage.

None of the 12 lesions histologically assessed were transmural despite the fact that in five of them the sub-endocardial temperatures reached values of and above 50 °C (55.9 ± 6.5 °C).

3.2. RF ablations in mitral patients

Histologically the lesions showed similar features to the in vitro ones, but in mitral patients (Fig. 2 left) the myocardial interstitium at the damaged area contained hemorrhagic foci and thrombosis of the small vessels (Fig. 2 right).

Table 2 shows the thickness of the epicardium, the depth of the myocardial lesion as well as the total depth of the lesion following epicardial RF applications at 70, 80, 85 and 90 °C. For each value of the application temperature, results are split between the cases in which the lesion was confined to the epicardium and those in which the lesion reached the whole epicardium and variable portions of the myocardium (Figs. 2 and 3). Out of the 38 lesions histologically assessed only 3, induced by applications at 70, 80 and at 85 °C, were transmural.

4. Discussion

The goal of a RF ablation is to produce a transmural lesion in order to provide a barrier to reentry currents believed to be responsible for the maintenance of atrial fibrillation. Although lesion of the epicardium is inevitable in RF epicardial ablation, transmurality can be achieved without damage of the endocardium provided that there is damage of the whole myocardium. Since the epicardial fat absorbs part of the RF energy it can be regarded as a barrier to the transmission of energy into the myocardium, during RF epicardial ablation.

4.1. RF ablations in vitro

Our results show that for the same ablation temperature the sub-endocardial temperatures are usually (but not consistently) higher in ablations performed on thinner walls than on thicker walls. Moreover, on atrial walls of similar thickness the sub-endocardial temperatures tend to increase with the set ablation temperature but not consistently. In other words, the sub-endocardial temperatures do not depend uniquely on the atrial wall thickness and on the ablation temperature. This is in agreement with our findings concerning endocardial ablations in human tissues [12].

Histology showed that there is a large variability associated to the depth of the epicardially induced lesions. Although the deepest lesions tend to occur at higher application temperatures, it cannot be stated that the average depth of the lesions increases with the application temperature. It seems that the ablation temperature and the intra-tissue temperatures are not the only factors that influence the lesion depth. In fact, sub-endocardial temperatures above 50 °C were measured in five epicardial applications without achieving transmurality. We encountered a similar situation in previous in vitro studies of endocardial ablations on human tissues [12] and again this raises the issue of whether 50 °C as the temperature threshold between viable and non-viable tissue [2,13,14] is true for all biological tissues. It is worth noting that all the referred studies were performed in healthy canine
ventricular myocardium and rabbit skeletal muscle and not in human myocardial tissue. Further studies are needed to clarify this issue.

It might be argued that the temperatures opposite the application surface were incorrectly measured during the epicardial applications that led to the non-transmural lesions. We have no reason to believe that this was the case. The very thin thermocouples were always visible under the endocardium and were inserted at three different points approximately 5 mm apart (to measure the tissue temperature under the area ablated by the same electrode) in order to compensate for the inevitable imprecision in their insertion.

4.2. RF ablations in mitral patients

The thickness of the normal human left atrial wall, and the proportion of its three layers, vary from person to person, depending on age and on related diseases but its average value is approximately 3 mm. In the majority of patients with mitral valve pathology the endocardium is thicker than in normal subjects due to a higher content in elastic fibers, in collagen, in fat content and to eventual smooth muscle cells hyperplasia. The thickness of the myocardium is also highly variable, due to myocyte hypertrophy and fibrosis or lipomatosis of the interstitium. The thickness of the epicardium depends on its fat content.

Histology showed that RF epicardial applications in mitral patients at 70, 80 and 85 °C led, in each case, to myocardial lesions of variable depth:

Half of the ablations at 70 °C produced lesions that were confined to the epicardium (Group A, Table 2), whereas the remaining cases produced lesions that damaged the epicardium and variable portions of the myocardium (Group B, Table 2). It is interesting to note that the average thickness of the epicardium pertaining to samples from the two groups was similar. Furthermore, the two deepest myocardial lesions did not occur in walls with the thinnest epicardium as might be expected. In one of them the epicardium measured 1.00 mm and in the other one it was

