Direct Measurement of the Lethal Isotherm for Radiofrequency Ablation of Myocardial Tissue

Mark Wood, MD; Scott Goldberg, BA; Melissa Lau, MD; Aneesh Goel; Daniel Alexander, DO; Frederick Han, MD; Shawn Feinstein, BA

Background—The lethal isotherm for radiofrequency catheter ablation of cardiac myocardium is widely accepted to be 50°C, but this has not been directly measured. The purpose of this study was to directly measure the tissue temperature at the edge of radiofrequency lesions in real time using infrared thermal imaging.

Methods and Results—Fifteen radiofrequency lesions of 6 to 240 seconds in duration were applied to the left ventricular surface of isolated perfused pig hearts. At the end of radiofrequency delivery, a thermal image of the tissue surface was acquired with an infrared camera. The lesion was then stained and an optical image of the lesion was obtained. The thermal and optical images were electronically merged to allow determination of the tissue temperature at the edge of the lesion at the end of radiofrequency delivery. By adjusting the temperature overlay display to conform with the edge of the radiofrequency lesion, the lethal isotherm was measured to be 60.6°C (interquartile ranges, 59.7° to 62.4°C; range, 58.1° to 64.2°C). The areas encompassed by the lesion border in the optical image and the lethal isotherm in the thermal image were statistically similar and highly correlated (Spearman ρ=0.99, P<0.001). The lethal isotherm temperature was not related to the duration of radiofrequency delivery or to lesion size (both P>0.64). The areas circumscribed by 50°C isotherms were significantly larger than the areas of the lesions on optical imaging (P=0.002).

Conclusions—By direct measurement, the lethal isotherm for cardiac myocardium is near 61°C for radiofrequency energy deliveries <240 seconds in duration. A 50°C isotherm overestimates lesion size. Accurate knowledge of the lethal isotherm for radiofrequency ablation is important to clinical practice as well as mathematical modeling of radiofrequency lesions. (Circ Arrhythm Electrophysiol. 2011;4:373-378.)

Key Words: radiofrequency ablation ■ lethal isotherm

Radiofrequency ablation affects tissue necrosis primarily through thermal injury.1–3 For myocardial tissue, the minimal tissue temperature necessary to produce permanent tissue destruction around the radiofrequency electrode, the lethal isotherm, has only been estimated indirectly.1–3 Using spaced thermocouples and the mathematical relationship of tissue temperature to distance from the radiofrequency electrode, the lethal isotherm for intact myocardium has been estimated to be 47.9° to 53.6°C.1–3 On the basis of these studies, a tissue temperature of 50°C has been widely accepted to represent the critical temperature for irreversible myocardial injury during radiofrequency ablation.1–3 A precise measurement of the lethal isotherm for radiofrequency ablation is important to clinical practice and the development of new ablation technology. However, the value of the lethal isotherm has not been measured directly. Infrared thermal imaging allows for the measurement of surface temperatures with high precision and spatial resolution. The objective of this study was to use infrared thermal imaging to directly measure the lethal isotherm for myocardial tissue during radiofrequency ablation.

Methods

Tissue Preparation

The tissue model used in this protocol was the intact perfused pig heart. The experimental protocol was approved by our institution’s animal use committee. Yorkshire pigs (weight, 35 to 45 kg) were sedated with ketamine (25 mg/kg), xylazine (2 mg/kg), and propofol (3 mg/kg) and were then intubated and ventilated with 1% to 3% isoflurane. Heparin (10 000 U) was given intravenously, and a cardiectomy was performed via median sternotomy. The heart was immediately submerged in iced saline. A cannula was secured in the aortic root above the level of the coronary artery ostia. The heart was then perfused with warmed (40°C), oxygenated (95% O₂, 5% CO₂) Krebs-Henseleit solution (pH 7.35 to 7.45) at 50 mL/min and placed in a warmed (40°C) fluid bath with a portion of the epicardial left ventricle exposed above the bath fluid level. The exposed tissue would receive the radiofrequency lesion (see below). The higher perfusate and bath temperatures were used to maintain the temperature of the exposed tissue at 38°C. A wedge-shaped section of myocardium (2 cm edges by 1 cm deep) was excised to accommodate the ablation catheter tip perpendicular to the cut surface of the myocardium. The tissue preparation and ablation protocol will be described in greater detail below. The average left ventricular size was 100 cm² with the average weight of the perfused hearts at approximately 250 g. The tissue was immediately placed in the bath fluid, and the temperature of the exposed tissue was maintained at 37°C at all times. The perfusate temperature was maintained at 37°C at all times. The perfusion pressure was maintained at 80 mm Hg, and the temperature of the perfusate and the bath fluid were maintained at 40°C at all times.

