Ethanol Infusion in the Vein of Marshall Leads to Parasympathetic Denervation of the Human Left Atrium

Implications for Atrial Fibrillation

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Objectives
This study sought to determine whether ethanol infusion in the vein of Marshall (VOM) can ablate intrinsic cardiac nerves (ICN).

Background
ICN cluster around the left atrial epicardium and are implicated in the genesis of atrial fibrillation (AF).

Methods
Patients undergoing catheter AF ablation underwent adjunctive ethanol injection in the VOM. A multipolar catheter was introduced in the VOM and used for high-frequency stimulation (HFS), either as HFS with P-wave synchronized (SynchHFS), 30 pulses, 100 Hz (n = 8) or as HFS with 3 to 10 s bursts (BurstHFS), 33 Hz (n = 72) at 25 mA for 1-ms duration. Atrioventricular (AV) nodal conduction slowing (asystole >2 s or R-R interval prolongation >50%) and AF inducibility were assessed before and after VOM ethanol infusion. Up to 4 1-ml infusions of 98% ethanol were delivered via an angioplasty balloon in the VOM.

Results
SynchHFS induced AF in 8 of 8 patients. In 4 of 8 AF initiated spontaneously without VOM capture. No parasympathetic responses were elicited by SynchHFS. BurstHFS was performed in 32 patients undergoing de novo AF ablation (Group 1) and 40 patients undergoing repeat ablation (Group 2). Parasympathetic responses were found in all 32 Group 1 patients and in 75% of Group 2 patients. After VOM ethanol infusion, parasympathetic responses were abolished in all patients (both groups). There were no acute complications related to VOM ethanol infusion.

Conclusions
The VOM contains ICN that connect with the AV node and can trigger AF. Retrograde ethanol infusion in the VOM reliably eliminates local ICN responses. The VOM is a vascular route for ICN-targeting therapies. (J Am Coll Cardiol 2014;63:1892–901) © 2014 by the American College of Cardiology Foundation

Intrinsic cardiac nerves (ICN) can modulate atrial muscle physiology in a pro-fibrillatory manner (1). ICN ablation has been proposed as an adjunctive (2) or stand-alone (3) therapy for atrial fibrillation (AF). Strategies for ICN ablation have involved the use of radiofrequency at endocardial sites where parasympathetic reflexes were elicited or epicardial ablation during surgery or epicardial access. To date, the clinical impact of ICN ablation in the overall procedural success is unclear, in part because of unreliable ablation techniques.

ICNs cluster in discrete ganglia located in the proximity of the pulmonary veins (PV). The ligament of Marshall is considered part of the ICN (4). It has been shown to contain sympathetic (5) and parasympathetic (6) innervation, and it coincides with regions known to harbor ICN, specifically the left dorsal nerve (7). It becomes the vein of Marshall (VOM) caudally as it connects with the coronary sinus. The ligament of Marshall has been implicated in the genesis of AF by multiple mechanisms: as a source of ectopic beats initiating AF (8–10), as a connection pathway with
neighboring myocardium and left PV (5,11), and as a source of arrhythmogenic autonomic innervation (5,6). Animal (12) and human (3) surgical open-chest studies have shown that high-frequency electrical stimulation (HFS) at the ligament of Marshall area may induce parasympathetic responses characterized by significant slowing of atrioventricular (AV) nodal conduction and AF induction. It is not known whether the VOM can anatomically connect with the ICN associated with the ligament of Marshall. If so, then the VOM could be used as a closed-chest endovascular route to the ICN for therapeutic purposes. We have developed a technique for retrograde VOM ethanol infusion and have shown its feasibility and safety in humans (13,14). We hypothesized that: 1) HFS performed within the VOM can elicit parasympathetic responses; and 2) the VOM can be used as a vascular route to target these epicardial ICN and regionally denervate the LA with chemical ablation.

Methods

Patients. We enrolled 133 patients in the VOM ethanol infusion procedure. Patients were undergoing clinically indicated PV antral isolation (PVAI) and gave consent for adjunctive ethanol injection in the VOM in a protocol that was approved by the local institutional review board, overseen by the Food and Drug Administration (IND #105083), and an external Data Safety Monitoring Board.

