Effects of Radiofrequency Catheter Ablation on Regional Myocardial Blood Flow
Possible Mechanism for Late Electrophysiological Outcome

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**Background**
We postulated that the late electrophysiological effects of radiofrequency (RF) ablation may be related to microvascular injury extending beyond the region of acute coagulation necrosis.

**Methods and Results**
Eighteen RF lesions created in the left anterior descending coronary artery (LAD) perfusion bed of seven open chest anesthetized dogs were studied. The ablation electrode and surrounding myocardium were imaged using high-resolution two-dimensional echocardiography at 4x magnification. After 60 seconds of RF delivery, sonicated albumin microbubbles (mean size, 4.3 μm) were injected into the LAD to measure regional myocardial perfusion, and time-intensity plots were generated from simultaneously acquired two-dimensional echocardiography images. The regions with persistent contrast effect on two-dimensional echocardiography were larger than the pathological lesions (mean cross-sectional area, 48.3±6.3 versus 19.3±4.7 mm², respectively; P<.0001). The mean contrast transit rate in the area corresponding to the pathological lesion was 25±12% of that in the normal myocardium, but it was also reduced beyond the lesion, being 48±27% and 82±28% of normal, respectively, in the 3-mm and 3- to 6-mm circumferential rims surrounding the pathological lesion (P<.05). Electron microscopy performed in two additional dogs with similar lesions demonstrated the presence of ultrastructural damage to the microvascular endothelium well beyond the pathological lesion edge.

**Conclusions**
RF catheter ablation not only results in a marked reduction in blood flow within the acute pathological lesion but also causes reduced flow beyond the borders of the acute lesion because of microvascular endothelial cell injury. The progression or resolution of tissue injury within the region beyond the border of the pathological lesion may explain the late electrophysiological effects of RF ablation. *(Circulation, 1994;89:2667-2672.)*

**Key Words**
- radiofrequency
- catheters
- ablation
- microvasculature

**Methods**

**Animal Preparation**
Seven mongrel dogs weighing 22 to 30 kg were anesthetized with 30 mg/kg pentobarbital sodium IV (Abbott), intubated, and ventilated with a dual-phase control respirator pump (Harvard Apparatus). Additional anesthesia was given as needed during the experiment. An 8F catheter was placed in the left femoral vein for the administration of intravenous fluids and drugs as needed, and a similar catheter was placed in the left femoral artery to measure arterial pressure and blood gases during the experiment. This catheter was connected to a multichannel physiological recorder (model 4568C, Hewlett Packard) via a fluid-filled transducer (model 1280C, Hewlett Packard).

A left thoracotomy was performed, and the heart was suspended in a pericardial cradle. A 4F catheter was inserted into the left atrium for measurement of left atrial pressure and connected to the multichannel recorder. The proximal portion of the left anterior descending coronary artery (LAD) was dissected free from the surrounding tissues, and two silk ties were placed loosely around it. The left carotid artery was exposed, and a 12F plastic cannula was introduced into its lumen. This cannula was connected to plastic tubing that was placed in a constant-flow roller pump. The other end of this tubing was connected to a custom-designed stainless-steel cannula that had a side-arm that was connected to the
physiological recorder to measure LAD pressure. A Y-connector was placed in this tubing and connected to a power injector for introduction of the contrast agent. The entire system was primed with heparinized saline.

After heparinization and administration of lidocaine, we started the roller pump at a slow speed to prime the extracorporeal circuit with arterial blood. The proximal portion of the LAD was ligated, and a small incision was made in its wall for introduction of the tip of the metal cannula, which was secured in place with a silk tie. The roller pump was adjusted to maintain a mean LAD pressure of 100 to 110 mm Hg, which was measured through the side-arm of the metal cannula.

RF Ablation

All RF ablations were performed in a unipolar fashion from the tip electrode of the ablation catheter to an indifferent dispersive electrode applied to the shaved skin of the thorax. A 500-KHz RF energy source (RFG-3AV, Radionics, Inc) was used, and root-mean-square current and voltage were continuously recorded. Power and impedance were calculated from measured values. The ablation catheter used was 6F in diameter and 4 mm in length. The tip electrode was made of stainless-steel alloy and had a thermistor embedded at the apex of the dome that had a time constant of less than 1 second and accuracy of ±1°C (Radionics, Inc). A small puncture was made in the left ventricular apex, and the RF ablation electrode was positioned against the endocardium of the LAD perfusion bed under echocardiographic guidance.

