In vivo preliminary test of a new biparietal bidirectional bipolar radiofrequency magnetic coupling system

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Abstract

Background: To test in vivo a new design prototype for radio frequency (RF) ablation.

Methods: A prototype based on a concept of endo-epicardial biparietal bipolar RF ablation with the atrial tissue interposed and consisting of two specular endocardial-epicardial catheters was tested in four pigs (80±5 kg). The endocardial catheter was introduced into the left atrium through the left atrial appendage on the beating heart. The epicardial counterpart was placed manually on the atrial epicardial surface. The coupling of the two catheters was achieved using a neodymium magnet around the gold plate electrode, and RF was applied to the interposed tissue. The hearts were excised, and the lesions were examined using morphometric evaluation.

Results: The RF application resulted in transmural lesions in all of the four animals tested. In these animals the maximum endocardial width (W_{endo}) was 6.34±0.25, 6.54±0.33, 6.36±0.57, and 6.49±0.96 mm. The pericardial width (W_{epi}) was similar: 6.37±0.47, 6.58±0.32, 6.35±0.56 and 6.53±0.94 mm. The lesion area was 924.78, 949.25, 944.25, and 926.05 mm², and the lesion volume was 92.47, 94.92, 94.42, and 92.60 mm³, respectively.

Conclusions: The idea of an endocardial-epicardial bidirectional biparietal bipolar radiofrequency tool such that the atrial tissue is fully interposed between the two RF poles might be promising for future clinical applications. Further research is warranted.

KEYWORDS
atrial Fibrillation, bipolar radiofrequency, radiofrequency ablation

1 INTRODUCTION

There is unanimous consensus among experts on the need for new optimized tools to improve the atrial fibrillation (AF) ablation success rate and, therefore, to ensure long-term stable sinus rhythm.1

A few years ago, our group pioneered the one-step hybrid AF ablation technique2 that has recently been included in the 2020 European Society of Cardiology (ESC) Guidelines for the diagnosis and management of atrial fibrillation.3 Nonetheless, results show that there is still room for improvement,4 especially regarding bipolar radiofrequency tools.5

In this regard, we have recently started developing a proof-of-concept for an endo/epicardial biparietal bidirectional bipolar radiofrequency (RF) tool prototype such that the atrial tissue is fully interposed...
between the two RF poles. The advantage of this tool is a lower amount of tissue interposed between the catheters and a lower chance of creating folds, thus increasing the likelihood of lesion transmurality. In this preliminary study, the effectiveness, feasibility, and safety of a new RF biparietal bipolar prototype was tested in four pigs to evaluate the potential of this novel technology and explore the possibility of future developments and the possible need of more extensive targeted animal tests.

2 | METHODS

2.1 | Animal preparation and handling

The ten minimum basic items of the ARRIVE guidelines were met. The study was approved by the Institutional Animal Welfare Committee of the University of Maastricht, The Netherlands. The principal investigator (S.G.) was responsible for the animal welfare and carried out all the experiments according to the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996) and European Union Directive 2010/63/ on the protection of animals used for scientific purposes. All experiments were carried out with the supervision of the Institutional Animal Welfare veterinarian.

Four Landrace pigs (mean weight 80 ± 5 kg) were used for this study. Animals were premedicated with i.m. 10 mg/kg, i.m. ketamine (Alfasan, Woerden, The Netherlands), 0.4 mg/kg, i.m. midazolam (Actavis, Munich, Germany), and 0.05 mg/kg i.m. atropine (Centrafarm BV, Etten-Leur, The Netherlands). Anesthesia was induced by i.v. injection of 4 mg/kg thiopental (Rotexmedica GmbH, Trittau, Germany) and maintained using an i.v. bolus of 0.5 mg/kg midazolam, followed by a continuous infusion of 0.5–0.6 mg/kg/h. Analgesia was achieved using an i.v. bolus of 5 μg/kg sufentanil citrate (Hameln Pharmaceuticals GmbH, Hameln, Germany), followed by a continuous infusion at 6–7 μg/kg/h. Neuromuscular block was provided by infusion of 0.1 mg kg/h pancuronium bromide (Inresa Arzneimittel GmbH, Freiburg, Germany). Animals were maintained under general anesthesia throughout the experiment and killed using Euthasol 20% when the experiment was completed (pentobarbital 150 mg/kg; AST Farma, Oudewater, The Netherlands). Cannulation of the common carotid artery was performed using a 16-gauge cannula (B. Braun Medical Inc.) for systemic arterial pressure monitoring.