Procedural strategy. After obtaining informed consent, patients were subjected to general anesthesia, and vascular access was obtained. A quadripolar catheter was positioned in the His bundle and a decapolar catheter in the coronary sinus via a femoral vein.

The VOM was cannulated as previously described (13,14). Briefly, the right internal jugular vein was accessed with a 9-F sheath. Then, a sheath designed for left ventricular pacing lead delivery was inserted in this sheath and into the coronary sinus (CPS sheath, St Jude Medical, Minneapolis, Minnesota). A subselector catheter (LIMA angioplasty guide) was then inserted through the CPS sheath and manipulated so that its tip faced posteriorly. Depending on the length of the VOM, up to 4 injections of 98% ethanol (1 cc over 2 min each) were delivered. Starting in the most distal VOM, the balloon was slightly retracted sequentially after each injection so that the last injection was given from the most proximal VOM. After ethanol infusion, the angioplasty wire and balloon were retracted, and the quadripolar catheter was reinserted into the VOM to record signals and repeat HFS with the same protocol used prior to ethanol administration. Ethanol levels were measured in mixed venous blood at the end of the procedure. Beyond VOM instrumentation, the procedure continued with radiofrequency ablation using a Thermocool catheter ( Biosense-Webster) navigated with the Artisan robotic sheath (Hansen Medical, Mountain View, California). Radiofrequency ablation with a power of 25 to 35 W and saline irrigation at a rate of 17 to 30 cc/min were performed as needed in each case to isolate the PVs, ablate complex-fractionated potentials, or LA flutters if present.
Statistical analyses. Data are mean ± SD. Student *t* test was used to compare means. Proportions were compared with chi-square or Fisher exact test where appropriate. A *p* value of <0.05 was considered significant.

Patient follow-up. Patients were followed clinically at 1, 3, 6, and 12 months post-procedure and as needed for clinical recurrences. Continuous 4-week event monitors were connected routinely at 3 and 12 months and as needed for symptomatic arrhythmias.

**Results**

All patients were brought to the electrophysiology laboratory for clinically indicated catheter ablation of AF after being treated with antiarrhythmic drugs and cardioversions as necessary prior to the procedure. The VOM was successfully cannulated in 119 of 133 patients (89%). We were able to complete either of the HFS protocols before and after VOM ethanol infusion in a total of 80 patients (8 in protocol 1 and 72 in protocol 2), which are reported in this study. In the remaining patients, HFS protocols were not completed due to inability to introduce a quadripolar catheter in the VOM (Pathfinder mini-catheter was not manufactured after 2010) or to complete the pre- and post-ethanol VOM HFS protocols. Figure 1 summarizes the results.

**Protocol 1.** A total of 8 patients who had previously undergone catheter ablation procedure underwent SynchHFS, which induced AF in all patients when atrial capture was obtained. In 4 of 8 patients, AF spontaneously initiated after noncapturing, short SynchHFS trains, and in all 4 cases, AF-initiating atrial premature contractions were recorded first in the VOM (Fig. 2). VOM ethanol infusion abolished AF induction upon repeating SynchHFS (Fig. 2B). Because of the inability to assess parasympathetic responses (which relied on effects on AV conduction) with this protocol, it was abandoned for subsequent patients.

**Protocol 2.** **GROUP 1: DE NOVO AF ABLATION.** Group 1 included 32 patients undergoing AF catheter ablation for the first time. AF was paroxysmal in 19 of 32 patients and persistent in 13. Mean age was 63 ± 8 years, and 9 patients were female. BurstHFS led to AF induction in all patients. Parasympathetic responses were assessed during induced AF and were elicited in all patients, either as an asystolic response (4.7 ± 2.3 s) in 23 of 32 patients or as

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**Figure 1 Results Summary**

(A) P-wave-synchronized high-frequency stimulation (SyncHFS) protocol. (B) BurstHFS = high-frequency stimulation with 3- to 10-s bursts, 33 Hz (BurstHFS) protocol. See text for details. *p < 0.05 compared with distribution in Group 1. #p = NS compared with Group 1. ¶p < 0.05 compared with Group 1.
an RR prolongation (of 228 ± 70%) in 9 of 32 patients. Figures 3 and 4 show examples of asystole and RR prolongation elicited upon VOM BurstHFS, respectively. AF was induced in all patients during HFS and subsequently terminated in 27. After VOM ethanol infusion and AF, or any atrial arrhythmia, was not reinducible by VOM HFS in those 27 patients (Figs. 3B and 4B), and parasympathetic responses were not obtained in any of the 32 patients.

