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ORIGINAL ARTICLE

Characterization of Phrenic Nerve Response to Pulsed Field Ablation

Brian Howard, PhD; David E. Haines, MD; Atul Verma, MD; Nicole Kirchhof, DVM; Noah Barka, DVM; Birce Onal, PhD; Mark T. Stewart, BS; Daniel C. Sigg, MD, PhD

BACKGROUND: Phrenic nerve palsy is a well-known complication of cardiac ablation, resulting from the application of direct thermal energy. Emerging pulsed field ablation (PFA) may reduce the risk of phrenic nerve injury but has not been well characterized.

METHODS: Accelerometers and continuous pacing were used during PFA deliveries in a porcine model. Acute dose response was established in a first experimental phase with ascending PFA intensity delivered to the phrenic nerve (n=12). In a second phase, nerves were targeted with a single ablation level to observe the effect of repetitive ablations on nerve function (n=4). A third chronic phase characterized assessed histopathology of nerves adjacent to ablated cardiac tissue (n=6).

RESULTS: Acutely, we observed a dose-dependent response in phrenic nerve function including reversible stunning (R^2=0.965, P<0.001). Furthermore, acute results demonstrated that phrenic nerve function responded to varying levels of PFA and catheter proximity placements, resulting in either: no effect, effect, or stunning. In the chronic study phase, successful isolation of superior vena cava at a dose not predicted to cause phrenic nerve dysfunction was associated with normal phrenic nerve function and normal phrenic nerve histopathology at 4 weeks.

CONCLUSIONS: Proximity of the catheter to the phrenic nerve and the PFA dose level were critical for phrenic nerve response. Gross and histopathologic evaluation of phrenic nerves and diaphragms at a chronic time point yielded no injury. These results provide a basis for understanding the susceptibility and recovery of phrenic nerves in response to PFA and a need for appropriate caution in moving beyond animal models.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: animals ■ catheter ■ diaphragm ■ electroporation ■ phrenic nerve

Pulsed field ablation (PFA) is an emerging energy modality for catheter-based treatment of cardiac arrhythmias and is currently under clinical evaluation.[1,2] PFA therapy involves the application of hundreds to thousands of volts to tissues to induce hyperpermeabilization of cell membranes and subsequently lead to cell death through the mechanism of irreversible electroporation (IRE).[3–5] The lesion formation in response to PFA is a function of the electric field distribution of the pulsed field waveforms applied to the ablation electrodes.[3,5–7] Unlike radiofrequency ablation or cryoablation, PFA induces only a minimal postdelivery temperature rise at the delivery electrodes, indicating that the mechanism of action is largely nonthermal.[8] This mechanism is linked with potential clinical benefits, including possible avoidance of severe complications such as pulmonary vein (PV) stenosis,[9] esophageal fistula, and other collateral damage.[8]

Diaphragmatic paralysis due to phrenic nerve injury is a well-known complication for existing thermal cardiac ablation modalities such as radiofrequency ablation and cryoablation.[10–12] The proximity of the phrenic nerves to

Correspondence to: Daniel C. Sigg, MD, PhD, Medtronic, MVS46, 8200 Coral Sea St, NE, Mounds View, MN 55112. Email daniel.c.sigg@medtronic.com
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WHAT IS KNOWN?

- Diaphragmatic paralysis due to phrenic nerve injury is a well-known complication for existing catheter ablation modalities.
- Pulsed field ablation (PFA) is an emerging energy modality for catheter-based treatment of cardiac arrhythmias and is currently in clinical development. The effect of varying doses of PFA on phrenic nerve function has not been systematically or quantitatively studied.

WHAT THE STUDY ADDS

- The impact of pulsed field ablation on phrenic nerve function was investigated in an in vivo model with a novel accelerometer-based experimental design. A 3 part investigation was completed that tested (1) the effect of increased voltage levels of PFA on the phrenic nerve, (2) the effect of increased repetitive deliveries of PFA at a single dose, and (3) the chronic function of phrenic nerves at 4 weeks after dosing experiments.
- Acute results demonstrated that phrenic nerve function responded to varying levels of PFA and catheter proximity placements, resulting in responses characterized as either no effect, a modulated effect, or temporary stunning. Gross and histopathologic evaluation of phrenic nerves and diaphragms at the chronic assessment yielded no injury.
- A phrenic nerve function dose response curve was generated and validated. This investigation quantified the relative susceptibility of the phrenic nerve to PFA with the intent to steer cardiac ablation towards a safer space that actively seeks to eliminate the potential for phrenic nerve injury.

Nonstandard Abbreviations and Acronyms

IRE	irreversible electroporation
IVC	inferior vena cava
PFA	pulsed field ablation
PV	pulmonary vein
SVC	superior vena cava

anatomic targets of cardiac ablation, in particular the PVs as well as the superior vena cava (SVC), may result in direct damage of the phrenic nerve. While previous preclinical studies have found minimal impact on nerve function after delivery of IRE in cardiac tissue,[1,13] the effect of varying doses of PFA on phrenic nerve function has not been systematically or quantitatively studied. Furthermore, identifying PFA levels capable of avoiding phrenic nerve injury is an important aspect to understanding the safety margin of this emerging therapeutic cardiac ablation modality.

