Selective Ablation of the Ligament of Marshall Reduces the Prevalence of Ventricular Arrhythmias Through Autonomic Modulation in a Cesium-Induced Long QT Canine Model

Songyun Wang, MD, Zhibing Lu, MD, PhD, Wenbo He, MD, PhD, Bo He, MD, PhD, Jing Xie, PhD, Xiaomei Yu, PhD, Hong Jiang, MD, PhD

ABSTRACT

OBJECTIVES The goal of this study was to investigate the effect of selective ablation of the ligament of Marshall (LOM) on ventricular arrhythmias (VAs).

BACKGROUND Previous studies have shown that selective stimulation of sympathetic elements of the LOM, the distal segment of the ligament of Marshall that extends beyond the left superior pulmonary vein (LOM-LSV), might induce VAs.

METHODS In protocol 1, the blood pressure and ventricular effective refractory period changes as a response to LOM-LSV stimulation and left stellate ganglion (LSG) stimulation were measured before and after LOM-LSV ablation in 8 anesthetized dogs. In protocol 2, a total of 24 dogs were randomly divided into group 1 (cesium alone, n = 8), group 2 (cesium combined with LSG stimulation, n = 8), and group 3 (cesium combined with LSG stimulation after LOM-LSV ablation, n = 8). Early afterdepolarization amplitude, VA prevalence, and the tachycardia threshold (measured according to the dose of cesium administered) were compared among the groups.

RESULTS In protocol 1, both LOM-LSV stimulation and LSG stimulation significantly increased blood pressure and shortened the ventricular effective refractory period, both of which were significantly attenuated by LOM-LSV ablation. In protocol 2, compared with group 1, the prevalence of VAs and the early afterdepolarization amplitudes were significantly augmented in group 2 and were maintained at a comparable level in group 3. Furthermore, the tachycardia threshold in group 2 (0.625 mmol/kg) was significantly lower than that noted in groups 1 and 3 (both 1.000 mmol/kg; p < 0.05).

CONCLUSIONS LOM-LSV ablation reduced the prevalence of the VAs induced by cesium in combination with LSG stimulation, and the antiarrhythmic effect may involve the blockade of the sympathetic conduit between the LSG and the ventricles. (J Am Coll Cardiol EP 2016;2:97–106) © 2016 by the American College of Cardiology Foundation.
Horner’s syndrome have limited the clinical usefulness of LSG interventions (6,7). It is therefore necessary to explore alternative methods of treating LQTS.

The ligament of Marshall (LOM), a remnant of the left superior vena cava that extends into the coronary sinus, may be subdivided into a proximal portion that connects to the muscle sleeves of the coronary sinus (LOMCS), a distal portion that extends toward the left superior pulmonary vein (LOMLSPV), and a middle portion (8). Histological analysis has shown that the LOM has a rich autonomic innervation; the LOMCS is innervated primarily by the sympathetic nerves, and the LOMCS is innervated primarily by the parasympathetic nerves (9). Studies have also shown that stimulation and ablation of the parasympathetic elements in the LOM induces and eliminates atrial fibrillation, respectively (10). However, few studies have focused on the sympathetic element of the LOM and its significance. In a canine model, Lin et al. (11) found that the injection of norepinephrine into the LOMCS and the application of high-frequency stimulation (HFS) at this site, which is located centimeters away from the ventricles, caused accelerated junctional rhythms, accelerated idioventricular rhythms, and monomorphic and polymorphic ventricular tachycardia (VT) by activating the sympathetic element of the LOM. We therefore hypothesized that LOMCS ablation may suppress VAs. In the present study, the sympathetic connections among the LSG, the LOM, and the ventricles were investigated with respect to blood pressure (BP), heart rate, and ventricular electrophysiology. We also evaluated the effect of LOMCS ablation on VAs in a widely used long QT canine model.

METHODS

ANIMAL PREPARATION. This study was reviewed and approved by Wuhan University (permit number 2014-0216), and it conformed to the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Thirty-two adult mongrel dogs weighing 17 to 24 kg were anesthetized with 3% Na-pentobarbital at a basal dose of 30 mg/kg and an additional maintenance dose of 2 ml/h. All dogs were ventilated with a positive pressure ventilator, and their core body temperature was maintained at 36.5 ± 1.5°C with a heating pad. Body surface electrocardiograms and arterial BP were recorded continuously throughout the procedure by using a computer-based Lab System (Lead 2000B, Jingjiang Inc., Chengdu, China). The QT interval and QTc interval were measured by using the methods described in a previous study (12). A bilateral thoracotomy was performed at the fourth intercostal space, and the pericardium was hung to expose the heart.