2.2 | The biparietal bipolar prototype

The system consists of two identical catheters, which includes a handle, a plastic cylindrical connector, a hollow flexible element, and a tip (Figure 1A). The handle was made by modeling playdough (Plastilina Maimeri, Industria Maimeri S.p.A. Via G. Maimeri 1 - Bettolino di Mediglia - 20060 Mediglia (MI) – Italy) to create a comfortable anatomic shape. The holder was modeled surrounding a hollow plastic cylinder (12 mm diameter x 18 length) with a double purpose: first, to secure the wires coming from the tip; second, to offer robust support for the flexible hollow aluminum (3.50 mm width x 2.50 height) element connected to the tip. This was obtained by filling the hollow plastic cylinder with epoxy glue (Pattex Power Epoxy, Henkel AG & Co. KGaA Henkelstraße 67, 40589 Düsseldorf, Germany). For the alignment of the catheters, we chose an epoxy coated neodymium magnet (NdFeB) (30L x 7W x 2.5H mm) (SuperMagnete, Webcraft GmbH Industriepark 206, 78244 Gottmadingen, Germany), with a relative strength of magnetization along the 2.5 mm axis of 2.1 kg (approx. 20.6 N). The coating was essential for electric isolation between the magnet and the conductor. The magnetic elements were certified to keep their properties up to a maximum of 120°C. The electrode was represented by a gold-coated parallelepiped (20L x 5W x 1H, mm, Figure 1B). After welding the electric wires to the electrode, it was glued to the magnet. The connection between the flexible element and the magnet/electrode group
was reinforced with epoxy resin around the insertion of the two elements.

A specifically built console-setup measured the tissue conductance and impedance throughout the ablation cycle (50 times per second) and controlled the application of energy to the tissue. The output of the power generator was fixed in bipolar mode to 28.5 W at 114 Ω. The maximum energy delivery was reached along a constant power zone, where the tissue impedance was set at 30–70 ohms and the conductance at 15–30 mS. The RF delivery automatically stopped when a stable low level of conductance was reached, indicating that a lesion had been created.

2.3 | Surgical and ablation procedure

After median sternotomy, the pericardium was opened and retracted with exteriorized 2–0 Ethibond sutures (Ethicon Inc., Sommerville, NJ, USA), allowing access to the heart and great vessels. A double 4-0 purse-string prolene suture (Ethicon Inc., Sommerville, NJ, USA) was placed on the left atrial appendage (LAA) and secured with a tourniquet. The LAA was opened within the purse-string suture with an 11-knife blade, and one of the prototype’s catheters was moved via the purse-string suture into the left atrium using gentle traction. The maneuver was completed as quickly as possible to prevent possible embolus formation. Furthermore, the device was positioned towards the posterior atrial wall. The heart was gently lifted to visualize the pulmonary veins. The second catheter was manually placed on the epicardium, opposing the endocardial catheter, and obtaining perfect alignment through the magnetic coupling (Figure 2).

2.4 | Samples

After sacrificing the animals, the hearts were harvested, and the left atrial ablated area isolated. The samples were cleaned with NaCl 0.9% (B. Braun, Melsungen, Hessen, Germany) and immersed in a transport medium RPMI 1640 (Sigma-A Samples Aldrich, St. Louis, MO, USA). The atrial wall thickness for each heart was measured with a digital caliper (IP67 ABS COOLANTPROOF Mitutoyo Nederland B.V. Wiltonstraat 25 3905 KW Veenendaal). Furthermore, a full-thickness sample (5 × 18 mm LxW) was obtained from each ablated area. In total, we obtained four parallelepiped shaped samples ready to be analyzed (Figure 3A). Considering that the size of the sample blocks and the layer setting thickness were being fixed, we obtained from each animal a total of 50 layers, and thus in total, we gathered 2000 layers to be analyzed.

2.5 | Morphometric evaluation of myocardial tissue ablation

The specimens were put in 10% neutral buffered formalin for 48 h. Then, they were included in the Tissue-Tek OCT compound, and 100 µm thick slices were obtained using a Leica CM 3050 cryostat (Leica Biosystems, Wetzlar, Germany) at −20°C (Figure 3B). These slices were positioned on transparent A4 acetate sheets and desiccated at room temperature for 1 hour and then were covered with an additional transparent acetate sheet and digitalized at 600 dpi with a flatbed scanner (Perfection V39 Scanner, Epson America, Inc. 3840 Kilroy Airport Way, Long Beach, CA, USA).