We aimed to precisely identify the impact of PFA on phrenic nerve function in an in vivo model using a 3-part investigation with a novel accelerometer-based experimental design: (1) acute assessment of phrenic nerve function in response to increasing PFA therapy deliveries, (2) acute phrenic nerve response to repeated energy deliveries, and (3) chronic assessment of phrenic nerve function followed by gross examination and histopathologic analysis of both phrenic nerves including pathology of the diaphragm after delivery of IRE.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request. The detailed methods are described in the Supplemental Material.

Experimental Design: Overview

A summary of the experimental design is provided in the Table. This research protocol was approved by the Institutional Animal Care and Use Committee of the University of Minnesota (Acute Studies) and of Medtronic Physiological Research Laboratories (Chronic Studies). A previously described initial feasibility PFA system was reported[1]; this and other preclinical studies evaluated a distinct PFA system[8,9] and waveforms.

In the first part of the investigation (Acute Phase 1: Dose-Response) 6 animals were subject to PFA deliveries with ascending voltage levels at the SVC and inferior vena cava (IVC) near the phrenic nerve (n=12 experiments). Each applied voltage level is defined as a PFA therapy dose. Phrenic nerve function was evaluated in response to PFA dose using accelerometers placed near the diaphragm (Figure 1) and pacing thresholds. The catheter's proximity to the phrenic nerve was characterized by the preablation phrenic nerve pacing threshold. The accelerometer response characterizes phrenic nerve function for 3 cases: normal phrenic nerve function, reduced or modified function, and the absence of phrenic nerve function (stunning for any period of time) which are quantitatively correlated with the associated PFA dose which produced the response. Figure 2 represents one experiment where increasing PFA voltage eventually led to a temporary decrease in function (*) as measured by reduced accelerometer response to diaphragmatic movement (measured in g). In this experiment, first stunning is seen at 1200 V (†).

In the second part of the investigation (Acute Phase 2: Repetition), a single PFA dose was delivered repeatedly with single placements in the SVC and IVC of 2 animals to monitor the nerve response due to repeated ablations (n=4 experiments). Dose levels for this experiment were also predictively selected to elicit the range of phrenic nerve responses observed in the first phase.

In the third part of the investigation (chronic phase), ablations were delivered near the phrenic nerve in the SVC (n=6 experiments). The doses delivered were determined based on data from acute phase 1 and acute phase 2. Isolation of the SVC was tested for entrance block to verify that PFA deliveries targeting the phrenic nerve were also capable of effective cardiac ablation. Phrenic nerve function was assessed at 0 weeks and at 4 weeks via phrenic nerve thresholds and

Table. Experimental Design

	Acute phase 1: dose response (n=12)	Acute phase 2: repetition (n=4)	Chronic phase: (n=6)
Objective	Evaluate phrenic nerve function in response to PFA dose and pacing threshold	Evaluate phrenic nerve function in response to repeated energy deliveries Validate phrenic nerve response based on acute phase 1 data	Evaluate chronic phrenic nerve function Evaluate histopathology of phrenic nerve and pathology of diaphragm at 4 wk
Intervention	Repeated ablations at SVC and IVC with single catheter placements	Repeated ablations at SVC and IVC with single catheter placements	8 distinct ablation placements in SVC
Dosing	Increasing dose levels in all locations	Single dose per site chosen for range of predicted responses	Single dose level in all locations
Accelerometer measurements	Acute phrenic nerve pacing, function, and stunning.	Acute phrenic nerve pacing, function, and stunning.	Phrenic nerve function at 0 and 4 wk

IVC indicates inferior vena cava; PFA, pulsed field ablation; and SVC, superior vena cava.

accelerometers and pathological evaluation of the diaphragm (ie, atrophy).

Gross and Histopathologic Analysis

During necropsy, the SVC, the juxtaposed right and left phrenic nerves as well as the diaphragm were assessed for gross lesions in all 6 chronic animals, collected and immersed in 10% neutral buffered formalin. All specimens were routinely processed and embedded in paraffin. Blocks were cut at 3 to 5 µm and a pair of serial sections was with Hematoxylin and Eosin and Masson trichrome.

In addition, the SVC/phrenic nerve histopathology was assessed in 6 weight matched porcine subjects (6 female Yorkshire pigs, mean body weight 78.8±3.7 kg) who have not undergone PFA.

Statistical Methods

Statistical analysis was conducted with GraphPad Prism (version 9.01). The relationship between pacing threshold and ablation voltage for dose response were assessed with nonlinear regression. Response was modeled with a log function. Linear regression was used to assess the relationship between voltage and phrenic nerve stunning time. Repeated measures ANOVA was used to evaluate differences between phrenic nerve thresholds and peak-to-peak accelerations. Statistical significance was defined as a $P<0.05$.

