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# Epicardial Ablation of Rotors Suppresses Inducibility of Acetylcholine-Induced Atrial Fibrillation in Left Pulmonary Vein–Left Atrium Preparations in a Beagle Heart Failure Model

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**Objectives** The purpose of this study was to provide direct evidences that rotor ablation suppresses atrial fibrillation (AF) inducibility.

**Background** Micro–re-entrant wavefronts have been suggested to serve as sources of rapid activations during AF. Whether AF inducibility is suppressed by elimination of rotors remains unknown.

**Methods** We used optical mapping to study Langendorff-perfused left pulmonary vein (PV)–left atrium (LA) preparations from 13 dogs with pacing-induced heart failure. Atrial arrhythmias were induced by pacing and mapped during acetylcholine infusion (1 $\mu$mol/l). Rotors were identified from optical recordings. Epicardial ablation was performed targeting the rotor anchoring sites in preparations with sustained (>10 min) or incessant spontaneous AF. Non-rotor ablation was performed in 4 preparations. Repeated pacing was performed to test the AF inducibility after ablation.

**Results** Sustained AF (n = 12) and incessant spontaneous AF (n = 1) were induced after acetylcholine infusion. Pulmonary vein focal discharge was found in 9 preparations (9.2 ± 4.2 beats/s), and rotor anchoring was found at the left superior PV-LA junction in 13 preparations (9.1 ± 4.6 beats/s) and at the ligament of Marshall-PV-LA junction in 1 preparation. Epicardial rotor ablation successfully inhibited the inducibility of sustained AF in 12 of 13 preparations ($p < 0.01$), including 4 with the maximal dominant frequency sites located on the PV-LA junctional rotor zones (direct elimination of mother rotors). The longest AF duration was shortened significantly by rotor ablation (Wilcoxon Z = 3.60, p = 0.002, n = 13), but not by non-rotor ablation (Wilcoxon Z = 1.00, p = 0.317, n = 4).

**Conclusions** Epicardial ablation of the rotor anchoring sites suppresses AF inducibility. The arrhythmogenicity at the maximal dominant frequency sites is directly/indirectly suppressed by the rotor ablation. (J Am Coll Cardiol 2011;58: 158–66) © 2011 by the American College of Cardiology Foundation

Although pulmonary vein (PV) bursting appears to play a role in atrial fibrillation (AF) initiation and maintenance (1,2), this bursting behavior in turn depends on the atrial input and largely disappears when PVs are disconnected from the left atrium (LA) (3). We have previously shown that the left superior pulmonary vein (LSPV)-LA junction is a privileged site for anisotropic re-entry (4). Acetylcholine infusion facilitates both PV focal discharge and micro–re-entry at the LSPV-LA junction to perpetuate AF, and pharmacological suppression of PV focal discharge may inhibit the AF inducibility in a heart failure canine model (5). Whether radiofrequency ablation (RFA) targeting at the rotor anchoring sites is effective in suppressing AF inducibility remains unknown. In this study, we performed high-density optical mapping to test the hypothesis that RFA of the re-entry anchoring sites prevents AF inducibility.

## Methods

The research protocol was approved by the Institutional Animal Care and Use Committees of Chang Gung Memo-

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rial Hospital and conformed to the guidelines of American Heart Association on research animal use. Thirteen beagle dogs (weight 8 to 14 kg) were used in the study.

**Surgery and rapid ventricular pacing-induced heart failure.** Dogs were pre-medicated with intramuscular injection of ketamine (10 mg/kg) and xylazine (4 mg/kg), intubated, and anesthetized with isoflurane. When the dogs were fully anesthetized and nonresponsive to physical stimuli, the chests were opened through a right thoracotomy. An epicardial lead was placed in the free wall of the right ventricle and connected to a Medtronic Itrel III neurostimulator (Medtronic, Minneapolis, Minnesota) for chronic pacing (250 beats/min for 1 month). Ventricular function was assessed by echocardiography at the baseline and after 1 month of pacing.

**Optical mapping studies.** LANGENDORFF-PERFUSED PV-LA PREPARATION. The hearts were rapidly excised under general anesthesia. All ventricular branches were tied off, and the left coronary artery was immediately cannulated and perfused with cold cardioplegic solution (4°C). Ventricular tissue was quickly removed. The preparation was then placed in a temperature-controlled tissue bath (36.5 ± 0.5°C). It was both perfused and superfused with oxygenated Tyrode's solution (composition in mmol/l: NaCl 125, KCl 4.5, $MgCl_2$ 0.25, $NaHCO_3$ 24, $NaH_2PO_4$ 1.8, $CaCl_2$ 1.8, glucose 5.5, and albumin 50 mg/l in deionized water), equilibrated with 95% $O_2$ and 5% $CO_2$ to maintain a pH of 7.4. Coronary perfusion pressure was regulated between 80 mm Hg and 85 mm Hg. Two hook electrodes were inserted into the LA appendage (LAA) for recording and pacing.