Later, the images were imported into an image processing software (Image J version 1.48 software; National Institutes of Health, Bethesda, MD, USA) and measured. Every slice was investigated for the ablated area, volume, and maximum width of the lesion. The total area (A_{tot}) of the lesion was obtained by the sum of the single areas from each layer. The volume of each layer was calculated by multiplying the area and its thickness (fixed to 0.1 mm). The sum of all the volumes produced the total volume (V_{tot}). The maximum endocardial and epicardial width (respectively W_{endo} and Epi-W_{epi}) of the lesion was calculated by the mean or median of the single measurements.
3 | STATISTICAL ANALYSIS

The normality of the data was evaluated with the Shapiro-Wilk normality test, and data were expressed as mean with standard deviation in case of normal distribution or median with interquartile range in case of a nonnormal distribution. The comparison of the measurements between the groups was tested through the one-way ANOVA test. Post hoc testing was performed using the Tukey correction. The correctness and objectiveness of the measurements were assessed using all the samples to test the intra-observer and inter-observer variability between two readers (F. M. and M. L. M.). The agreement between the readers was evaluated with κ-statistics. The parameters used were the A tot and the maximum width. A κ of 1 indicates a perfect agreement, while 0 means agreement equivalent to chance. All the statistical analyses were performed using SPSS v.18.0 (IBM Corp., Armonk, NY, USA).

4 | RESULTS

4.1 | Intra- and interobserver variability

The κ-statistic was low for both the parameters assessed (0.98/0.92 and 0.96/0.91, for Intra/interobserver variability during A tot and W tot evaluation, respectively).

4.2 | Ablated tissue characteristics

The thickness of the sample was 4.17 ± 0.10 mm. During the experiments, only one steam pops were audible, but no further holes were visible in the tissue. The animal was monitored, and no significant and durable ECG ST- elevations were observed during the experiments. The ablation times ranged from 12 to 30 s. After the procedure, we could observe an ablated area on the posterior left atrial wall (Figure 4A, 4C). After slicing the samples, transmurality was visually assessed through the evidence of a pale lesion area extending from the endocardium to epicardium (Figure 4B, Figure 5).

The overall ablated area (A tot) in animal 1 (924.78 mm²) was comparable with that of animals 2, 3, and 4 (respectively 949.25, 944.25, and 926.05 mm², p = .29). Likewise, the overall volume (V tot) did not show significant differences between the animals: (92.47, 94.92, 94.42, and 92.60 mm³, respectively in animals 1, 2, 3, and 4, p = .29). (Table 1)

The maximum endocardial width (W endo) in animal 1 was 6.34 ± 0.25 mm, and it was comparable with that of the other animals 2, 3, 4 (6.54 ± 0.33, 6.36 ± 0.57, and 6.49 ± 0.96 mm, respectively, p = .25).

Moreover, the maximum epicardial width (W epic) was comparable in all the animals (6.37 ± 0.47, 6.58 ± 0.32, 6.35 ± 0.56, and 6.53 ± 0.94 mm in animals 1, 2, 3, 4, p = .15, respectively).

In Table 1, we also reported lesion depth (A tot and V tot) which was indexed by the thickness of the sample, even though it was not statistically different among the groups.

5 | DISCUSSION

In this study, we presented the first results of a new biparietal bidirectional bipolar prototype for one-time (simultaneous) endocardial-epicardial ablation in animals. RF application with this prototype resulted in fully transmural ablation lesions. To the best of our knowledge, this is the first attempt to test this configuration in vivo.

This study is part of a broader project that started with in vitro studies and is ending with the onset of in vivo animal models to further validate our findings for clinical use. In this first attempt, we focused more on the technical feasibility of magnetic coupling and the evaluation of the lesions produced with this new method. As an important confirmation test of our in vitro results, the next step will be that of achieving a deeper involvement of electrophysiologists who may assess in real-time the conduction block over the lesions being produced.
In vitro, we have recently confirmed both that a biparietal bipolar mode yields a higher transmurality rate compared to a simultaneous uniparietal bipolar set-up and that different time sequences of combined epi-endo ablation do not result in an acceptable transmural lesion rate.\(^6,7\) In contrast with the latter evidence, in the hybrid approach with either a single or two-stage procedures,\(^8\) the standard procedure prescribes that the electrophysiologist, although he or she can make lesions independently from the surgeon, should make lesions after the surgeon has completed the epicardial surgical ablation and only if a residual gap is being detected within the lesion area. Nonetheless, although the hybrid technique results in an increased rate of freedom from AF recurrence, compared to endocardial catheter and surgical epicardial ablation alone,\(^2\) undetected reconnections may occur after some time even though a complete conduction block should be detected immediately after the procedure.\(^9\) For this reason, some authors support the staged approach claiming that a catheter ablation performed in a second stage (maximum six months after surgery) significantly increases the likelihood of identifying conduction gaps in surgical lesions, which would potentially translate into a higher mid-term success rate.\(^10\)