RESULTS

Acute Phase 1: Dose-Response Results

A representative individual dose response experiment is shown in Figure 2. In this experiment, the first effect on the phrenic nerve was observed at 900 Volt deliveries (Figure 2, *). As PFA therapy deliveries increased to 1200 Volts, we observed stunning (Figure 2, †). These dose response data sets were combined and analyzed to produce a comprehensive dose response curve across all experiments.

The response of the phrenic nerve was evaluated as a function of the applied PFA therapy dose and proximity to the phrenic nerve. When the PFA catheter was very close to the phrenic nerve, such as in the thin vein tissue of the IVC, a threshold was measured between 0.3 and 0.8 V. In the SVC, where the PFA catheter was placed on cardiac tissue and further from the nerve, a pacing threshold of 1.3 to 2.8 V was observed. Voltage levels at which no effect and stunning of the phrenic nerves were observed were correlated with the phrenic nerve pacing threshold which is treated here as representative of the ablation catheter's proximity to the phrenic nerve (Figure 3). These results indicated that as the catheter was closer to the phrenic nerve, lower PFA therapy levels were more likely to affect the function of the phrenic nerve.

The differential between delivered voltage and the threshold voltage for initial stunning was calculated as (Figure 4):

$$\Delta \text{ Voltage} = \text{Delivered voltage} - \text{threshold voltage}$$

The Δ Voltage was directly proportional to stunning time (R^2=0.965, linear regression). These results indicated that as PFA therapy levels increased, stunning time of the phrenic nerve increased in a dose-dependent fashion. For example, if the first stunning point was 900 Volts for one animal, then a 1000 Volt delivery in that animal resulted in 0.63 minutes of stunning time. If the first stunning point was 1200 Volts for another animal, then a 1300 Volt delivery in that animal also resulted in 0.63 minutes of stunning time (Figure 4).

All phrenic nerves were assessed to be functional at the end of the acute procedure.

Acute Phase 2: Repetition Results

PFA Repeated Ablations

Based on calculated predictions shown in Figure 3 and a measured phrenic nerve threshold for the specific site, repetitive ablation levels were chosen to cause no effect on phrenic nerve function (Figure 5A), modulation of phrenic nerve function (Figure 5B), and temporary stunning of the phrenic nerve (Figure 5C) based on a measure phrenic nerve threshold when the ablation catheter was placed. Follow-up ablations at 1500 Volts then were expected and confirmed to temporarily stun the phrenic nerve and served to verify the placement of the catheter was placed near the phrenic nerve.