RF energy was delivered in a random sequence at three or four endocardial sites per dog. Sixty seconds after initiation of RF delivery, contrast agent was injected into the LAD during simultaneously performed echocardiographic imaging that was recorded continuously during and for 5 minutes after the RF ablation. A 5-minute recovery interval was allowed between ablations. At the end of the experiment, the dog was killed, and the heart was processed for pathological analysis.

Histopathology

RF lesion size was determined by nitroblue tetrazolium (NBT) histochemical staining. Grossly nonviable regions were pale yellow and were sharply demarcated from viable regions that stained purple. Individual RF lesions were identified by examining the endocardial surface of the explanted heart. Transmural tissue blocks containing each lesion were excised and bisected in a plane similar to that in which the echocardiographic images were obtained. The bisected specimens were incubated in an NBT solution of 0.5 mg/mL of 0.2 mol/L Sorenson's buffer for 15 minutes at 37°C. Each NBT-stained lesion was then photographed for later analysis. Fig 1A illustrates an example of a typical lesion.

MCE

MCE was performed using a 128-channel, phased-array imaging system with a 7.5-MHz transducer (128XP, Acuson Corp). A saline bath acted as an acoustic interface between the hand-held transducer and the anterior surface of the heart. The smallest imaging depth of 4 cm and the broadest dynamic range of 70 Db were used. The gain and power settings were optimized at the beginning of each experiment and were held constant throughout. The images were recorded on 1.25-cm video tape (VHS) for later analysis, using a video recorder (Panasonic model NV-8950, Matsushita Corp).

The contact point between the RF ablation catheter tip and the endocardium was identified by scanning the anterior surface of the heart with the hand-held transducer. The part of the image showing the tip of the RF electrode and the surrounding myocardium was then magnified fourfold to provide detailed spatial resolution of the region of interest. Because the imaging sector encompassing this region was significantly smaller than the standard 90° sector, the frame rate was 60 Hz instead of the standard 30 Hz, providing a temporal resolution that was twofold that which is normally used.
Soni cated albumin microbubbles (Al bunex, Molecular Bio systems, Inc) were used as the contrast agent. We have shown that these microbubbles have no effect on coronary blood flow or systemic hemodynamics and that their intravascular rheology resembles that of red blood cells, thus acting as markers of red blood cell flow. One milliliter of this agent (mean diameter, 4.3 μm; concentration, 0.48 billion bubbles per milliliter) was diluted with 3 mL of 5% human albumin (American Red Cross, New York), and 0.75 mL of this mixture was introduced into the plastic tubing connected to the power injector. At each stage of the experiment, the contrast agent was injected into the LAD at a rate of 4 mL/s, with 3.25 mL of 0.9% saline acting as flush.

The recorded images were transferred from video tape to the video memory of a dedicated off-line computer (Mipron System, Kontron Electronics) for analysis as previously described. In brief, end-diastolic frames from immediately before contrast injection until its disappearance from the myocardium were selected and aligned using cross-correlation techniques. Regions of interest were identified from a contour drawing of the pathological section of the RF lesion and were planarized on the MCE image where contrast had disappeared from the normal myocardium but persisted around the site of the lesion (Fig 1B).

Four regions of interest were identified after scaling the pathological contour to the echocardiographic image (Fig 1C). Zone 1 was the central zone of pathological necrosis. Zone 2 was a circumferential area extending 3 mm beyond the pathological lesion edge. This region included the echo bright halo surrounding the central zone. Zone 3 was a circumferential rim extending 3 to 6 mm beyond the lesion edge. Normal myocardium was defined as a region farther than 6 mm from the lesion border. The region encompassing the halo (Fig 1B) was planimetered.

The mean pixel intensity within each region of interest was measured in each aligned end-diastolic frame in the injection sequence. Mean video intensities in these regions before appearance of contrast were subtracted from all values after its appearance. Background-subtracted time-intensity plots then were generated. To derive transit rates of microbubbles from different regions, the time-intensity plots from these regions were subjected to curve-fitting using a gamma-variate function: y=Ae^(-x), where A is a scaling factor, and α is equal to transit rate. Previous studies from our laboratory using a similar animal model have demonstrated that α is proportional to myocardial blood flow.