Our idea was first driven by the aim of performing an epicardial-endocardial lesion in one stage. This could be obtained by interposing the atrial tissue between the ablation poles of two catheters placed on the epicardial and endocardial surfaces. A similar set-up employing ventricular tissue has demonstrated that the perfect alignment of the electrodes and the contact friction force of tissue are the key to obtaining effective transmural lesions.\(^11\) This finding might also represent a relevant aspect in atrial lesion making. To this end, in our prototype, the neodymium magnets placed around the two electrodes were accordingly used.

Another aim was to make a "bidirectional biparietal bipolar" system. Indeed, the tools we currently employ in our clinical practice are not truly bidirectional, especially in endocardial ablation, because these devices contain side-by-side electrodes instead of the tissue being interposed between the two poles. It is well known that bipolar RF clamps are the most reliable devices for creating transmural lesions.\(^12\) However, our starting idea was strongly supported by the excellent efficacy described using RF clamps either in open procedures with one jaw placed in the endocardium and the outer jaw on the epicardial surface or in an epi-epicardial closed procedure.

Another point was that, in endocardial ablation, the tools in use provide only a small catheter tip-to-tissue contact\(^,13\) and, as a result, only a fraction of all power is effectively delivered to the tissue.\(^14\) The bidirectional bipolar ablation system would offer the advantage of applying high-dose current to both the endo- and epicardial surfaces. The "bidirectional" design plays a crucial role in ensuring symmetrical endo-epicardial lesions that would not otherwise be ensured if performed only by epi/endocardial application.\(^11\)

Furthermore, our purpose was to make linear lesions in a single step and to obtain transmurality along the lesions. The importance of linear lesions in patients with persistent AF, as well as the need to obtain a bidirectional conduction block of these lesions, have been confirmed.\(^15\) Unsatisfactory results have been obtained with the use of surface bipolar RF devices.\(^16\) Indeed, the Coolrail Linear Pen (AtriCure, Inc. Mason, OH, USA) did not result in an effective conduction block,\(^17\) and it reached only 76% transmurality after a second ablation performed at the lesion site.\(^16\) Moreover, with the use of the Isolator linear pen (AtriCure, Inc. Mason, OH, USA), only 63% of the lesions were transmural throughout their entire length.\(^18\) In addition, the use of the COBRA Fusion device (AtriCure, Inc. Mason, OH, USA) led to 68% of transmural lesions throughout their entire length.\(^19\) Finally, the Cardioblade XL irrigated pen (Medtronic, INS. Minneapolis, MN, USA) is reported to be tedious to use in creating long linear lines, and its efficacy on the beating heart has never been tested.\(^16\) Better results have been obtained using cryoablation energy, which has the disadvantages of a long ablation time and lack of effectiveness in performing epicardial

### TABLE 1 Lesion features

| Animal | Transmurality % | Tissue thickness mm | \(A_{tot}\) mm\(^2/\text{Indexed}\) mm\(^2/\text{mm}\) | \(V_{tot}\) mm\(^3/\text{Indexed}\) mm\(^3/\text{mm}\) | \(W_{endo}\) mm | \(W_{epi}\) mm |
|--------|-----------------|---------------------|-----------------------------|--------------------------|-----------------|-----------------|
| 1      | 100%            | 4.05                | 924.78228.34                | 92.47228.34              | 6.34 ± 0.25     | 6.37 ± 0.47     |
| 2      | 100%            | 4.28                | 949.25221.78                | 94.92221.78              | 6.54 ± 0.33     | 6.58 ± 0.32     |
| 3      | 100%            | 4.25                | 944.25222.17                | 94.42221.21              | 6.36 ± 0.57     | 6.35 ± 0.56     |
| 4      | 100%            | 4.12                | 926.05224.77                | 92.60224.77              | 6.49 ± 0.96     | 6.53 ± 0.94     |