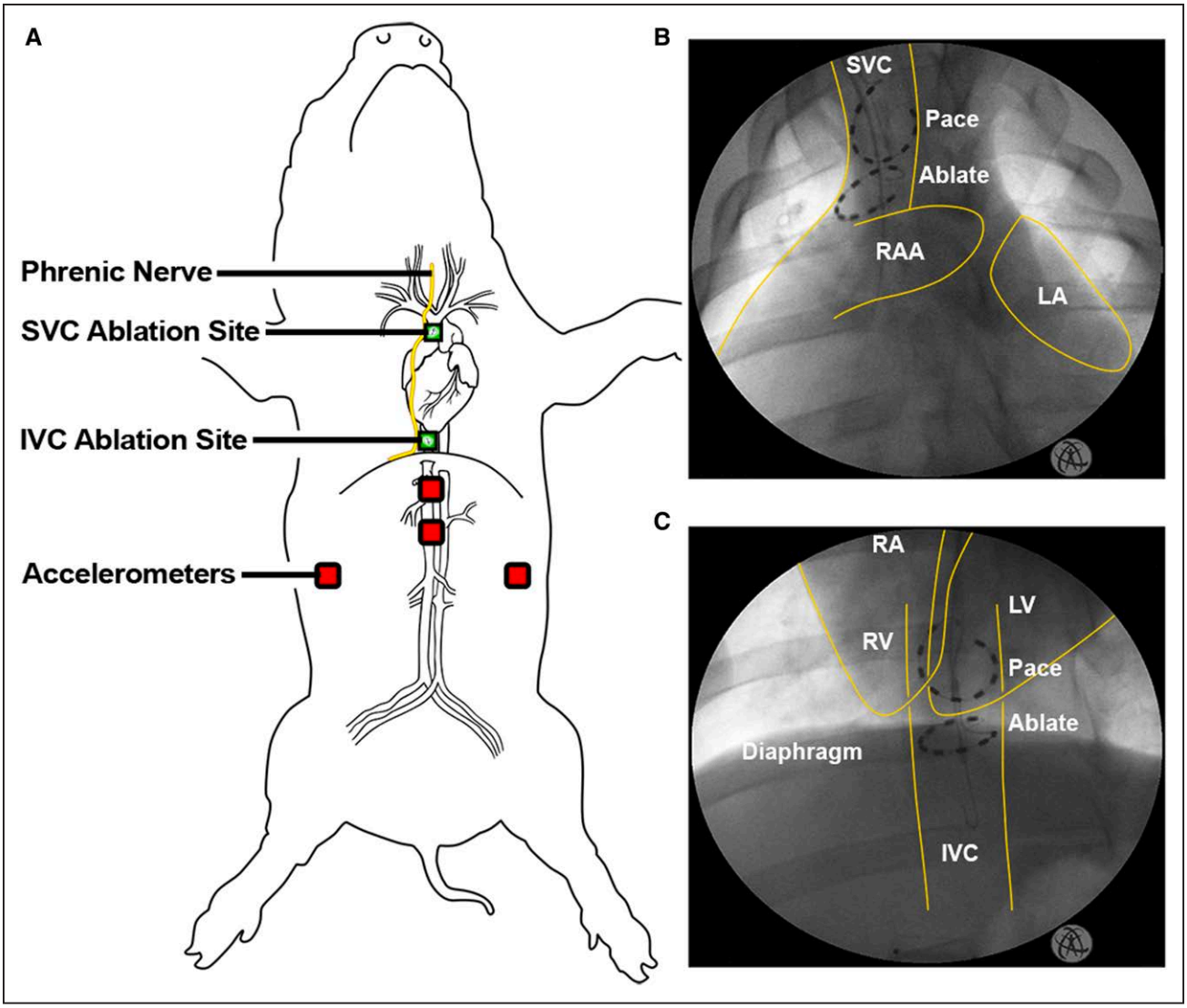

Figure 1. Experimental setup.
Two catheter sites and 4 accelerometer sites were used to capture and measure phrenic nerve function and subsequent diaphragmatic activity (**A**). Two catheters were placed in the superior vena cava (SVC; **B**). One catheter was placed in a more superior position to pace the phrenic nerve. The second catheter was placed in an inferior position for ablation. Two more catheters were placed in the inferior vena cava (IVC), with the superiorly positioned catheter used to pace the phrenic nerve and inferiorly positioned catheter used to ablate (**C**). This experimental model was leveraged for all 3 stages of this investigation. LA indicates left atrium; LV, left ventricle; RA, right atrium; RAA, right atrial appendage; and RV, right ventricle.

Chronic Phase: Results

Phrenic Nerve Function

In the chronic animals all treated with the 700 V level and multiple placements in the SVC, all animals demonstrated complete entrance block and functional phrenic nerves both acutely after the initial procedure and chronically at termination. The phrenic nerve stimulation threshold was measured before and after PV ablation. Only small changes in phrenic nerve stimulation threshold were observed; the threshold remained ≤1.1 V in all cases (Figure 6A). Accelerometer data correlated well with phrenic nerve thresholds, indicating a healthy diaphragmatic response in subjects (Figure 6B).

Phrenic Nerve Pathology

Gross and histopathologic evaluation of chronic phrenic nerves 4 weeks after SVC ablations did not reveal lesions to the axons, the myelin, or the endoneural, epineural, or perineural connective tissue. There was also no evidence of inflammation (Figure 7).

Diaphragm Pathology

None of the diaphragms were associated with any gross or histopathologic changes, for example, muscular atrophy as evidence of phrenic nerve damage.

SVC Ablation Outcomes

All SVCs were successfully isolated acutely and remained isolated at 4 weeks. Gross pathological examination of

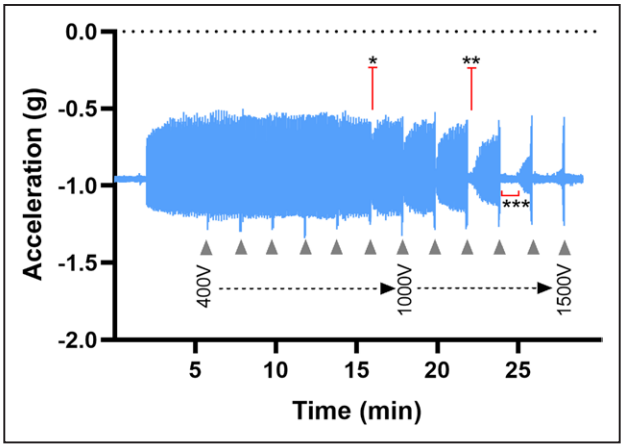

Figure 2. Sample accelerometer data demonstrated increasing pulsed field ablation (PFA) voltage eventually leads to a temporary decrease in function (*) as measured by reduced accelerometer response to diaphragmatic movement (measured in g).
In this experiment, first stunning is seen at 1200 V (†). Stunning time is measured as shown (‡). No effect was observed at low levels of therapy delivery (time <15 min).