GROUP 2: REPEAT AF ABLATION. Group 2 included 40 patients undergoing a repeat AF catheter ablation procedure. AF had been paroxysmal in 19 of 40 and persistent in 21. Mean age was 64 ± 9 years, and 12 patients were female. Except for the proportion of persistent versus paroxysmal AF (p < 0.001), none of the patient characteristics was statistically different from those of Group 1. Clinical failures of a previous catheter ablation procedure (1.3 ± 0.5 prior procedures) had been due to flutter in 22 of 40 patients.

BurstHFS in the VOM during sinus rhythm or atrial flutter led to AF induction in all patients at the initiation of HFS (4 required previous cardioversion for AF). Parasympathetic responses were triggered by BurstHFS in 30 of 40 patients: in 9 patients an asystolic response was obtained (4.5 ± 1.7 s, p = nonsignificant compared to asystolic responses of Group 1 patients); and in 21 patients, an RR prolongation was obtained (194 ± 21 %, p < 0.05 compared to RR prolongation obtained in Group 1 patients). Figures 5 and 6 show examples of asystolic and RR-prolongation responses, respectively. The distribution of asystolic versus RR prolongation responses of Group 2 contained a greater proportion of RR prolongation responses (and of no parasympathetic responses) than that of Group 1 patients (p < 0.05).

Local AF inducibility with VOM HFS was reassessed post-ethanol injection. Thirteen patients remained in sustained AF after ethanol injection in the VOM, and inducibility could not be tested with repeat HFS (cardioversion was not performed). In the other 27 patients who had either cardioversion performed or the initial AF was nonsustained, inducibility was reassessed. In this group, AF, or any atrial arrhythmia, remained noninducible via VOM HFS in 27 of 27 patients (100%) (Figs. 1, 5B, and 6B). Parasympathetic responses were eliminated in all patients after VOM ethanol injection.

Acute procedural outcomes. There were no complications directly attributable to VOM instrumentation or ethanol infusion. Ethanol levels measured in mixed venous blood at the end of the procedure were undetectable in all patients. Fluoroscopy and procedure times required to complete the VOM component of the procedure were 9.6 ± 5.2 min and
Ethanol led to elimination of VOM signals in all patients and to the creation of a low-voltage area (bipolar voltage < 0.1 mV) in the endocardial surface of 8.8 ± 4.1 cm², located between the coronary sinus and the left inferior pulmonary vein (Fig. 7). The left inferior pulmonary vein was disconnected in 18 of 32 patients in Group 1, solely with VOM ethanol infusion. In Group 2, 32 of 40 patients had left inferior pulmonary vein (LIPV) reconnection, and in 23 of 32 patients VOM ethanol led to re-disconnection (16). Overall, average radiofrequency time required to disconnect the LIPV was 2.9 ± 1.2 min, compared with 16.1 ± 8.2 min required to isolate each of the remaining veins (p < 0.05) (17).

**Patient follow-up.** Patients were followed for a mean of 24.6 ± 12.5 months. On follow-up, 1 patient developed pleuropericarditis similar to Dressler’s syndrome: at 2 weeks post-ablation, the patient presented with bilateral pleural effusions and chest pain consistent with pericarditis but without pericardial effusion or any other abnormal findings on chest contrast computerized tomography. He responded to anti-inflammatory agents and colchicine. Two patients had chest pain and pericardial effusions requiring drainage at 2 and 4 weeks post-ablation. All patients had received conventional radiofrequency ablation concomitantly (with the use of a robotic sheath) that may have contributed to pericardial effusions. Seventy-eight percent of all patients completed electrocardiographic monitoring as requested (100% of 14 patients with symptoms). AF recurrence occurred in 6 of 32 patients in Group 1, and all had pulmonary vein reconnections in a repeat procedure, including 1 patient who had the LIPV disconnected with ethanol. In Group 2, 8 of 40 patients had recurrent atrial tachyarrhythmia episodes, 2 had AF, and 6 had atrial flutters (roof-dependent, LA appendage atrial tachycardia, and right atrial flutter, 2 each) which underwent successful repeat ablation.