LOMLSPV STIMULATION AND ABLATION. The LOM, which stretches from the coronary sinus toward the left superior pulmonary vein and left atrial appendage, was clearly visualized. Multielectrode catheters were held and sutured to the LOM and left atrial appendage, respectively (Figures 1A to 1C). Only the distal portion (LOMLSPV), which was innervated primarily by sympathetic nerves, was stimulated and ablated. The threshold was defined as the voltage required to induce an increase in BP. HFS (20 Hz, 0.1-ms duration, square waves) at a quadruple threshold was applied to the LOMCS via a stimulator (Grass-S88; Astro-Med, West Warwick, Rhode Island). Each HFS lasted <30 s, and the next measurement was delayed until the BP returned to a normal level. To ablate the LOMCS, radiofrequency ablation (50–300 W) was performed at the LOMCS. The endpoint of ablation was the elimination of the BP elevation response induced by stimulation along the LOM, with minimal damage to the underlying atrial tissue (13).

MEASUREMENT OF LSG FUNCTION. LSG function was measured according to the maximal change in BP in response to direct electrical stimulation of the LSG (14). HFS (20 Hz, 0.1-ms pulse duration, 10 to 70 V, increased by 10 V) was applied to the LSG by using a stimulator (Grass-S88; Astro-Med). The maximal BP increase induced by LSG stimulation, an indicator of the adrenergic influence from the LSG, was recorded at the end of each HFS before and after LOMCS ablation. A voltage/BP response curve was subsequently constructed, and the slope of the curve was calculated as described in the previous study (14). Each HFS was <30 s, and the next measurement was delayed until the BP returned to normal.

MEASUREMENT OF THE VENTRICULAR EFFECTIVE REFRACTORY PERIOD. A multielectrode catheter was sutured to both the left and right ventricular free walls. The ventricular effective refractory period (ERP) was recorded at the following 6 sites (Figure 1): the left ventricular apex, the left ventricular base, the median area of the left ventricle, the right ventricular apex, the right ventricular base, and the median area of the right ventricle. The ERP at each site was determined according to programmed pacing that
consisted of 8 consecutive stimuli (S1–S8, 350-ms cycle length) followed by a premature stimulus (S9). The S1–S2 interval was progressively decreased until refractoriness was achieved. The ERP was defined as the longest S1–S2 interval that failed to capture the ventricles (15).

**MONOPHASIC ACTION POTENTIAL RECORDING.**
Monophasic action potentials at the median of the epicardium of the left ventricle and the right ventricle were steadily recorded at baseline and after each cesium chloride (CsCl) injection by using a custom-made Ag-AgCl catheter (16). The monophasic action potential signals were amplified and filtered at 1 to 1,200 Hz. Furthermore, both the duration and the amplitude of the monophasic action potentials were measured, and the presence or absence of afterdepolarization was noted. The monophasic action potential recordings were analyzed by using the LEAD 2000B workstation (Jingjiang Inc.) system. The definitions and methods used to analyze the monophasic action potentials and early afterdepolarization (EAD) have been described in detail elsewhere (17). EAD was defined as either a delay in repolarization or true depolarization occurring during phase 2 or phase 3 of the monophasic action potential. The amplitude of the monophasic action potential was defined as the difference between phase 2 and the diastolic resting potential. The amplitude of the EAD, expressed as a percentage of the monophasic action potential amplitude, was defined as the potential difference between the diastolic resting potential and the first deviation from the smooth contour during either phase 2 or 3 of the repolarization (17).

**TACHYCARDIA THRESHOLD DOSE.** To evaluate the effect of the aforementioned autonomic interventions...
on ventricular arrhythmogenesis, increasing doses of CsCl were administered until a “threshold dose” produced sustained VT or ventricular fibrillation (VF). CsCl was administered as a rapid intravenous bolus (over several seconds) at 15-min intervals in 7 successive escalating doses as follows: 0.125, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, and 2.5 mmol/kg (17). The following definitions were used for the CsCl-induced VAs recorded on the electrocardiogram (18): VT, ≥3 consecutive ventricular premature ventricular beats (VPBs); nonsustained VT, VT terminating spontaneously within 30 s; sustained VT, VT persisting for 30 s; and VF, a tachycardia with a random electrocardiogram morphology associated with the loss of arterial BP that degenerated into ventricular asystole.