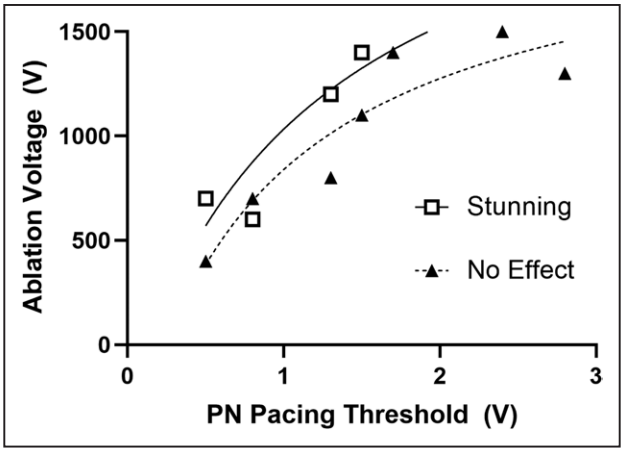

Figure 3. Ablation threshold levels from acute phase 1 data.
Shown are nonlinear regression curves (modeled as a log function) based on the data set. Pacing threshold is representative of the current delivered to the electrode array. The dashed regression line represents the maximum voltage applied without any observation of phrenic nerve (PN) dysfunction, while the solid line represents the first-dose level that elicited a stunning response from the nerve (R^2 for stunning =0.869, R^2 for no effect =0.866).

the SVCs of all 6 animals supported the electrical findings as all lines were placed in the muscular portion of the SVC and were continuous and transmural. These results have been reported previously.[8]

Distance SVC to Phrenic Nerve

The distance between epineural layer of the phrenic nerve and endocardial layer of the SVC in animals not undergoing PFA was 1.4±0.31 mm.

DISCUSSION

Knowing that unique catheter design and PFA therapy dose are key components of safety in electroporation, we sought to precisely identify the impact of PFA on phrenic nerve function for a specific PFA system using a novel experimental design. This study demonstrated that acute and chronic phrenic nerve function in response to PFA was quantifiable, modifiable, and predictable. These results and the usage of accelerometer data as an indicator of phrenic nerve function are novel compared with prior investigations that have only performed a binary phrenic nerve function assessment at the end of preclinical procedures.[13,14] This was an exploratory preclinical study, performed before other preclinical investigations with a similar system.[8,9] The doses presented in these experiments were tested with the intention of future clinical use and are currently under evaluation in an ongoing clinical study (URL: https://clinicaltrials.gov/ct2/show/NCT04198701; Unique identifier: NCT04198701).

This investigation generated and validated a phrenic nerve function dose response curve. Using dose response studies involving voltage and phrenic nerve thresholds, we calculated and tested PFA doses that resulted in no stunning, induced an effect or lead to stunning. Such predications were validated both in acute as well as chronic studies. The results validated the operating parameters at which phrenic nerves demonstrated no effect or stunning. The combined phrenic nerve pacing and accelerometer data analysis characterized brief (>2 seconds) period of stunning after deliveries. This continuous method of phrenic nerve evaluation may be more sensitive than clinical procedures, during which operators pace to assess phrenic nerve function before or after ablation.

All phrenic nerve function impairment was reversible in the acute phases of this experimental design. The chronic phase demonstrated no gross or histopathologic

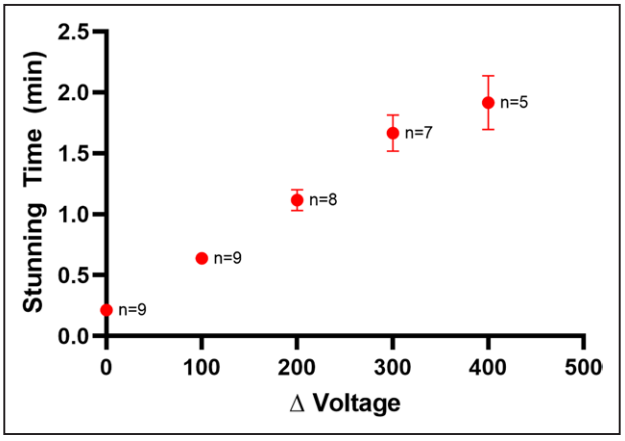

Figure 4. Dose-dependent increase in stunning time.
Linear regression used to assess relationship (R^2=0.965, slope significantly nonzero [P<0.001]). Data were normalized to accommodate different experimental thresholds to first stunning occurrence (see Figure 2). Shown are means and SEM. N indicates the number of experiments at which a threshold determination was made.