**Discussion**

The main findings of our study are: 1) the VOM and its neighboring atrial myocardium contain ICN that can reach the AV node and induce parasympathetic responses;
**Figure 4** Bradycardic (RR Prolongation) Response to BurstHFS in a Patient Undergoing De Novo AF Ablation (Group 1)

(A) Catheter location in right anterior oblique view. A septal occluder device was also present. (B) Pre- and post-ethanol recordings. Atrial fibrillation (AF) had been previously induced, and significant RR prolongation was induced by BurstHFS. Such a response was abolished after ethanol injection.

**Figure 5** Asystolic Response to BurstHFS in a Patient Undergoing Repeat AF Ablation (Group 2)

(A) Catheter is shown in the right anterior oblique view. (B) Pre- and post-ethanol recordings. AF had been previously induced, and a 5.35-s asystole was induced by BurstHFS. Such a response was abolished after ethanol injection.
2) stimulation of VOM-associated ICN can induce AF without local atrial capture; 3) VOM HFS-induced parasympathetic responses are less common in patients with prior catheter AF ablation, suggesting that neuronal damage can occur during a PVAI procedure; and 4) VOM ethanol infusion eliminates parasympathetic responses and AF induction, consistent with regional left atrial denervation. These findings support the feasibility of VOM cannulation as a percutaneous technique for endovascular delivery of ICN-targeted therapies.

The anatomical distribution of the ICN is complex. ICNs are reported to cluster in distinct ganglionated plexi in the vicinity of the PV ostia. It is important to recognize the distinction between the extracardiac ligament of Marshall, a remnant of the left superior vena cava, and the intracardiac VOM. The ligament of Marshall is known to be a conduit for sympathetic (5) and parasympathetic (6) extrinsic cardiac nerves, and it coincides with the left dorsal cardiac nerve. Stimulation of the ligament of Marshall can induce atrial fibrillation and even ventricular arrhythmia (15) presumably by direct activation of such extrinsic nerves. The VOM, although in continuity with the ligament of Marshall, is an intracardiac structure in direct connection with the coronary sinus. ICN reached by VOM HFS may include not only those ICN clustered in the VOM-posterolateral left atrial ganglionated plexus (18) but also those associated with the inferior left ganglionated plexus. Depending on the VOM length, even extrinsic cardiac nerves of the ligament of Marshall may be reached. Regardless of which ICN are stimulated by VOM HFS, in order to lead to AV node conduction slowing, those VOM-associated ICN need to communicate with the right inferior PV plexus, which is the one directly communicating with the AV node (19,20). Thus, eliciting a parasympathetic response in the AV node by VOM HFS proves not only local ICN stimulation but also the existence and activation of interneuronal communications between different ICN. These findings are consistent with those of Lin et al. (12), who found, in a canine study using HFS, that both the ligament of Marshall and the inferior left ganglionated plexus modulate AV nodal conduction.

Induction of AF by local VOM HFS without local atrial capture is a more complex phenomenon. A conceivable pathophysiological conjecture is that SynchHFS can selectively stimulate local ICN leading to acetylcholine release and action potential shortening. Coincidental stimulation of local adrenergic nerves could lead to local after-depolarization and AF induction, as postulated by Patterson et al. (21). The relative inconsistency of AF induction by this SynchHFS (4 of 8 patients) may thus illustrate the multiple pathophysiological steps required in its mechanism.