EXPERIMENTAL PROTOCOLS. Protocol 1: effect of LOM LSPV stimulation and LSG stimulation on BP and ventricular ERP before and after LOMCS ablation. In 8 anesthetized dogs, BP, heart rate, and ventricular ERP changes in response to LOMLSPV stimulation were measured before and after LOMCS ablation. Furthermore, BP and the ventricular ERP changes induced by LSG stimulation were also measured pre- ablation and post-ablation of the LOMLSPV. Each HFS was <30 s, and the next measurement was delayed until the BP and ERP returned to normal.

Protocol 2: effect of LOM ablation on VA-induced by CsCl in combination with LSG stimulation. Twenty-four dogs were divided into group 1 (CsCl alone, n = 8), group 2 (CsCl combined with LSG stimulation, n = 8), and group 3 (CsCl combined with LSG stimulation after LOMLSPV ablation, n = 8). LSG stimulation was initiated at 15 s before and maintained for 3 min after each CsCl injection. Adequate LSG stimulation was defined as a 30% increase in systolic BP compared with baseline.

**FIGURE 2** Effect of LOMLSPV Ablation on Dynamic and Electrophysiological Changes Induced by LOM Stimulation

Effect of LOMLSPV ablation on (A to C) dynamic and (D to I) electrophysiological changes induced by LOMLSPV stimulation. *p < 0.05, **p < 0.01 versus pre-ablation. BP = blood pressure; ERP = effective refractory period; other abbreviations as in Figure 1.
STATISTICAL ANALYSIS. The continuous variables are presented as mean ± SD and were analyzed by using t tests, 1-way analysis of variance, or 2-way repeated measures analysis of variance with a Bonferroni post-hoc test. To compare the tachycardia thresholds among the groups, the nonparametric Wilcoxon signed rank test was used. To evaluate the incidence of sustained VT/VF, a chi-square test was used. All data were analyzed by using GraphPad Prism version 5.0 software (GraphPad Software, Inc., San Diego, California), and a 2-tailed p value < 0.05 was considered statistically significant.

RESULTS

EFFECTS OF LOM<sub>LSPV</sub> STIMULATION AND ABLATION ON BP AND VENTRICULAR ERP. Figure 1D shows the activation sequence of LOM. The potential of the LOM was recorded clearly in most cases (28 out of 32 dogs) (Figure 1E); in the other cases, however, the LOM was fused with the potential of either the left atrium or the pulmonary vein, and rapid atrial pacing was required to isolate it. All LOM were isolated (Figure 1F). During LOM<sub>LSPV</sub> stimulation, the voltage required to induce BP elevation ranged from 8.9 to 12.7 V. Compared with baseline, LOM<sub>LSPV</sub> stimulation at a quadruple threshold, ranging from 35.6 to 50.8 V, resulted in a significant increase in BP (systolic BP 96 ± 10 mm Hg vs. 132 ± 18 mm Hg; diastolic BP 66 ± 8 mm Hg vs. 78 ± 12 mm Hg; both p < 0.05) (Figures 2A and 2B), a slight but not significant increase in heart rate (164 ± 24 beats/min vs. 80 ± 32 beats/min; p > 0.05) (Figure 2C), and a significant shortening in ventricular ERP at all 6 sites (Figures 2D to 2I). After LOM<sub>LSPV</sub> ablation, however, no significant effects of LOM<sub>LSPV</sub> stimulation were observed regarding BP (systolic BP 96 ± 10 mm Hg vs. 102 ± 18 mm Hg; diastolic BP 66 ± 8 mm Hg vs. 68 ± 12 mm Hg; both p > 0.05), heart rate, or ventricular ERP.

EFFECT OF LOM<sub>LSPV</sub> ABLATION ON LSG FUNCTION. As a result of LSG stimulation, systolic BP and diastolic BP were both significantly increased and stabilized at a high level after the voltage reached 50 V (systolic

![Figure 3: Effect of LOM<sub>LSPV</sub> Ablation on LSG Function](image)

A and B represent typical examples of the maximal BP elevation induced by LSG stimulation before and after LOM<sub>LSPV</sub> ablation, respectively. C and D depict the corresponding response curves. *p < 0.05, **p < 0.01 compared with pre-ablation. LSG = left stellate ganglion; other abbreviations as in Figures 1 and 2.)
BP 144 ± 20 mm Hg vs. 94 ± 14 mm Hg; diastolic BP 78 ± 14 mm Hg vs. 60 ± 10 mm Hg; both p < 0.05 compared with baseline). Furthermore, both the maximum systolic and the maximum diastolic BP changes in response to LSG stimulation at voltages ≥30 V were significantly attenuated by LOMLSPV ablation (Figures 3). In addition, the voltage/systolic BP response curve was significantly flattened as a result of LOMLSPV ablation.