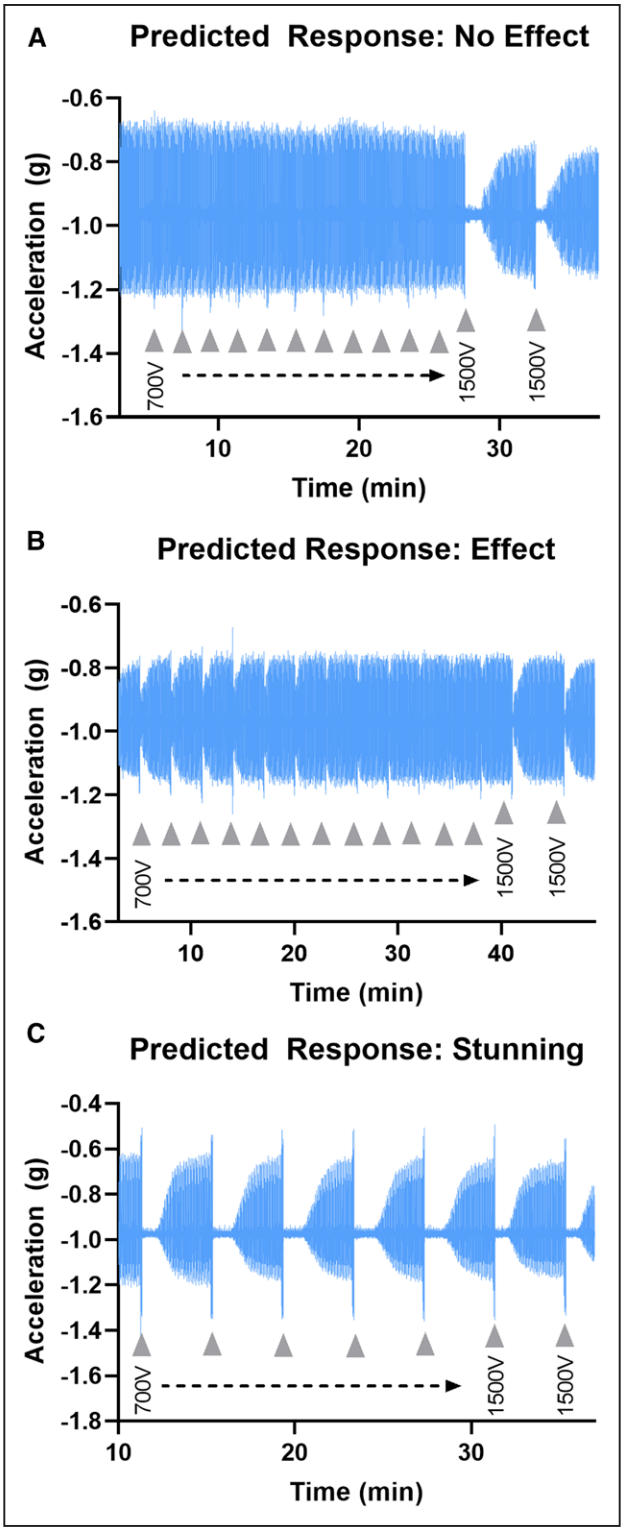

Figure 5. Predicted responses of phrenic nerve function based on experimental data collected previously and a measure of the minimum phrenic pacing threshold as a metric for proximity to the nerve.
Repeated ablations at 700 V cause no effect on phrenic function ([**A**] predicted response: no effect, phrenic nerve threshold 1.8 V), modulation of phrenic function ([**B**] predicted response: effect, phrenic nerve threshold 0.7 V), and temporary stunning of the phrenic ([**C**] predicted response: stunning, phrenic nerve threshold 0.6 V). At the end of each series, 2 ablations at 1500 V were performed.

changes indicative of phrenic nerve injury. In the chronic phase, complete ablation of cardiac tissue was also achieved without transient modification of the phrenic nerve response. Repeated PFA deliveries may result in a cumulative effect on cardiac lesion creation due to the mechanism of IRE. However, this study evaluated the impact of PFA on reversible phrenic nerve function. A gross cumulative effect on phrenic nerve function was not observed with repeated energy deliveries. Additional studies could be performed to elucidate the impact of repeated long-term PFA applications on the same subject over time to assess the cumulative adverse functional effect on the phrenic nerve, using PFA and other energy sources. Overall, these results indicated that the positioning of the catheter and the PFA dose level were critical for phrenic nerve response.

Identifying therapy levels capable of avoiding phrenic nerve injury is an important aspect to understanding the safety margin of this emerging therapeutic cardiac ablation modality. Other catheter ablation energy modalities may use electromyographic phrenic nerve monitoring using the surface compound motor action potential to help identify impending phrenic nerve injury.[15] The development of the PFA energy source, with curated therapy dosing profiles that are demonstrated to avoid phrenic nerve stunning, reduces the need to vigorously monitor phrenic nerve function throughout catheter ablation procedures using pace-mapping.[16]

In the porcine model, PFA applied in the SVC and IVC has a more direct impact on the phrenic nerve than when delivered in the PVs. This is due to the anatomic proximity of the SVC to the phrenic nerve compared with the SVC proximity to the PVs. In this context, this investigation represented a high-impact scenario of PFA with regards of proximity of the electrodes to the phrenic nerve. In human and porcine subjects, the anatomic relationship between the SVC and the phrenic nerve, and the SVC and the right PVs, is comparable. More specifically, the distance between the SVC-right atrium junction and the phrenic nerve in humans was measured as 0.27±0.68 mm. The distance from the right superior PV to the phrenic nerve in humans was measured as 1.4±1.7 mm.[17] In porcine subjects, in our study, the distance between the endocardial layer of the SVC muscle sleeve and the epineural layer of the phrenic nerve was 1.4±0.31 mm. The distance between right superior PV and phrenic nerve in pigs has not been quantified in the literature to our knowledge but is considered to be similar to what is observed in humans.[18] While the findings described in these experiments are relevant for SVC ablations, they also elucidate the potential impact of PFA on the phrenic nerve when PFA is applied to the PVs.