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tissue (see below). The ablation site was chosen remote from epicardial vessels. The heart was not stimulated to prevent tissue motion during image acquisition.

Radiofrequency Lesion Formation
Radiofrequency lesions were created with a closed-irrigation, 8F, 4-mm-tip catheter (Chili catheter and model 8004 generator, Boston Scientific, Natick, MA). A closed-irrigation catheter was used to prevent irrigation fluid from spraying onto the tissue surface and interfering with thermal measurements. The catheter tip was positioned against the tissue at the level of the bath fluid perpendicular to the excised area and 2 mm below the epicardial surface of the tissue (Figure 1). The catheter was irrigated with room temperature saline at 36 mL/min. An electrode in the fluid bath completed the electric circuit. Radiofrequency energy was delivered beginning at 20 to 25 W for durations sufficient to create a visible semicircular lesion. The duration of radiofrequency energy delivery varied from approximately 10 seconds to 240 seconds to emulate the ablation times common to clinical applications and to explore the time-temperature relationship between the duration of tissue heating and the temperatures required for lethal injury within these time boundaries. For radiofrequency durations ≤11 seconds, radiofrequency delivery to the excised surface of the tissue produced very shallow epicardial lesions that could not be measured. Therefore, for very short radiofrequency deliveries, the electrode was positioned directly on the epicardial surface to create a circular lesion. To ensure the viability of the tissue preparation only 1 or 2 lesions were created in each heart.

Tissue Staining
Immediately after radiofrequency delivery, a cotton ball saturated with 2% triphenyltetrazolium chloride was placed over the lesion for 5 to 10 minutes such that the lesion edges were sharply demarcated from the remaining tissue. Triphenyltetrazolium chloride differentiates viable from nonviable myocardial tissue.4,5 The tetrazolium staining method has been used extensively to evaluate radiofrequency lesion size.1,3,6 In preliminary work, the lesion edge determined by superficial triphenyltetrazolium chloride staining matched the lesion edge by formal tissue fixation in paraffin, sectioning and staining with hematoxylin and eosin, or with phosphotungstic acid hematoxylin followed by examination with light microscopy.

Imaging
The surface temperature of the tissue exposed above the level of the fluid bath was imaged continuously during lesion formation with an infrared thermal imaging camera (model T400, Flir, Inc, Danderyd, Sweden). The thermal camera measures surface temperature only; thus, the lesion site was exposed above the fluid bath to avoid recording the temperature of fluid overlying submerged tissue. Similarly, a closed irrigation catheter was used to prevent irrigation fluid from spraying onto the tissue surface and interfering with thermal measurements. The camera was held over the tissue on a photographic copy stand. The thermal camera has a temperature range of −20°C to 120°C, image resolution of 320×240, sensitivity of 0.05°C, and accuracy of ±2% of reading.7 Simultaneous with the termination of radiofrequency delivery, a thermal image of the lesion was taken. Preliminary work demonstrated the absence of continued increase in the tissue surface temperature (thermal latency) after the end of energy delivery. After acquisition of the thermal image, the tissue was stained (see above) and the thermal camera replaced with an optical camera (Nikon model 990, 3.34 megapixel resolution, Nikon, Inc, Melville, NY) and an optical image of the tissue was acquired. Preliminary work demonstrated the absence of tissue

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**Figure 1.** Schematic of tissue preparation. The heart is perfused via a cannula in the aorta and is partially submerged in the tissue bath. A wedge of tissue has been excised to accommodate the ablation catheter. Three hollow 20-g needles serve as fiducial points for image registration. A ruler is included in the imaging field for reference. The infrared and optical cameras are held over the tissue with a photographic copy stand.

