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A Novel Nonthermal Energy Source for Surgical Epicardial Atrial Ablation: Irreversible Electroporation

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ABSTRACT

Background. All currently used energy sources in surgical ablation for atrial fibrillation create lesions via thermal injury. We report for the first time the in vivo results of a new nonthermal modality of epicardial atrial ablation called irreversible electroporation (IRE). IRE utilizes a sequence of electrical pulses that produce permanent nonthermal damage to tissue in a few seconds with sharp borders between affected and unaffected regions.

Methods. Five pigs underwent beating heart surgical epicardial ablations of their right and/or left atrial appendages, utilizing a sequence of 8, 16, or 32 direct current pulses of 1500 to 2000 V, 100 μs each, at a frequency of 5 per second, applied between two 4-cm long parallel electrodes with an IRE pulse generator. Local temperature measurements were performed during ablations followed by electrical isolation testing by pacing. Animal hearts were excised 24 hours after surgery and processed histologically to evaluate the degree of myocardial tissue necrosis and transmurality.

Results. A clear demarcation line between ablated and normal tissue, with no tissue disruption or charring, was observed on gross inspection of all lesions. Staining results showed complete transmural destruction of atrial tissue at the site of the electrode application in all 10 atrial lesions, measuring a mean of 0.9 cm in depth. Each 3- to 3.5-cm long lesion was created in 1 to 4 seconds with no local temperature change and with demonstration of electrical isolation.

Conclusions. We propose a new modality to perform atrial ablations, which holds the potential of providing very swift, precise, and complete transmurality with no local heating effects.

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INTRODUCTION

The recent resurgence of the field of surgical ablation for the treatment of atrial fibrillation has been predominantly based on a renewed interest in energy sources that create lesions via thermal injury. The majority of currently used energy sources utilize hyperthermic injury by obtaining a tissue temperature of 50°C, which has been shown to be the temperature at which electrophysiologic disruption occurs. A variety of energy sources are used to induce hyperthermic damage including radiofrequency (RF), microwave, laser, and high-intensity focal ultrasound devices [Viola 2002; Cummings 2005; Ninet 2005]. Alternatively, hypothermic injury of the atrial tissue has long been used with cryoablation devices, achieving injury at a tissue temperature of –55°C [Viola 2002; Cummings 2005]. While all of these energy sources have been widely utilized with varying results [Barnett 2006], they do not always produce the required transmural lesion. Furthermore, their use is time consuming in procedures in which time is of the essence. In addition, local complications due to overheating, tissue coagulation, and the variable temperature distribution in the treated tissue, which is typical to the fundamental physical characteristics of the heat-transfer process, have been reported [Doll 2003; Fayad 2003; Manasse 2003].

Recently, our group has begun studying the effects of irreversible electroporation (IRE) on tissues [Davalos 2005; Miller 2005; Edd 2006]. IRE is a modality in which microsecond electrical pulses are applied across the cell to generate a destabilizing electric potential across biological membranes and cause the formation of nanoscale defects in the lipid bilayer; these defects are permanent and lead to cell death. In our preliminary research, we have shown that IRE is an independent modality from thermal modalities and that it affects tissue in a way that is different from conventional thermal ablation modalities. IRE leads to tissue necrosis through an unusual path by producing nanoscale defects in the cell membrane only and sparing all other tissue components, including macromolecules, proteins, connective tissue, and cell and tissue scaffold. The cell death is caused by the departure from homeostatic conditions inside the cell. The parameters of IRE are precise; ie, an electrical pulse either causes IRE on the cell membrane or not, thereby producing sharp, cell-scale

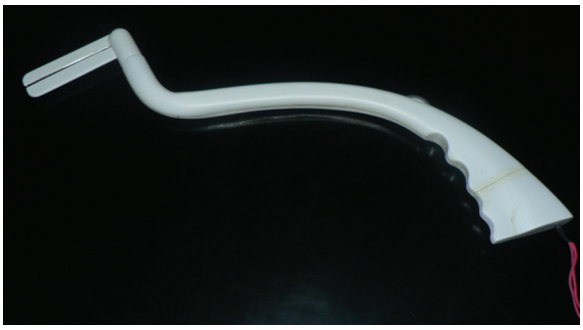

Figure 1. A specially designed hand-held clamp containing 2 parallel electrodes embedded in its end jaws, which can be rotated at their handle articulation up to 270° to allow shaping of the device as the surgical situation requires and provide a clear visual field of the ablation site.

borders between affected and unaffected regions of tissues. It is not affected by blood flow and is capable of producing permanent nonthermal damage to tissue within a fraction of a second [Davalos 2005; Miller 2005; Edd 2006].

