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

Running title: Wood et al.; Radiofrequency Lethal Isotherm

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Abstract:

Background - The lethal isotherm for radiofrequency (RF) 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 RF lesions in real time using infrared thermal imaging.

Methods and Results - Fifteen radiofrequency lesions of 6 - 240 seconds in duration were applied to the left ventricular surface of isolated perfused pig hearts. At the end of RF 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 RF delivery. By adjusting the temperature overlay display to conform with the edge of the RF lesion, the lethal isotherm was measured to be 60.6°C (interquartile ranges 59.7, 62.4°C, range 58.1 – 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’s rho = 0.99, p <0.001). The lethal isotherm temperature was not related to the duration of RF 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 RF energy deliveries ≤ 240 seconds in duration. A 50°C isotherm overestimates lesion size. Accurate knowledge of the lethal isotherm for RF ablation is important to clinical practice as well as mathematical modeling of RF lesions.

Key words: radiofrequency ablation, lethal isotherm
Radiofrequency (RF) ablation affects tissue necrosis primarily through thermal injury. (1,2,3) 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. (1,2,3) Using spaced thermocouples and the mathematical relationship of tissue temperature to distance from the RF electrode, the lethal isotherm for intact myocardium has been estimated to be 47.9 to 53.6°C. (1,3) Based on these studies a tissue temperature of 50°C has been widely accepted to represent the critical temperature for irreversible myocardial injury during RF ablation. (1,2,3) A precise measurement of the lethal isotherm for RF 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 utilize infrared thermal imaging to directly measure the lethal isotherm for myocardial tissue during RF 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 (35 - 45 kg) were sedated with ketamine (25 mg/kg), xylazine (2 mg/kg) and propofol (3 mg/kg) then intubated and ventilated with 1-3% isofluorane. Heparin (10,000 units) was given intravenously and 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 7.35-7.45) at 50 cc/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 RF lesion (see below). The higher perfusate and bath temperatures were utilized 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 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 4mm 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 cc/minute. An electrode in the fluid bath completed the electrical circuit.

Radiofrequency energy was delivered beginning at 20-25 W for durations sufficient to create a visible semi-circular lesion. The duration of RF 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 RF durations ≤ 11 seconds, RF delivery to the excised surface of the tissue produced very shallow epicardial lesions that could not be measured. Therefore, for very short RF deliveries the electrode was positioned directly on the epicardial surface to create a circular lesion. To insure the viability of the tissue preparation 1 or 2 lesions were created in each heart.
**Tissue Staining:** Immediately following RF delivery, a cotton ball saturated with 2% triphenyltetrazolium chloride was placed over the lesion for 5–10 minutes such that the lesion edges were sharply demarcated from the remaining tissue. Triphenyltetrazolium chloride differentiates viable from non-viable 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 x 240, sensitivity of 0.05°C and accuracy of ± 2% of reading. (7) Simultaneous with the termination of RF 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. Following acquisition of the thermal image the tissue was stained (see below) 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 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, three 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 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’s rank correlation coefficient (rho). Comparisons between paired data were performed using the Sign test. A p 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 RF energy duration was 55 seconds (interquartile ranges 39, 70 seconds, range 6 – 240 seconds). The lethal Isotherm was 60.6°C (interquartile ranges 59.7, 62.4°C, range 58.1 – 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 60.5, 64.2°C, range 59.2 – 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 RF 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 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, 0.95 cm²) was not significantly different from the area encompassed by the lethal isotherm (0.49 cm², interquartile ranges 0.35, 1.03 cm², p = 0.71). The correlation coefficient for the relationship between the area of the optical lesion and the area of the lethal isotherm temperature was 0.99 (p <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, 1.45 cm²) was significantly larger than the area of the optical lesion (0.49 cm², interquartile ranges 0.39, 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 that is widely quoted in clinical practice and in mathematical modeling significantly overestimates RF lesion size.(1,2,3,8,9) The estimate of 50°C was based primarily on 3 studies.(1,2,3) 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 - 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 utilized radially arranged fluoroptic thermometry probes at 2.5 mm increments from the radiofrequency electrode in an isolated porcine right ventricle preparation.(1) 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 RF ablation. In the third study, Haines et al. 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.(3) 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 technologic 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. (2) 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 RF ablation. (10)

Another possible source of differences from prior studies involves the time-temperature relationship for thermal injury to biological tissue. (8,11,12) For many tissue types treated with hyperthermia, it is estimated that each 1°C temperature rise above 43°C, there is a doubling of the biological effect of the hyperthermia. (8,11) 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, RF durations
of 120 to up to 300 seconds were used. (1,3) Despite showing the expected trend, we do not feel that longer RF 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 range of RF deliveries from 6 – 240 seconds. Second, no time-temperature relationship was reported in the previous studies despite using wide ranges of RF deliveries. Third, In skeletal muscle, the lethal temperature at an RF lesion edge is about 60°C for RF deliveries of 3 minutes in duration. (11) To be associated with a 50°C lethal temperature, RF deliveries of 20 minutes duration is required.(11) It is likely that for RF energy durations relevant to cardiac applications such as examined in our work, the duration of RF delivery is too short to demonstrate a significant time temperature relationship. (8,11,12) 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 are a potential source of error. The use of sharply delineated fiducial points and electronic merging of the images is felt 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 histochemical methods to define the anatomic rather than the electrophysiologic lesion boundaries. Work from our laboratory with a similar preparation has shown that loss of electrical activity occurs within 0.1 mm from the anatomically defined edge of the acute radiofrequency lesion, however. (6) The relevance of the ablation model to the clinical situation can be questioned. While the methods used here likely 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 physiologic 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 RF delivery as was demonstrated in this study.

Clinical Implications: Underestimating the lethal myocardial temperature may lead to inadequate RF power delivery during clinical use. The ability to monitor myocardial temperature in vivo, in real time during RF ablation has been demonstrated using MRI thermography. Clearly, knowledge of the lethal isotherm for cardiac tissue is needed to utilize such technology. Mathematical modeling has been an important tool 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 underestimates lesion size.

Conflict of Interest Disclosures: None

References:


Table: Results of Experiments

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*RF lesion applied to epicardial surface

Figure Legends:

Figure 1. Schematic of tissue preparation. The heart 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 the 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.

**Figure 4.** Left Panel: Determination of the lethal isotherm by point determination of the temperature at 5 locations about the edge of the lesion. The temperatures at each location are shown. The lethal isotherm as determined by this method was 60.3°C. Right Panel: Demonstration of the 50°C isotherm in the merged image by adjusting the displayed temperature scale to a lower limit of 50°C. The 50°C isotherm overestimates radius of the lesion boundary by 2 - 3 mm. The measured lethal isotherm (broken line) is shown for reference.

**Figure 5.** A. Lethal isotherm temperature versus duration of RF energy delivery for all 15 experiments. The lethal isotherm was determined by adjusting the temperature display on the merged optical and thermal images to best match the edge of the optical lesion. Results were similar for the lethal isotherm determined by averaging 5 temperature points from the edge of lesion (Spearman’s rho = 0.13, p = 0.657). B. Lethal isotherm temperature versus area of the visible lesion for all 15 experiments.

**Figure 6.** Relationship between lesion area determined on the optical image and lesion area circumscribed by the lethal isotherm in each experiment.
Spearman's rho = 0.99, p < 0.001
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