There is limited data available in the literature quantifying the effects of PFA on phrenic nerve stunning and injury. In a study investigating a focal PFA catheter in an acute porcine model, supraclinical doses of PFA

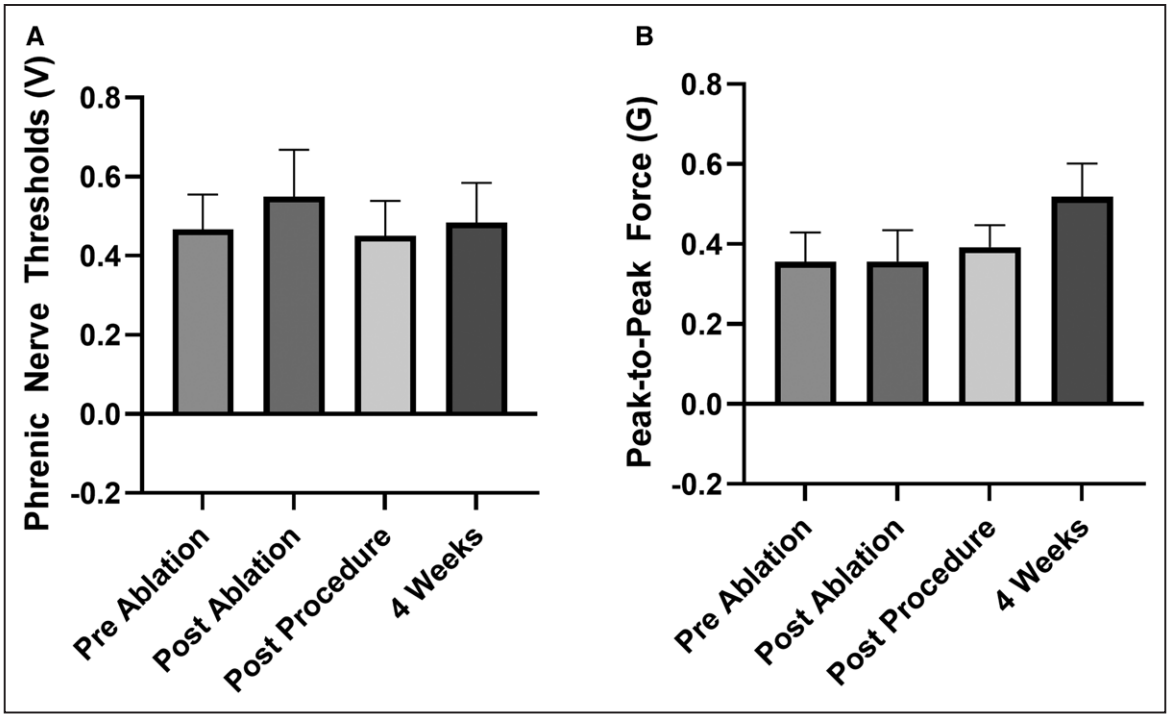

Figure 6. Normal phrenic nerve thresholds (A) and maintained peak-to-peak acceleration (measured via abdominal accelerometer, as a surrogate for diaphragmatic function, B) demonstrate intact phrenic nerve function at all time points in chronic study.

No significant differences were detected between groups (repeated measures ANOVA, *P*=not significant).

energy administered via an experimental focal catheter the right atrium resulted in transiently reduced or absent diaphragmatic contraction for 5 minutes before returning to baseline.[19] In the same study, right atrial endocardial delivery of regular doses of PFA energy did not result in any detectable chronic nerve injury via histopathology nor