**Figure 2.** Image merge. The infrared image (upper left) is obtained immediately before the end of radiofrequency energy delivery. The color-coded temperature scale is shown. The 3 fiducial points are indicated by arrowheads. After tissue staining, the optical image (upper right) is taken. The fiducial points are again shown. The bottom figure shows the merged optical and infrared images with the lower limit of the displayed temperature adjusted to correspond to the edge of the lesion. In this experiment, the lethal isotherm is 59.7°C.

**Figure 3.** Magnified view of radiofrequency lesion and the merged image temperature adjusted to correspond to the edge of the lesion. The broken line in the optical image corresponds to the lethal isotherm of 59.7°C.
contraction between the time points of acquisition of the thermal image and the acquisition of the optical image. Tissue contraction in this time frame would spuriously skew the measurement of the lethal isotherm toward higher values.

Protocol and Data Analysis

After placing the heart in the tissue bath, 3 18-gauge needles devoid of their hubs were placed perpendicularly in the tissue within the imaging field. The needle lumens provided fiducial points for registration of the thermal and optical images. A millimeter grid was included in the image field for calibration of distances. The lesion was then created and the thermal image recorded. After staining the optical image was recorded. Using commercially available software (ThermaCam Quickreport, Flir, Inc, Danderyd, Sweden) the thermal image was registered to the optical image and electronically merged as an overlay with the optical image (Figure 2). By adjusting the range of the temperatures displayed in the thermal image overlay, the tissue temperature that most closely fit the contour of the lesion by visual inspection was determined (Figure 2 and Figure 3). This single temperature that most closely fit the visible lesion boundary was taken to be the lethal isotherm. In addition, 5 temperature points were taken equally spaced about the edge of the optical lesion image and averaged as alternate method of measuring the lethal isotherm (Figure 4). The area of the lesion on the optical image and the tissue area circumscribed by the lethal isotherm were determined using the ThermaCam software. Fifteen experiments were performed.

Statistics

All data are presented as median and interquartile ranges. The correlations between lesion areas determined by optical and thermal imaging were analyzed using Spearman rank correlation coefficient ($\rho$). Comparisons between paired data were performed using the Sign test. A probability value <0.05 was considered significant. Statistical analysis was performed using SPSS 13.0 (SPSS Inc, Chicago, IL).

All authors had full access to the data and approved the manuscript.

Results

The results for all 15 experiments are shown in the Table. The median radiofrequency energy duration was 55 seconds (interquartile ranges, 39 to 70 seconds; range, 6 to 240 seconds). The lethal isotherm was 60.6°C (interquartile ranges, 59.7° to 62.4°C; range, 58.1° to 64.2°C) determined by adjusting the temperature overlay to conform to the edge of the visible lesion. The lethal isotherm was 62.1°C (interquartile ranges, 60.5° to 64.2°C; range, 59.2° to 64.6°C) determined by averaging 5 temperature points about the edge of the lesion. There was no significant difference in the value of the lethal isotherm determined by these 2 methods ($P=0.18$). The lethal isotherm temperature was not related to the duration of radiofrequency energy delivery ($P=0.86$) or optical lesion area ($P=0.64$, Figure 5).