OPTICAL MAPPING. The preparations were stained with a voltage-sensitive dye (RH 237, Molecular Probes, Eugene, Oregon) and excited with laser light at 532 nm (4). The emitted fluorescence was filtered through a 710-nm long-pass filter and was then acquired with a charge-coupled device camera (CA-D1-0128T, Dalsa, Waterloo, Ontario, Canada) at 269 frames/s. The digital images (128 × 128 pixels) were gathered from the epicardium of the left PVs and adjacent LA over a 30 × 30 $mm^2$ area, resulting in a spatial resolution of 0.24 × 0.24 $mm^2$ per pixel. Motion artifacts were suppressed by 5 $\mu$mol/l cytochalasin D (Sigma Aldrich, St. Louis, Missouri). To create color maps, the average fluorescence level ($\bar{F}$) over the entire data window was first calculated for each pixel. At each pixel, the change in fluorescence (the difference between fluorescence level and the $\bar{F}$) at each time point was color coded to generate the color maps. Two-dimensional color maps of the membrane potential (Vm) were constructed to illustrate the Vm changes during paced rhythm and arrhythmia. Individual Vm maps showed the depolarized areas in shades of red and repolarized areas in shades of blue and were animated to show the patterns of propagation in the mapped region.

**Acetylcholine infusion and pacing protocols.** We infused acetylcholine (1 $\mu$mol/l) for 15 min. Atrial electrical activities were recorded continuously, and spontaneous atrial arrhythmias were mapped when possible to determine the patterns of initiation and the sources of focal discharge. We paced the LAA with 5-ms pulse width and twice diastolic threshold current. Atrial arrhythmias were induced by standard $S_1S_2$ pacing and/or burst atrial pacing protocols (pacing cycle length [CL] 50 ms, 5-ms pulse duration, and 5-mA current for 3 to 5 s, 8 to 12 times). The inducibility of sustained AF was defined as any episode of induced AF >10 min. Three episodes of AF were mapped consecutively within the first 30 s after induction. These episodes, each containing 1,000 frames of optical images, were used for dominant frequency (DF) analyses and identification of micro–re-entrant wavefronts anchoring. After AF sustained for >10 min, defibrillation was performed with 3 to 5 J biphasic shocks delivered by epicardial patch electrodes.

**Abbreviations and Acronyms**

- **AF** = atrial fibrillation
- **CL** = cycle length
- **DF** = dominant frequency
- **$DF_{max}$** = maximal dominant frequency
- **LA** = left atrium
- **LAA** = left atrial appendage
- **LOM** = ligament of Marshall
- **LSPV** = left superior pulmonary vein
- **PV** = pulmonary vein
- **RFA** = radiofrequency ablation
- **Vm** = membrane potential

**Stepwise catheter ablation to suppress AF.** Epicardial RFA was performed in preparations with sustained AF (>10 min) and/or incessant spontaneous AF bursts after acetylcholine infusion. In the first 9 preparations, RFA was performed at the sites that fit the phase singularity clustering (by phase singularity maps) and the re-entry rotor anchoring (by activation movies). We used a thermo control 8-mm ablation catheter (St. Jude Medical, Minneapolis, Minnesota) with a temperature setting of up to 60°C and maximum power of 70 W RF energy (HAT300, Osypka, Berlin, Germany) and delivered multiple burns (2 min/time, range 3 to 6 times) until no further rotor anchoring could be induced at the ablation sites. If sustained AF was still inducible, the post-RF AF episodes were analyzed to determine if other focal mechanisms were present for further ablation. In the remaining 4 preparations, RFA was first performed at non-rotor anchoring sites (LAA), then at the rotor anchoring sites.

**Histological examination.** The hearts were fixed in 4% formalin for 1 h and stored in 70% alcohol. The proximal LSPV and ablated tissues overlying the LSPV-LA junction were selectively excised and paraffin embedded. Sections (5 $\mu$m) were stained with hematoxylin and eosin for light microscopic examinations.

**Data analysis.** Focal discharge was defined as an activation propagating centrifugally from a central site. Alternatively, an activation originating from the distal end of the PV muscle sleeve and propagating only in the direction toward the LA was also considered a focal activation. The frequency of focal discharge and micro–re-entry were calculated by the average of total number of focal discharges in