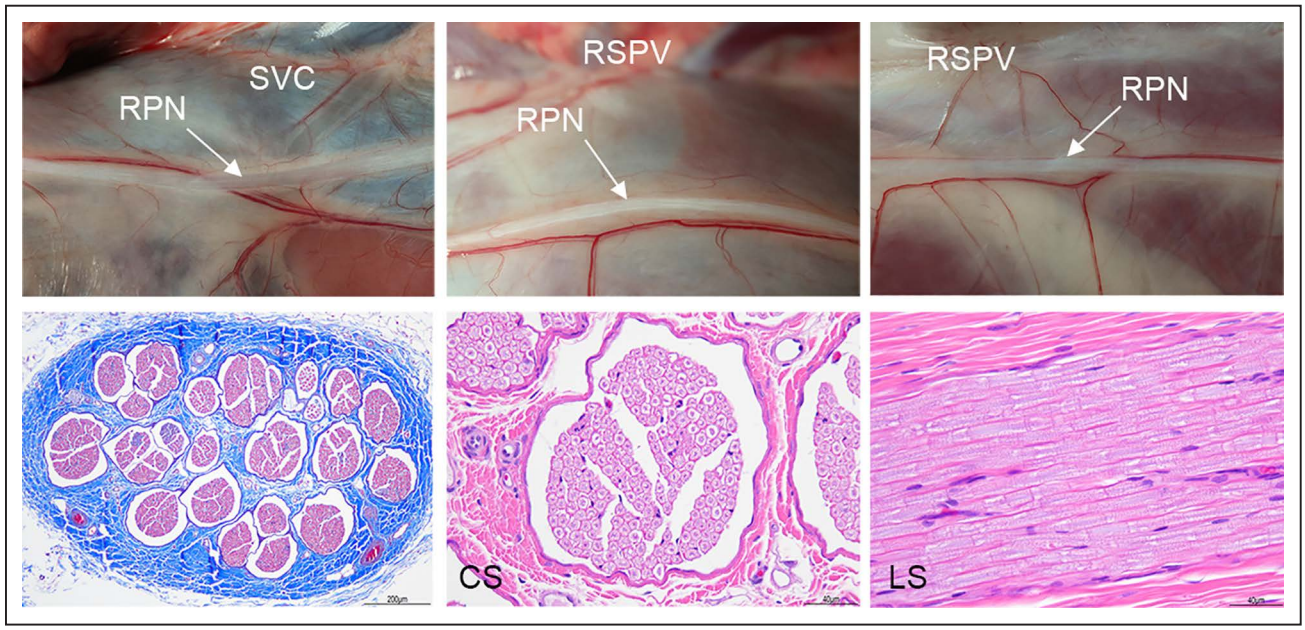

Figure 7. Gross images of the right phrenic nerve (RPN, top row, arrows) over the treated superior vena cava (SVC) or beneath the treated right superior pulmonary vein (RSPV).

All nerves were grossly normal after pulsed field ablation to these vascular structures about 4 wk earlier. Photomicrographs of randomly chosen phrenic nerves from this study (**bottom** row) illustrate that there were no histopathologic changes. The Masson trichrome stain indicates no increase in epineural, perineural, and endoneural connective tissue around the entirety of the nerve fascicles at low magnification in this cross-section (CS) view. The 2 hematoxylin and eosin stains showcase a CS and longitudinal section (LS) through a single nerve fascicle. Note the absence of inflammation, Wallerian degeneration, or atrophy of the nerve fascicle. Scale bars inserted.

acute phrenic nerve dysfunction (measured via phrenic nerve pacing not via the ablation site but via the IVC/SVC before and after ablation).

Preclinical studies of IRE in tumors showed that it may take up to 24 hours for first damages to nerves to occur, highlighting the importance of evaluating phrenic nerve injury not only acutely, but also chronically.[20] While direct application of IRE to myelinated nerves such as sciatic nerves has the potential to damage nerves, preservation of endoneurium architecture and proliferation of Schwann cells may suggest the potential for axonal regeneration.[21] Consistent with that hypothesis, it has been shown that direct application of IRE energy to sciatic nerves can result in near complete dysfunction of nerve function after ablation (up to several weeks), however, with full recovery of function at 7 weeks postablation.[22] Such regenerative capacity may be explained by the preservation of nervous tissue architecture (ie, endoneural and epineural extracellular matrix) after IRE application facilitating axonal regeneration via Schwann cells.[21,23]

Transitioning to human subjects with these identified effects on phrenic nerve should be done carefully, but these results are encouraging about the dosages that elicit no stunning of the phrenic nerve. Future studies evaluating the difference between phrenic nerve function in animal and humans would be valuable.

Limitations

This study was performed in porcine subjects, which may not capture all representative physiological responses when ablations are performed in humans in atrial tissue. Because of anatomic differences in pigs compared with humans, testing the effects of PFA on phrenic nerve function was better with SVC ablation than PV ablation, but the findings may not be entirely representative of phenomena in human patients. Evaluating irreversible phrenic nerve function was not achieved with this experimental design but would be of interest for future studies. Evaluating acutely stunned nerves in a chronic manner, and long-term phrenic nerve recovery dynamics, is also of interest for future studies but was not within the scope of this study. This investigation was performed with a specific catheter and PFA generator/waveform combination in a limited number of porcine subjects. As such, these results may not be universally applicable to other PFA systems. We are limited with regards to accessing and testing other PFA systems but hope that this model and new evidence can serve as a basis for others working in the field.

Conclusions

A 3-part novel experimental design with acute and chronic assessment of phrenic nerve response to a variety of PFA doses and repetition was performed. Positioning of the catheter and the PFA dose level were critical to phrenic nerve response. Gross and histopathologic evaluation of phrenic nerves and diaphragms at the chronic assessment yielded no injury. This investigation helped quantify the relative susceptibility of the phrenic nerve to PFA with the intent to steer cardiac ablation towards a safer space that actively seeks to eliminate the potential for phrenic nerve injury.

ARTICLE INFORMATION

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Affiliations

Medtronic, Minneapolis, MN (B.H., N.K., N.B., B.O., M.T.S., D.C.S.). Beaumont, Royal Oaks, MI (D.E.H.). McGill University Health Center, McGill University, Montreal, Quebec, Canada (A.V.).

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