Electron Microscopy

Five RF lesions were created in two additional dogs. The dogs were immediately killed, and the RF lesions were processed for electron microscopy using standard techniques. A minimum of four electron micrographs (final magnification ×60,000) from zones 2 and 3 of each lesion were analyzed for microvascular endothelial injury and compared with normal myocardium. Specific ultrastructural features of evaluated endothelial cells included plasma membrane, nuclear membrane, nuclear chromatin, transport vesicles, endoplasmic reticulum, mitochondria, and cell junctions. In addition, integrity of endothelial cell basement membranes and extent of extravasation of red blood cells were analyzed for each region. The degree of microvascular endothelial cell injury was then scored for each zone by an observer blinded to the results of the MCE findings.

Statistical Analysis

All values were expressed as mean±1 SD unless otherwise specified. Comparisons of α between different zones were made using a two-way ANOVA, whereas those between pathological and MCE data were made using a paired Student's t test. Statistical significance was defined as P<.05 (two-sided).

Results

Twenty-five RF lesions were created in the seven dogs used for MCE. Five lesions were eliminated from analysis because of image attenuation during peak contrast effect, and two lesions were discarded because of unsatisfactory image alignment during subsequent analysis. Data from the remaining 18 lesions are presented. The mean current required to maintain an electrode-tissue interface temperature of 85°C was 217±31 mA. The mean lesion dimensions were a depth of 3.8±1.0 mm, a width of 6.1±1.1 mm, and a cross-sectional area of 19.3±4.7 mm².

MCE Findings

After the injection of microbubbles following 60 seconds of RF energy delivery, minimal contrast effect was observed in the center of the lesion. Adjacent to the central region was a zone of intense contrast effect that persisted for several minutes after microbubble injection. The mean cross-sectional area of this region was 48.4±6.3 mm², which was significantly larger (P<.0001) than the mean cross-sectional area of the pathological lesion (19.3±4.7 mm²). Beyond this zone, relatively normal washout of the microbubbles (within several seconds) was noted (Fig 1B).

The time-intensity plots constructed from the different zones of interest depicted in Fig 1C for a typical RF lesion are illustrated in Fig 2. The mean value for α in the normal zone was 0.71±0.49 in the 18 injection sequences analyzed. Zone 1, which corresponded to the area of pathological necrosis, had minimal contrast effect and significantly delayed microbubble washout. The mean value of α in this zone in the 18 injection sequences studied was 0.20±0.20, which was 25±12% of normal. Zone 2, which immediately surrounded the pathological lesion, also had significantly slow contrast washout. The mean value of α in this zone was 0.39±0.38, which was 48±27% of normal. Zone 3 also had delayed transit of microbubbles but not as slow as the inner zones. The mean value of α in this zone was 0.66±0.51, which was 82±28% of normal (Fig 3).
Electron Microscopy Findings

The electron microscopic findings from five representative RF lesions are summarized in the Table. The prominent ultrastructural damage to the microvascular endothelium observed in zone 2, which included loss of the basement membrane, disruption of the plasma and nuclear membranes, and extravasation of red blood cells, confirmed the presence of significant microvascular injury within this region (Fig 4C). The microvasculature in zone 3 was also abnormal, but the extent of endothelial cellular injury was less marked than in zone 2 (Fig 4B).

Discussion

After delivery of RF energy, loss of conduction may be delayed for as long as several hours.8-7 There also may be late recovery of conduction after an initially successful ablation.8 We postulated that these phenomena may be partly related to microvascular injury extending beyond the site of coagulation necrosis. In certain instances, the damage may be progressive, leading to ongoing myocardial necrosis and loss of conduction. In some cases, the injury may be transient, resulting in recovery of regional perfusion and restoration of electrophysiological function.

The present study demonstrated that myocardial blood flow was diminished markedly within the acute pathological RF lesion. Because this region was subject to coagulation necrosis, this was not an unexpected finding. A significant reduction in myocardial blood flow, however, extended beyond the border of the pathological lesion, indicating that the region of acute myocardial injury produced by RF ablation may be more extensive than that demonstrated by histochemical techniques. Electron microscopy showed the presence of ultrastructural damage to the microvascular endothelium extending outside of the pathological lesion.