<table>
<thead>
<tr>
<th>Set T (°C)</th>
<th>Group</th>
<th>N</th>
<th>Epicardial wall (mm)</th>
<th>Myocardial lesion (mm)</th>
<th>% DM</th>
<th>Total lesion depth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>A</td>
<td>13</td>
<td>0.89 ± 0.32</td>
<td>0.00 ± 0.00</td>
<td>0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>12</td>
<td>0.75 ± 0.56</td>
<td>0.36 ± 0.33</td>
<td>40.0</td>
<td>0.87 ± 0.29</td>
</tr>
<tr>
<td>80</td>
<td>A</td>
<td>2</td>
<td>0.81 ± 0.44</td>
<td>0.00 ± 0.00</td>
<td>0.0</td>
<td>0.81 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>6</td>
<td>0.79 ± 0.50</td>
<td>0.62 ± 0.19</td>
<td>53.0</td>
<td>1.27 ± 0.44</td>
</tr>
<tr>
<td>85</td>
<td>A</td>
<td>1</td>
<td>1.63</td>
<td>0.00</td>
<td>0.0</td>
<td>1.63</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>3</td>
<td>0.58 ± 0.30</td>
<td>0.72 ± 0.55</td>
<td>57.1</td>
<td>1.29 ± 0.44</td>
</tr>
<tr>
<td>90</td>
<td>B</td>
<td>1</td>
<td>1.50</td>
<td>0.50</td>
<td>30.8</td>
<td>2.00</td>
</tr>
</tbody>
</table>

A, group of lesions that did not show myocardial damage; B, group of lesions with myocardial damage; T, temperature; and % DM, percentage of damaged myocardium.

Fig. 2. Non-transmural lesion induced by epicardial RF in a mitral patient. Left: Epic., epicardium; Myoc., myocardium; * shows damaged area of the myocardium; and F shows interstitial fibrosis. Right: Arrows show thrombosis of small interstitial vessels in the damaged area of the myocardium. (Gomori’s trichrome × 100 and × 400).

Fig. 3. Transmural lesion induced by epicardial RF in a mitral patient. Endoc, endocardium; and Myoc., myocardium. Left: Note very thickened endocardium. Right: Arrows show thrombosis of small interstitial vessels next to the endocardium confirming the transmurality of the lesion. (Gomori’s trichrome × 100 and × 400).
showed lesions confined to the epicardium leaving the myocardium undamaged, and one of the remaining lesions was transmural.

At 85 °C a similar proportion of the lesions was confined to the epicardium and one of the remaining lesions was also transmural. Again there is no correlation between the depth of the myocardial lesion and the thickness of the epicardium.

Similarly to the in vitro applications, it cannot be stated that increasing the application temperature will increase the average depth of the myocardial lesion. Nevertheless, in mitral patients the number of lesions confined to the epicardium appears to decrease when the temperature of RF application is raised.

When lesions obtained in vitro are compared with the ones similarly obtained in mitral patients limitations of the in vitro model, i.e. the lack of microcirculation and the differences in blood and saline flow must be taken into account. Ablations in patients were performed under partial CPB meaning that the saline flow in vitro and its cooling effect were probably higher than the blood flow through the atrium. This may be the reason why transmurality was never achieved in vitro.

Another important difference between the in vitro model and the in vivo results is that the former used atrial tissue from organ donors without previous pathology, other than age-related alterations, whereas the in vivo results came from mitral patients that had disease atrial walls. This may explain why all the in vitro lesions damaged the whole epicardium plus variable portions of the myocardium, whereas a significant number of lesions in mitral patients were confined to the epicardium.

Our findings suggest that the thickness and the composition of the epicardium and of the myocardium play an important role in the formation of the myocardial lesion induced by epicardial RF applications and that this may account for the large variability in the clinical results reported by different groups [15–17]. Since the histological content varies from patient to patient it is logical to assume that this feature is one of the reason for such variability. From a biophysical point of view, it is likely that atrial tissues with different compositions will have different electrical properties, which will affect the conduction of RF electrical current in the tissue. For the aforementioned reasons areas of epicardial fat should be removed or avoided during RF epicardial ablation.

When we compare the depth of the epicardially induced lesions in mitral patients at 80, 85 and 90 °C (Table 2) with that of the endocardially induced lesion at 70 °C (1.06 ± 0.46 mm) also in mitral patients [12] it becomes apparent that the depth of the lesions is similar. It may therefore be argued that, from the point of view of the lesion depth, it does not matter whether the ablation is performed endocardially or epicardially. However, because the endocardium of mitral patients is often very thickened it is sometimes more likely to obtain a deep lesion epicardially than endocardially. An example of such a situation in which transmurality was achieved with an epicardial ablation is illustrated in Fig. 3. Had we attempted to induce such a lesion endocardially it is unlikely that transmurality would have been achieved due to the very thickened endocardium.