If the lethal isotherm temperature accurately reflected the edge of the optical lesion, it would be expected that the areas

Table. Results of Experiments

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>RF Power, W</th>
<th>RF Duration, s</th>
<th>Lethal Isotherm by Area, °C</th>
<th>Lethal Isotherm by Points, °C</th>
<th>Area Lethal Isotherm, cm²</th>
<th>Area Optical Lesion, cm²</th>
<th>Area 50°C Isotherm, cm²</th>
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<td>62.9</td>
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*Radiofrequency (RF) lesion applied to epicardial surface.
encompassed by these 2 boundaries would be similar. The area circumscribed by the optical border of the lesion (0.49 cm²; interquartile ranges, 0.39 to 0.95 cm²) was not significantly different from the area encompassed by the lethal isotherm (0.49 cm²; interquartile ranges, 0.35 to 1.03 cm²; \( P/H_{0.71} \)). The correlation coefficient for the relationship between the area of the optical lesion and the area of the lethal isotherm was 0.99 (\( P/H_{0.001} \), Figure 6). If the lethal isotherm was different from the accepted value of 50°C for myocardial tissue, it would be expected that the areas encompassed by the 50°C isotherm would differ from that encompassed by the edge of the visible lesion. The area circumscribed by the 50°C isotherm (0.98 cm²; interquartile ranges, 0.65 to 1.45 cm²) was significantly larger than the area of the optical lesion (0.49 cm²; interquartile ranges, 0.39 to 0.95 cm²; \( P=0.002 \)).

**Discussion**

The findings of this study suggest that the lethal isotherm of myocardium is near 61°C for durations of radiofrequency energy delivery that are commonly used in clinical practice. A 50°C value that is widely quoted in clinical practice and in mathematical modeling significantly overestimates radiofrequency lesion size.\(^3\) Fifty degrees centigrade was found to be the temperature above which heated superfusate produced irreversible loss of excitability in isolated guinea pig papillary muscles.\(^2\) Below 50°C, no preparation demonstrated irreversible loss of excitability. Between 50° and 56°C, 54% of preparations permanently lost excitability. The tissue was maintained at the target temperature for 60 seconds.

In the 2 remaining studies, the lethal isotherm was estimated from the mathematical relationship between tissue temperature and distance from the ablation electrode. Whaynes et al\(^1\) used radially arranged fluoroptic thermometry probes at 2.5-mm increments from the radiofrequency electrode in an isolated porcine right ventricle preparation. Radiofrequency energy was delivered for 60 to 300 seconds. The lethal isotherm was estimated by fitting the temperatures at each probe to the inverse proportion function characterizing the relation between tissue temperature versus distance from the thermal source. The lesion radius (defined as half the maximal lesion width) was entered into the best fit temperature-distance function to provide an estimate of the tissue temperature at the lesion edge. Using this methodology, the lethal isotherm was estimated to be 53.6±3.2°C for radiofrequency ablation. In the third study, Haines et al\(^3\) estimated the boundary of tissue viability to be at an average temperature of 47.9°C (range, 46.6° to 48.9°C) in an isolated perfused canine right ventricular preparation. Tissue temperature was measured with a single-needle thermistor probe at a fixed location from the ablation electrode. The lethal temperature was also derived from the mathematical relationship between tissue temperature and distance from the ablation catheter. The duration of lesion delivery was 120 seconds.

We believe that our results differ from prior studies primarily due to the advancements in temperature monitoring technology. Specifically, thermal imaging allows temperature measurement as a continuous variable over the tissue surface. This allows for temperature measurements at precise locations with spatial resolution not possible with individual
temperature probes. With the use of thermal imaging, the tissue temperatures at the lesion edge was measured directly in our study. With interspersed temperature probes, the lethal isotherm temperature is necessarily extrapolated from between discrete points of temperature measurement. In the process of extrapolation, sources of error are introduced due to curve fitting, estimation of lesion width, thermistor spacing, thermistor depth, and extraction of data from a best fit line. In the case of the isolated superfused papillary muscle preparation, the 50°C temperature resulted in irreversible tissue injury in only 54% of experiments. It is likely that the temperature needed to irreversibly injure all preparations would be higher. More similar to our findings, Simmers et al. using thermocouples, found that the tissue temperature required for permanent loss of conduction in isolated canine myocardium was 58.0±3.4°C during radiofrequency ablation.