In addition, the use of the COBRA Fusion device (AtriCure, Inc. Mason, OH, USA) did not result in an effective conduction block,\(^17\) and it reached only 76% transmurality after a second ablation performed at the lesion site.\(^16\) Moreover, with the use of the Isolator linear pen (AtriCure, Inc. Mason, OH, USA), only 63% of the lesions were transmural throughout their entire length.\(^18\) In addition, the use of the COBRA Fusion device (AtriCure, Inc. Mason, OH, USA) led to 68% of transmural lesions throughout their entire length.\(^19\) Finally, the Cardioblade XL irrigated pen (Medtronic, INS. Minneapolis, MN, USA) is reported to be tedious to use in creating long linear lines, and its efficacy on the beating heart has never been tested.\(^16\) Better results have been obtained using cryoablation energy, which has the disadvantages of a long ablation time and lack of effectiveness in performing epicardial
cryoablations on the beating heart due to the heat sink of the circulating blood volume.16

Putting all these observations together, we have developed an endo-epicardial biparietal and bidirectional prototype. In this preliminary animal experiment, we obtained 100% transmurality with large lesion volume and symmetric epi-endocardial ablation areas. Only one steam pop episode was audible during the experiments, but no damage was visible on the tissue. Concerning what has been said above, it is well known that high temperatures above 100°C are responsible for steam pops and charring during cardiac ablation. In particular, a power above 30 W and lasting more than 60 seconds are likely to provoke steam pops. Moreover, the perpendicular orientation of the electrodes also are likely to cause steam pops and perforations.20 We hypothesized that the very significant absence of steam pops during our experiments may be related to two different characteristics. First, the linear shape of the electrodes, rather than a more sharpened one, might spread the energy over a broader surface, resulting in a decreased impedance of the system and, consequently, producing less overheating effects on the myocardium.20 The second hypothesis is that the synergy of magnetic coupling and the biparietal configuration might have caused the RF spread to drive in a very focused way within the tissue. Indeed, the amount of contact force and transmurality of the lesions in cardiac ablation is still debated, but it is well known that by increasing the contact force the lesions become deeper and broader.20

Notably, transmurality was observed all along the linear lesions confirming our previous experience in vitro.7

We are already working on future developments of the bipolar biparietal concept and its potential application in clinical practice. The idea is to exploit this technology to make a system that can be used in an open surgery setting or for a minimally invasive approach, performed
as a "true" hybrid procedure by the surgeon and the electrophysiologist (EP) or only by the EP Electromagnetism could represent a good option for selected coupling, allowing targeted ablations. Finally, the bipolar biparietal proof-of-concept tool could also be applied for the ablation of other arrhythmias already treated with the hybrid approach by our group.21

Likewise, apart from cardiac ablation, RF is commonly used in other disciplines such as liver, kidney, lung, and bone tumor treatment, as well as aesthetic medicine. Our novel methods might be used in the treatment of intramural tumors in hollow organs such as the esophagus, stomach, intestine, and uterus.

Nonetheless, we are well aware of the difficulty of translating such a concept into an effective, maneuverable tool to be used on patients. There are several reasons for concern: (1) The miniaturization of the system for making its use minimally invasive. (2) The maneuverability and flexibility of the catheter, which are limited by the presence of magnets around the ablation electrode. (3) The interaction of a magnetic force with blood is still unknown and needs to be explored. In our study, we noticed that in vivo a higher magnetic force was necessary compared to the magnetic coupling at the bench. (4) Perfect alignment and coupling in the presence of fat may be difficult, and this is a factor that needs to be fully explored. (5) The increase in impedance with the thickness of fat could be clarified to develop a more tailored fat-based algorithm. Ongoing research is being focused on these aspects.

6 | LIMITATIONS

The first limitation is represented by the small number of animals. However, since our study was a first proof-of-concept experiment, the reason we used a small number of animals was, in agreement with Animal Welfare, to minimize the number of animals used for experiments. The next development steps will include more extensive animal studies.

In addition, tissue samples were not sent for histological examination. This decision was taken so that the results could be compared to our previous in-vitro studies. Therefore, we employed the same morphometric evaluation of the ablated tissue previously used.

In addition, as we planned to test our technique in acute experiments, the safety of the procedure has been deliberately left out. Indeed, animals were not investigated through brain MRI for one of the common adverse effects such as embolus formation. This limitation represents a future call for another set of experiments.

7 | CONCLUSIONS

Our first experience on the beating heart in animals has shown satisfactory results, and the biparietal bidirectional bipolar concept-of-proof protocol seems to be a promising design for future clinical applications. Nonetheless, multidisciplinary research is warranted to translate this preliminary evidence into a new reliable tool that completely isolates the left atrial tissue in a single step after it is tested more extensively in animal experiments before its application in humans.

AUTHORS’ CONTRIBUTIONS

All authors actively contributed to the paper in the following domains: Conception or design of the work, Data collection, Data analysis, and interpretation, Drafting the article, Critical revision of the article, Final approval of the version to be published.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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