Conversely, in re-operated patients the epicardium may be so thickened that an epicardial ablation will have little chance to be transmural. Although the future lies on epicardial ablations on beating heart these considerations will have to be taken into account.

The development of a definitive treatment of atrial fibrillation with radiofrequency requires that the ideal ablation lines be defined. Until transmurality is consistently achieved a scientific comparison between surgical techniques will remain a clinical challenge.

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Appendix A. Conference discussion

**Dr A. Morit (Frankfurt, Germany):** If it is obviously very uncertain that we achieve with various techniques true transmural lesions, then we should think about cutting down or decreasing the length of ablation lesions. When we saw the concept, for example, that Dr Schuetz used and others do also, they are using very long lines from mitral ring to one pulmonary vein, in, out and so on.

So if we now have the experience that at least with various energy sources we don’t reach maybe not a true linear transmural defect in the myocardium, then we should look out for the easiest or simplest ablation line, because then we at least are sure that we, for example, exclude the pulmonary veins in this box technique as it is now cited. Do you think that is reasonable?

**Dr Melo:** I don’t have the same opinion, because, there is not a relation with length unless you are using too long energy sources, which is not the case for any tool that is on the market. The depth is one issue, the length is another because the energy delivered is really proportional to the length of the tool you are using.

**Dr Moritz:** But the less perfect your transmurality is the more chance you have that it doesn’t work.

**Dr Melo:** Because I believe the minimal lesion set is bilateral isolation of the pulmonary veins, I should agree with you, but we have to go further because there are many patients, at least 30% of patients, in whom that is not enough.

**Dr F. Mohr (Leipzig, Germany):** I have a question and a remark. You maybe are the only one who can talk the same language in between all of surgeons and electrophysiologists, and I would like to make a comment, and it is a comment of your friend, Robert Dion, and I absolutely agree. Talking about your in vitro tests right now, others have done similar testing, comparing two radiofrequency probes, comparing the same probes you were using, and it was clear that contact to the tissue makes a major difference, and as soon as you don’t have close contact, it varies very much. So if you are transmural, how soon the epicardial or transmural temperature is reached, number one. Can you comment on that?

And number two, I just want to have your comment. Should the surgeons really think about a different concept as compared to the electrophysiologists, who have agreed upon a linear lesion, more or less, which is connected to the mitral valve? Should we go ahead and do something else as the electrophysiologists have defined in the catheter laboratory? Please comment on that.

**Dr Melo:** Answering to your first question, I fully agree that the contact pressure in between the probe and tissue is a major determinant of tissue temperatures. And because of the problems you have reported, we will be soon reporting experiments with varying pressures. If you make a lot of pressure, your temperatures will be rising very high, and mainly if you are near any other structures, the temperatures will spread. So pressure is really dangerous, especially if you are near a vital organ.

As for the second question, I must say that in the field we have two problems again: persistent atrial fibrillation and permanent atrial fibrillation. Until last year, cardiologists or the most advanced electrophysiologists would say that permanent atrial fibrillation was not amenable for the percutaneous approach.

The first group in the world to report outstanding results in this patients group was Carlos Pappone in San Raffaele, doing what is called the electroanatomical approach (more or less what we described 2 years ago by doing isolation of the pulmonary veins only). And he has reported in the setting of permanent atrial fibrillation without concomitant organic disease a success rate in the range of 70%. Last month at the European Society of Cardiology in Berlin, Michel Haissaguerre, showing exactly the same lines but together with a connecting line to the mitral annulus, has shown exactly the same results. So it is very clear in my mind that the pulmonary veins are a major component of permanent atrial fibrillation. In paroxysmal atrial fibrillation, we have either focal or single wavelength entries.

I think that we cannot address a patient in a different way as the cardiologists do. I know, that, the concept of your lines are slightly different, but they are affecting the pulmonary veins.

I don’t agree with the concept of performing the Maze operation in every patient. We have to develop a stepwise approach. There are completely different groups of patients. I think atrial fibrillation is a symptom.