**THE ROLE OF LOMLSPV ABLATION IN MODULATING VENTRICULAR ERP CHANGES INDUCED BY LSG STIMULATION.** As depicted in Figures 4A to 4F, LSG stimulation induced a significant shortening in ventricular ERP at all sites; this effect was attenuated by LOMLSPV ablation. For instance, the ERP at the right ventricular apex was decreased from 180 ± 15 ms to 166 ± 10 ms during LSG stimulation (p < 0.05). After LOMLSPV ablation, however, the LSG stimulation-mediated ERP shortening response was eliminated (182 ± 16 ms vs. 179 ± 15 ms; p > 0.05).

**EFFECT OF LOMLSPV ABLATION ON EAD AMPLITUDE AND THE TACHYCARDIA THRESHOLD DOSE.** Compared with baseline, both the QT interval and the QTc interval were significantly prolonged after bolus injections of 0.5 and 0.75 mmol/kg of CsCl, respectively (Figures 5A and 5B). The QT interval was prolonged by 20%, and the QTc interval was prolonged by 10% (both p < 0.05 compared with group baseline) at 30 s after the bolus injection of 0.75 mmol/kg of CsCl. Compared with group 1 (CsCl alone), the median tachycardia threshold dose was decreased in group 2 (CsCl + LSG stimulation) (0.625 vs. 1.000 mmol/kg; p = 0.01) and returned to a normal level in group 3 (LOM ablation + CsCl + LSG ablation, 1.000 mmol/kg) (Figure 5C). Figure 5D includes representative examples of the VPB, nonsustained VT, sustained VT, and VF induced by CsCl in combination with LSG stimulation.

As illustrated in Figure 6, no EADs were observed at baseline in any group. Compared with group 1, the EAD amplitudes at 15, 30, and 60 s after CsCl injection increased from 24.7 ± 8.9% to 57.3 ± 25.2% (p = 0.01), 35.3 ± 11.6% to 69.8 ± 31.7% (p = 0.02), and 29.1 ± 10.2% to 57.8 ± 21.4% (p = 0.02) in group 2, respectively. Compared with group 2, however, the increased EAD amplitudes were significantly attenuated in group 3 (15 s, 32.1 ± 13.2%; 30 s, 38.1 ± 15.6%; 60 s, 31.7 ± 16.4%; all p < 0.05 compared with group 2 at the same point). Doses >0.75 mmol/kg were not compared among these 3 groups because the onset of sustained VT prevented further analysis of the EAD amplitudes.

**EFFECT OF LOMLSPV ABLATION ON VAS.** Figures 7A to 7C depict the prevalence of the VAs induced by...
incremental injections of CsCl. Compared with group 1, the prevalence of VAs was significantly increased in group 2, an effect that was significantly attenuated by LOM-LSPV ablation in group 3. After the injection of 0.75 mmol/kg of CsCl, the number of VPBs in groups 1, 2, and 3 were 7.17 ± 4.26, 35.89 ± 18.76, and 12.68 ± 5.12 (p < 0.05), respectively, and the episodes of nonsustained VT totaled 1.65 ± 0.76, 3.54 ± 1.89, and 1.89 ± 0.98 (p < 0.05). Furthermore, the prevalence of sustained VT/VF was 12.5%, 100%, and 37.5% (p < 0.05).

**DISCUSSION**

**MAJOR FINDINGS.** The present study showed that: 1) LOM-LSPV stimulation significantly increased BP and decreased ventricular ERP; and 2) LOM-LSPV ablation significantly attenuated the effect of LSG stimulation on BP and ventricular ERP and decreased the prevalence of VAs induced by CsCl combined with LSG stimulation. These findings suggest that the LOM may serve as a sympathetic conduit between the LSG and the ventricles and that LOM ablation may reduce the prevalence of VAs induced by CsCl in combination with LSG stimulation by modulating the neural network between the LSG and the ventricles.

**THE ROLE OF LOM-LSPV IN THE NEURAL NETWORK BETWEEN THE LSG AND THE VENTRICLES.** The LOM, the residue of the embryonic left superior vena cava that originates from the left superior pulmonary vein and projects to the coronary sinus, is rich in sympathetic and parasympathetic nerves (8-10). The LOMCS is innervated primarily by parasympathetic nerves, whereas the LOM-LSPV is innervated primarily by sympathetic nerves. Previous studies have found that the ventricular rate slowing and atrial ERP shortening responses caused by vagal stimulation were blunted by LOM ablation,
FIGURE 6  Impact of LOM_{LSV} Ablation on EAD Amplitude After CsCl Injection

Impact of LOM_{LSV}-A on early afterdepolarization (EAD) amplitude at 15, 30, and 60 s after CsCl injection in groups 1, 2, and 3. *p < 0.05, compared with group 2. Abbreviations as in Figures 1, 4, and 5.