Previous Studies

The mechanism of reduced blood flow beyond the site of myocardial necrosis was probably due to RF heat-induced microvascular endothelial injury. It has been demonstrated that microvascular blood flow within rabbit granulation tissue begins to decline at temperatures above 45.7°C.13 Histological studies have demonstrated microvascular endothelial cell swelling and disruption, intravascular thrombosis, and neutrophil adherence to venular endothelium after thermal exposure.14,15 We have previously reported prolonged microbubble transit rates and decreased flow in two separate models of microvascular injury; we produced endothelial injury in one by hypoxia16 and in the other by coronary occlusion followed by reperfusion.17 In both, we noted prolonged microbubble transit rates and reduced flow. Although the mechanism of microvascular injury was different in the present study, the effect on microbubble transit rates was similar, suggesting that endothelial injury may be the cause. The presence of microvascular endothelial damage was demonstrated by electron microscopy in the present study.

Study Limitations

Because the region of coagulation necrosis and surrounding microvascular injury was very small, standard methods of measuring regional myocardial blood flow, such as using radionuclide microspheres, could not be used.18 For accurate count statistics to be obtained with microspheres, the smallest possible tissue sample would be larger than the entire site of injury produced in the current experiments. The demarcation between regions that were only a few millimeters apart therefore would not be possible. Because of this limitation, we elected to use MCE to measure relative changes in regional myocardial blood flow. The use of high-resolution imaging (7.5-MHz transducer with the tissue sample placed within the focal zone of the transducer) coupled with the on-line magnification function and twice the normal sampling (60 Hz) allowed us to examine changes in contrast transit rates in small regions within and surrounding the site of necrosis. Technical limitations of the technique, such as image attenuation and unsatisfactory frame alignment, precluded analysis of 7 of the 25 lesions. Furthermore, although a comparison of pathological and echocardiographic data was made regarding the size of the lesions, the precise echocardiographic plane transecting these lesions could not be determined. Because the lesions were smaller than the thickness of an echo beam, it is likely that the lesions were encompassed within the echo beam and had a topography similar to that noted on pathology. Finally, this study investigated only the acute effects of RF ablation on myocardial microvascular blood flow; the chronic effects of RF ablation on microcirculatory blood flow need to be further characterized.

Electron Microscopy Findings

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PM indicates plasma membrane; NM, nuclear membrane; NC, nuclear chromatin; TV, transport vesicles; ER, endoplasmic reticulum; MI, mitochondria; JX, cell-cell junctions; BM, basement membrane; EX, extravasation of red blood cells; ++ + , severe defect observed; ++ , moderate defect observed; +, minimal defect observed; and −, no defect observed.
Electron micrographs of microvascular endothelium at a magnification of ×55,000. Scaling bars indicate 0.5 μm. A, From healthy myocardium. Normal ultrastructural architecture of the endothelial cell is illustrated. B, From Zone 3. Notice disruption of the nuclear membrane, damage to the basement membrane, and adherence of a red blood cell to the luminal surface of the endothelial cell. C, From Zone 2. Again, complete loss of the normal ultrastructural architecture of the endothelial cell is shown. Arrows indicate a break in the inner plasma membrane of the cell. A red blood cell is seen adhering to the luminal surface of the endothelial cell. B indicates basement membrane; ER, endoplasmic reticulum; M, mitochondrion; N, nucleus; P, plasma membrane; R, red blood cell; and V, transport vesicles.
Conclusions

The present study demonstrated that RF ablation caused a significant reduction in microvascular blood flow well beyond the edge of the acute pathological lesion and was characterized by MCE findings consistent with microvascular endothelial injury. Electron microscopy showed the presence of ultrastructural damage to the microvascular endothelium well beyond the border of the pathological lesion. We speculate that progression or resolution of tissue injury outside the acute pathological lesion may explain the late electrophysiological effects of RF ablation.

Acknowledgments

This work was supported in part by a fellowship award from the North American Society of Pacing and Electrophysiology (S.N.); Grants-in-Aid from the National Center of the American Heart Association (D.E.H., S.K.); a grant from the National Institutes of Health (RO1-48890), Bethesda, Md. (S.K.); a grant from Molecular Biosystems, Inc, San Diego, Calif; and an Equipment Grant from Acuson Corp, Mountain View, Calif. S.K. is an Established Investigator of the American Heart Association. The authors appreciate the editorial services of Mr Jerry Curtis in the preparation of the manuscript.

References


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Circulation. 1994;89:2667-2672
doi: 10.1161/01.CIR.89.6.2667

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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