The purpose of the current report is to present our initial experience of utilizing the IRE modality in creating epicardial atrial transmural ablation lines in vivo, posing it as an ideal tool in the surgical treatment of atrial fibrillation.

MATERIALS AND METHODS

Experimental Protocol

Five domestic pigs weighing 43 to 46 kg were used in this pilot study. All animals received humane care from a properly trained professional in compliance with both the Principals of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals, published by the National Institute of Health (NIH publication No. 85-23, revised 1985).

Each animal was premedicated, intubated, anesthesized, and monitored continuously throughout the procedure. The heart was exposed through a median sternotomy. With the heart beating, a specially designed hand-held clamp (Figure 1), containing 2 parallel stainless steel electrodes, measuring 2 mm in diameter and 4 cm in length, was applied epicardially on both sides of the left atrial appendage and on the right atrial appendage in 2 pigs. A sequence of 8, 16, or 32 direct current pulses of 1500 to 2000 V, 100 μs each, at a frequency of 5 pulses per second (Table), was applied between the electrodes with an IRE pulse generator (Oncobionic, Rancho Santa Margarita, CA, USA). In 3 of the pigs, 2 ablations were performed on the left atrial appendage, 1 cm apart from each other, for a total of 10 atrial ablations. Temperature was measured at the left and right atrial appendages tips before, during, and after the pulse sequence applications with an EasyWay 15 Thermometer Datalogger (Extech Instruments, Waltham, MA, USA) and a type-K thermocouple. In 2 pigs, an electrical isolation study was performed following IRE ablation by pacing the tips of the left and right atrial appendages distal to the ablation lesions, at a stimulus strength of 20 mA, and capturing the heart response. The sternotomy incision was then closed, and the pigs were extubated and followed for 24 hours, at which point they were euthanized.

Histologic Assessment

Immediately following euthanasia, the animals' hearts were removed en bloc, and their left atrial appendages, as well as right atrial appendages where appropriate, were excised. Each lesion was sectioned 3 times, 1 cm apart, perpendicular to the long axis of the ablation line. The tissue samples from each site were fixed with a 5% formaldehyde fixative, embedded in paraffin, sectioned at 4-μm thickness, and stained with Masson trichrome staining to elucidate the myocardial damage. Degrees of myocardial damage (nuclear staining, homogeneity of myocytes striation), hemorrhage, or amount of inflammatory cells were studied. Complete myocardial necrosis was characterized by rounded, homogenous myocytes with loss of nuclear staining. Histological evaluation of lesion transmurality was based on maximum depth of myocardial necrosis. Complete transmural lesion was defined as myocardial necrosis throughout the atrial wall.

Summary of Ablation Sites, Parameters, and Dimensions*

Animal No.	Ablation Site	No. of Pulses	Pulse Voltage, V	Lesion Depth, cm	Lesion Length, cm	Lesion Width, cm
1	LA	8	2000	0.5	3.0	0.7
1	LA	8	2000	0.4	3.0	1.0
2	LA	8	2000	0.8	3.5	0.9
2	LA	8	2000	1.0	3.5	0.5
3	LA	8	2000	1.0	3.0	0.5
3	LA	8	2000	0.9	3.0	1.0
4	LA	16	1500	1.0	3.5	0.5
4	RA	32	1500	1.4	3.5	0.5
5	LA	16	1500	1.0	3.5	0.5
5	RA	16	1000	1.0	3.5	0.5

*Each pulse sequence was delivered at a frequency of 5 pulses per second, with each pulse being 100-μs long. LA indicates left atrial appendage; RA, right atrial appendage.

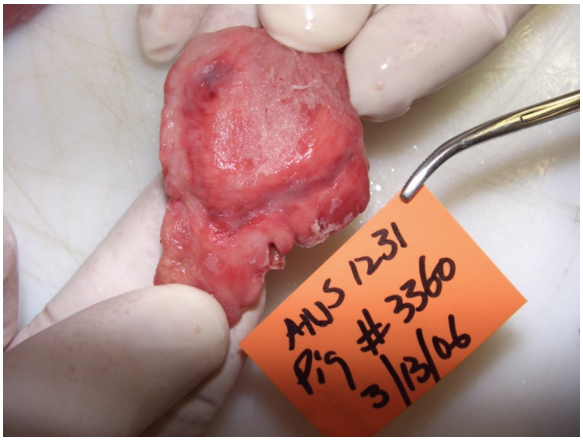

Figure 2. Gross specimen of the left atrial appendage spread open, showing 2 clear linear ablation lesions on both sides of the appendage.