AF induced by BurstHFS was a consistent phenomenon, but atrial capture is expected to have played a major role in this setting. Either by local ICN ablation or atrial tissue ablation, VOM ethanol eliminated AF inducibility by VOM HFS.
Reduction of parasympathetic responses by previous AF catheter ablation has been previously reported (22). Our data showing a reduced incidence of parasympathetic responses by VOM HFS is consistent with these previous observations. VOM ethanol successfully eliminated parasympathetic responses as well as AF inducibility. Selective radiofrequency ablation of ICN has been shown to abolish the inducibility of AF in humans (3) and to eliminate stimulation-induced vagal responses (22). Additionally, autonomic denervation with radiofrequency applications at sites that elicited a parasympathetic response considerably increased the effectiveness of PVAI in patients with paroxysmal AF (23). Even anatomically remote modulation of the autonomic nervous system such as renal denervation may have an impact on ablation success (24). Our study is the first to show abolition of parasympathetic responses by using chemical ablation rather than radiofrequency energy in humans. Ethanol is highly cytotoxic when infused intravascularly and creates a myocardial scar when delivered through either the coronary venous or arterial route. It has been therapeutically used in septal ablation for hypertrophic cardiomyopathy (25), ablation of ventricular tachycardia via the coronary arteries (26,27) or coronary veins (28,29), and ablation of the atrioventricular node (30). The vagal denervating effects of ethanol infusion in the VOM were initially studied by our group in dogs. We showed that it can selectively abolish vagal innervation of the left atrium by blunting vagally mediated decreases in LA effective refractory periods (13). We have subsequently shown the clinical usefulness of VOM ethanol infusion in humans on both AF recurrences after catheter ablation (16) and in the treatment of perimetal flutter (31). This is, to our knowledge, the first demonstration of percutaneous chemical denervation of the left atrium in humans. Clinical implications. Although still controversial, ablation of ICN is a potential target in AF ablation (2). Ethanol infusion in the VOM is attractive in ablation of AF because local toxicity of ethanol is limited to tissues in direct contact with it and spares neighboring structures such as the esophagus. VOM ethanol infusion directly addresses mechanistic sources of AF located in the VOM, including ectopic triggers (32). Our current data add atrial denervation as an additional therapeutic effect of VOM ethanol. Radiofrequency ablation can effectively eliminate parasympathetic responses; when identified (23), it is technically simpler than VOM cannulation, which does not eliminate its need. However, it is unclear whether ICN in the VOM region can be readily ablated conventionally, given the fact that VOM signals are often present after endocardial radiofrequency in this region. (16) The ultimate role of VOM ethanol infusion in the treatment of AF remains to be determined and would only be established in a randomized clinical trial assessing rhythm control outcomes.

An important implication of our study is that the VOM can be used as a vascular route to deliver therapies targeting the ICN. Ethanol is nonspecifically toxic to all tissues exposed. However, other pharmacological strategies...
specifically targeting the ICN could be used in the future, using the VOM as a route.

Study limitations. Due to its technical complexity, the protocol (VOM cannulation, HFS pre- and post-ethanol) could only be completed in 80 of 133 patients. Autonomic nervous system activity analysis with tools such as heart rate variability was not performed. The functional relevance of ICN ablation remains to be determined, but that was beyond the scope of our study. The physiological relevance of interrupting neuronal connections is unclear, and it may potentially be deleterious for AF (33) or ventricular arrhythmias (34), although these were not observed on follow-up. The patient group was widely heterogeneous and included a wide range of left atrium sizes and AF patterns. Although the acute procedural safety of VOM ethanol infusion is clear, the long-term effects of ethanol and the permanence of ethanol-induced ablation lesions are unknown. It is clear that VOM ethanol infusion is an unconventional procedure that requires additional experience. Most clinically relevant procedural endpoints achieved can currently be achieved by radiofrequency. Only a randomized clinical trial can prove whether VOM ethanol infusion adds clinical benefit to catheter ablation of AF.

Conclusions

The VOM contains ICN that connect with the AV node and can trigger AF. Retrograde ethanol infusion in the VOM causes regional LA parasympathetic denervation, by abolishing local ICN responses and AF inducibility in humans. The VOM is a vascular route for ICN-targeting therapies.

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