Another possible source of differences from prior studies involves the time-temperature relationship for thermal injury to biological tissue. For many tissue types treated with hyperthermia, it is estimated that for each 1°C temperature rise above 43°C, there is a doubling of the biological effect of the hyperthermia. For long periods of hyperthermia, lower tissue temperatures are needed to produce tissue injury. Conversely, shorter exposures to hyperthermia require higher temperatures to produce tissue damage. In 2 of the previous studies, radiofrequency durations of 120 to up to 300 seconds were used. Despite showing the expected trend, we do not think that longer radiofrequency delivery times are responsible for the lower lethal tissue temperatures reported in previous work. We base this conclusion on the following: First, our study did not demonstrate a time temperature relationship over a frequency deliveries of 20 minutes duration are required. It be associated with a 50°C lethal temperature, radiofrequency ablation using MRI thermography. Clearly, knowledge of the lethal isotherm for cardiac tissue is needed to use such technology. Mathematical modeling has been an important to understanding the biophysics of catheter ablation. Such modeling is critically dependent on the knowledge of the target temperature necessary for permanent tissue destruction. Such modeling has suggested that the 50°C isotherm for myocardial lesion formation overestimates lesion size.

Limitations

The accuracy of the thermal imaging is within 2% of the temperature reading, producing a potential error of about ±1.2°C for the value of the lethal isotherm. Imperfect registration between the optical and thermal images is a potential source of error. The use of sharply delineated fiducial points and electronic merging of the images is thought to minimize the potential for image registration errors. This is evinced by the close statistical similarity between the areas circumscribed by the visible lesion and the lethal isotherm temperature. It is uncertain if the isolated tissue preparation is more vulnerable to thermal injury than tissue in situ. Our study and previous work has used histo-chemical methods to define the anatomic rather than the electrophysiological lesion boundaries. Work from our laboratory with a similar preparation has shown that loss of electric activity occurs within 0.1 mm from the anatomically defined edge of the acute radiofrequency lesion, however. The relevance of the ablation model to the clinical situation can be questioned. Although the methods used here probably create lesions with different morphologies and energy requirements from those in clinical applications, these differences should not affect the value of the lethal isotherm which is a physiological property of the tissue. The value of the lethal isotherm should be independent of the efficiency of tissue heating, electrode contact pressure, lesion size, and characteristics of the radiofrequency delivery, as was demonstrated in this study.

Clinical Implications

Underestimating the lethal myocardial temperature may lead to inadequate radiofrequency power delivery during clinical use. The ability to monitor myocardial temperature in vivo, in real time during radiofrequency ablation, has been demonstrated using MRI thermography. Clearly, knowledge of the lethal isotherm for cardiac tissue is needed to use such technology. Mathematical modeling has been an important to understanding the biophysics of catheter ablation. Such modeling is critically dependent on the knowledge of the target temperature necessary for permanent tissue destruction. Such modeling has suggested that the 50°C isotherm for myocardial lesion formation overestimates lesion size.

Disclosures

None.

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**CLINICAL PERSPECTIVE**

At present, tissue temperatures during catheter ablation cannot be known. Electrode temperatures are only modestly predictive of lesion sizes (and thus tissue temperatures) in vivo. Current practice for energy delivery is based on prior estimates of lethal tissue temperatures and on empirical observation of “what works.” A precise estimate of the lethal isotherm for radiofrequency catheter ablation is important for accurate mathematical modeling of catheter ablation and the evolution of new technologies. Real-time imaging of tissue temperatures during catheter ablation is now feasible using MRI thermography. Clearly, applications of this technology require knowledge of lethal tissue temperatures. The present study provides new insights into lethal tissue temperatures in an experimental model.
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