RESULTS

On gross inspection of all 10 atrial lesions 24 hours after ablation, a clear demarcation line between ablated and normal tissue was observed, with no tissue disruption or charring (Figure 2). Masson trichrome staining results of all 10 atrial ablations showed continuous transmural destruction of the atrial tissue—from epicard to endocard—at the site of the electrode application (Figure 3). The 10 atrial lesions had a mean depth of 0.9 cm (range, 0.4-1.4 cm), mean width of 0.66 cm (range, 0.5-1.0 cm), and measured 3 to 3.5 cm in length (Figure 3, Table).

Higher magnifications of the ablated areas have repeatedly demonstrated a sharp demarcation line between the injured necrotic myocardial tissue and the surrounding normal myocardium (Figure 4).

All ablation pulse sequences were completed within 1 to 4 seconds, depending on the number of pulses within each sequence, and caused no permanent arrhythmia or any untoward rhythm disturbance apart from rapid atrial pacing during the pulse sequence application (Figure 5). Local heat measurements at the left and right atrial appendages tips showed no temperature changes during the pulse sequence applications in any of the pigs.

In the 2 pigs in which electrical isolation study was performed, complete electrical isolation was confirmed by pacing the tips of the left and right atrial appendages, distal to the ablation lesions, at a stimulus strength of 20 mA, and failure of the heart to capture.

DISCUSSION

This is the first report to demonstrate the ability of IRE to serve as a surgical modality to create epicardial atrial ablation. Each of the 3- to 3.5-cm long transmural ablation lines were created in 1 to 4 seconds and caused no peripheral thermal damage. These features demonstrate the potential superiority of the IRE nonthermal modality over the currently used hyperthermic or hypothermic energy sources for future clinical application in the surgical treatment of atrial fibrillation.

IRE is based on studies from the early seventies in which cell membrane permeability increased following application of microsecond electrical fields across a cell [Coster 1965; Neumann 1972; Crowley 1973], presumably by the formation of nanoscale defects or pores in the cell membrane [Weaver 2000]. Defects that do not reseal will cause eventual cell death due to the loss of the cell's homeostatic mechanisms in a process now known as IRE. The discovery of the membrane ability to reseal, known as "reversible electroporation" (RE) led to the use of this property for gene transfection in cells [Neumann 1982]. The application of RE to tissues led to important applications in biotechnology and medicine. Electrogenetherapy is the in vivo insertion of genes into cells in tissue through RE and presents an alternative to viral vectors [Gehl 2003]. The first use of electroporation as an ablation technology employed RE in combination with cytotoxic drugs and is named electrochemotherapy (ECT). ECT is accomplished by injecting drugs or macromolecules into a targeted area. Electrodes are then placed into or around that area to generate a reversible permeabilizing electric field in the tissue, thereby introducing the drugs or macromolecules into the cells of the affected area. ECT allows potent but normally impermeable anticancer drugs, such as bleomycin, to be used with electroporation to ablate tissue [Mir 1991]. In the above applications, the electroporation has to be reversible, and IRE is consciously avoided. Therefore, the electrical parameters that induce IRE were studied only as an upper limit to the range of electrical parameters that induce RE.

IRE, however, has been studied extensively in in vitro cellular systems as an effective means for killing both gram negative and positive bacteria responsible for water contamination [Rowan 2000]. When our group began developing the use of IRE in tissues, we were concerned that because IRE

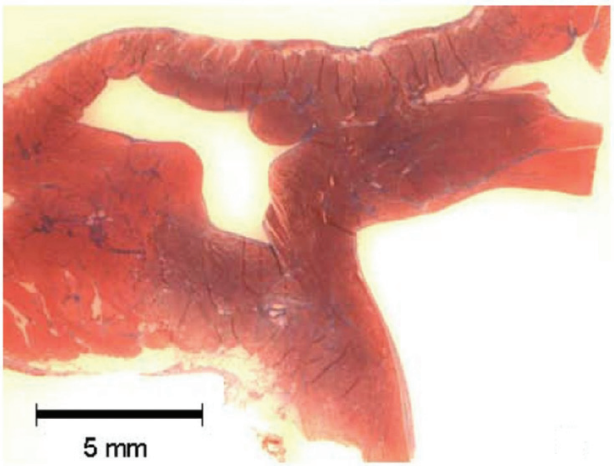

Figure 3. Stereomicroscopy pictures of Masson trichrome stainings (original magnification ×2). Left atrial appendage lesion showing transmural lesion (in purple) from epicard to endocard.

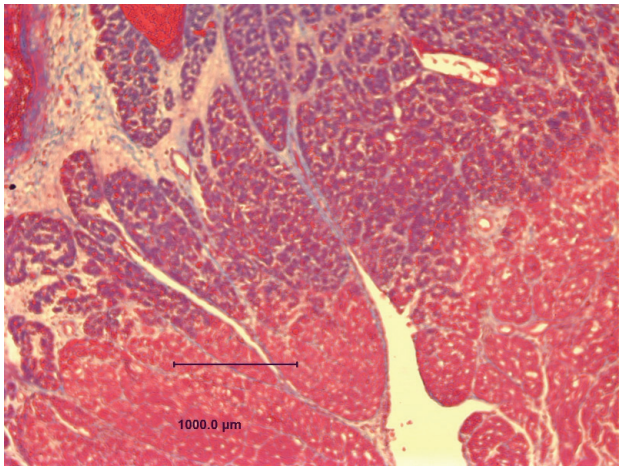

Figure 4. Higher magnification of an ablated area demonstrates a sharp demarcation line between the injured necrotic myocardial tissue (in purple) and the surrounding normal atrial myocardium (original magnification ×10).

employs larger electrical fields and is applied longer than RE, a Joule heating effect might occur if the IRE domain of electrical fields were to actually be mostly superimposed on the thermal ablation domain of these fields. Therefore, in our first published study in this field, we demonstrated with a mathematical model that IRE has the capability to ablate substantial amounts of tissue in a domain of electrical fields that do not overlap the thermal regime, proving that IRE can be used by itself for tissue ablation [Davalos 2005]. This was followed by an acute animal study in the rat liver demonstrating that substantial amounts of tissue can be ablated with IRE without thermal effects, and that the extent of tissue ablation is predictable with mathematical models [Edd 2006]. Furthermore, we found that the ablated area is continuously necrotic, with an abrupt transition, several cell layers thick, between necrotic hepatic parenchyma and the adjacent normal hepatic parenchyma, and that while sinusoids are ablated, the large blood vessels remain intact. An in vitro study with liver cancer cells demonstrated that IRE has the ability to completely ablate cancer cells in the nonthermal regime [Miller 2005].

Extending our previous experience with the IRE modality into the cardiac arena, we have shown in this pilot study that this new modality has the potential to serve as an ideal tool in cardiac ablation. The goal of the ablation procedure is to produce a transmural lesion to provide a barrier to re-entry currents, believed to be responsible for the maintenance of atrial fibrillation. The ideal lesion should penetrate deep enough to cause transmurality, but be as narrow as possible to limit redundant damage to viable myocardial tissue [Thomas 2003; Accord 2005].

Numerous animal and human studies utilizing RF, microwave, or argon-based cryoablation sources have shown that the goal of complete transmurality in atrial ablation is hard to achieve, especially when applied epicardially on a beating heart. Van Brakel et al [2004] have shown in a canine model that complete transmurality of the epicardial microwave ablation lesions is increased when evaluated after 1 to 3 weeks as compared to acute evaluation; however, even then it reached only 66% of the tissue depth compared to 33% in the acute lesions. Thomas et al [2003], performing RF epicardial and endocardial ablations in sheep hearts, have shown that lesions were unlikely to be transmural with either technique when the wall thickness was greater than about 4 mm and that the epicardial fat pad and endocardial cooling by circulating blood have an important negative effect on the epicardial lesion formation. In addition, they have shown that prolongation of the duration of ablation from 1 to 2 minutes does not significantly increase lesion depth. They have demonstrated that RF lesions were wider than they were deep, so deep penetration of lesions into the myocardium can only be achieved by producing broad lesions with large volumes. Gaynor et al [2006], working with microwave ablation in a pig model, have shown that while in the arrested heart transmural lesions can be reliably produced at 90 seconds, in the beating heart only 20% of the atrial lesions were transmural despite prolonged ablation times. Milla et al [2006], working with an argon-based cryoablation device in a beating heart canine model, have shown transmural lesions in 93% of their clamp device lesions and 84% of the linear device lesions, with confidence intervals reaching as low as 44% of transmurality for lesions performed on the left atrial appendage.

Santiago et al [2003] showed no transmurality in 12 lesions of human atrial tissue performed in vitro with epicardial RF with temperatures varying between 80°C and 90°C. Similarly they have shown that only 3 of 38 patients undergoing RF epicardial ablation concomitant with mitral surgery, at temperatures varying between 70°C and 85°C, showed histo-

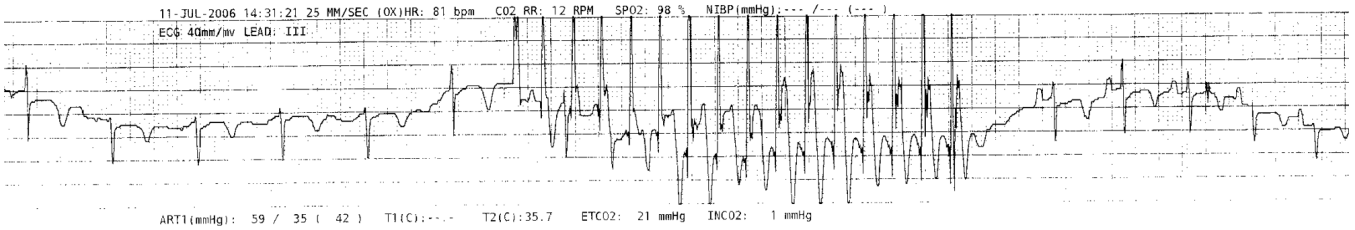

Figure 5. Electrocardiographic tracing showing rapid atrial pacing during a 16-pulse irreversible electroporation ablation sequence, with a frequency of 5 pulses per second, and immediate resumption of sinus rhythm following ablation.

logical transmurality. They concluded that the depth of the ablation lesion is not necessarily determined by the application temperature and that the thickness and composition of the epicardium and myocardium play an important role in the formation of the myocardial lesion. Accord et al [2005], evaluating the hearts of 3 nonablation-related deaths 2 to 22 days after microwave epicardial ablation, found transmural lesions in only 3 of the 13 tissue samples, no ablation damage in another 3 samples, and in the remaining 7 samples the transmural extent of myocardial damage ranged from only 48% to 82%. Denke et al [2005], studying 59 ablation lesions from 7 patients who died 2 to 22 days following RF cooled-tip ablation, found transmurality of the lesions in only 75% of the specimens.

Our preliminary results of complete transmurality in all the lesions performed epicardially by the IRE modality on a beating heart, with an unprecedented atrial tissue depth of 0.9 cm (range, 0.4-1.4 cm), raises our hopes that this new nonthermal energy source might provide a more complete clinical result when applied in patients. We speculate that the nonthermal mode of action of the IRE is the basis for the completeness of transmurality, as heat dissipation by the circulating blood plays no role in diminishing the depth of ablation in this modality as it does in all other thermally based techniques.

All currently used energy sources in atrial ablation are time consuming. Abreu Filho et al [2005], using a saline-irrigated cooled-tip RF ablation device, have shown that the additional mean time required for the left-sided ablation was 14 minutes, and for the right-sided ablation the mean time was 12 minutes. Melby et al [2006] reported requiring 44 minutes to complete both the left and right set of lesions. Mack et al [2005], using an argon-based cryoablation, have indicated an average of 17 minutes for the completion of the left-sided lesions. The new high-intensity focused ultrasound ablation technique takes an average of 12 minutes to perform [Ninet 2005]. The IRE modality is unique among all other sources in its swiftness of action—only 1 to 4 seconds are required to perform each 3- to 3.5-cm long lesion.

The nonthermal characteristic of the IRE modality, as evidenced by the constant temperature measured during our ablations, provides an additional safety feature to this source compared to the sometimes hazardous local heating problems created by the RF or microwave devices, with reported complications such as esophageal perforation [Doll 2003], left main coronary artery lesion [Manasse 2003], or circumflex coronary artery stenosis [Fayad 2003].

We are aware that our preliminary study results are limited by the fact that we have conducted so far only acute experiments and therefore have not yet demonstrated the mid-term or long-term durability of the IRE modality. However, given the 100% transmurality found in all our lesions in this acute study and confirmation of complete electrical isolation across the lesions, we foresee no reason to expect any inferior long-term results. Nevertheless, formal mid-term and long-term experiments with the IRE modality are currently planned. In addition, in our experimental protocol we did not duplicate all clinical sites of electrodes applications, such as the entry of the pulmonary veins into the left atrium, and have elected to perform the ablations at the atrial appendages, merely because of spatial limitations of our first generation hand-held electrodes prototype, which could not yet be easily maneuvered to the deep location of the pulmonary veins. However, as complete transmurality has been produced even in the thick appendages with lesion depth reaching 1.4 cm, we do not expect any limitations of the IRE modality once applied to the fat-padded pulmonary veins. As the IRE modality affects only cell membranes and spares all other tissue components, including macromolecules, proteins, and tissue scaffold, we foresee no reason to expect any undesired results, such as perforation, once the modality is applied to the thinner pulmonary veins.

In conclusion, we propose and demonstrate here a new and exciting modality to perform atrial ablation, which holds the potential of providing very swift, precise, and complete transmurality with no local heating effects.

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