FIGURE 7  Effect of LOM_{LSV} Ablation on the VAs Induced by CsCl in Combination With LSG Stimulation

*p < 0.05, **p < 0.01, compared with group 1; §p < 0.05, compared with group 2. Abbreviations as in Figures 4 and 5.
indicating that the LOM may act as an “integration center” in modulating the autonomic (parasympathetic) interactions between the extrinsic and intrinsic cardiac autonomic nervous system and atrioventricular conduction (9,19,20). Previous studies have also shown that sustained atrial fibrillation is accompanied by LOM hyperactivity (21) and that muscle bundles within LOM stimulation trigger either focal atrial fibrillation or maintain sustained atrial fibrillation in both human and animal models (11,15). LOM ablation via either radiofrequencies or retrograde ethanol infusions into the vein of Marshall, however, reduces atrial fibrillation inducibility by parasympathetic denervation (20,22).

In the present study, our results showed that stimulation at the LOM LSPV site significantly shortened the ventricular ERP and elevated BP, findings indicative of the activation of sympathetic elements within the LOM. We also observed that LOM LSPV ablation significantly attenuated the BP elevations and ventricular ERP shortening response induced by LSG stimulation. Together, these results imply that the LOM may serve as a sympathetic conduit between the LSG and the ventricles. This finding may be corroborated by the fact that the ventrolateral cardiac nerve, a major sympathetic nerve that originates from the middle cervical and stellate ganglia, may travel along the LOM to project to the ventricles (23,24). Furthermore, a previous study found that the stimulation of this large nerve trunk might elicit changes in the left ventricle, as well as minor changes in the right ventricle (24). We therefore hypothesized that ablation of the LOM could damage the sympathetic pathway traveling from the stellate ganglia to the ventricles.

**LOMLSPV ABLATION REDUCED THE PREVALENCE OF THE VAs INDUCED BY CsCL IN COMBINATION WITH LSG STIMULATION.** Activation of the sympathetic nervous system, including the LSG, seems to facilitate the initiation and maintenance of VAs in animals and patients with an abnormal myocardial substrate such as prior myocardial infarction, LQTS, or catecholaminergic polymorphic VT (1,2). Inhibition of the sympathetic nervous system, however, protects against VAs (17). Neural recordings from the LSG showed that most VTs (86.3%) and sudden cardiac deaths were preceded within 15 s by the discharge of an LSG spike, indicating that increased LSG activity may be the immediate triggering mechanism of VAs and sudden cardiac death (25). Hanich et al. (17) showed that LSG stimulation increased the amplitude of the CsCl-induced EADs, which had been proposed as the mechanism responsible for VPB and polymorphic VT, and decreased the dose of CsCl required to produce VT. The underlying mechanism of this phenomenon might entail activation of the sympathetic nervous system, increasing intracellular levels of cyclic adenosine monophosphate and stimulating calcium influx (26). Both experimental and clinical studies have indicated that LSG plays a more important role in modulating the electrophysiological properties of both ventricles than the right stellate ganglion, and LSG denervation or blocking is beneficial in the treatment of LQTS (3–5,27,28).

In the present study, LSG stimulation significantly increased the prevalence of VAs in the CsCl-induced long QT canine model; this effect was significantly attenuated by LOM LSPV ablation. Furthermore, the maximal BP acceleration in response to LSG stimulation, an indicator of the adrenergic influence of LSG, was significantly attenuated by LOM LSPV ablation, indicating that LOM LSPV ablation may exert an antiarrhythmic effect by antagonizing sympathetic activity, thereby increasing the tachycardia threshold and decreasing the prevalence of VAs. In the present study, we also observed that LOM LSPV ablation attenuated the ventricular ERP shortening induced by LSG stimulation, which supports the idea that the LOM may act as a sympathetic conduit between the LSG and the ventricles. Together, these findings indicate that the mechanism underlying the antiarrhythmic effect of LOM LSPV ablation in a drug-induced long QT canine model may involve the blockade of the sympathetic neural pathway between the LSG and the ventricles, thereby attenuating the effects of LSG stimulation on the ventricles.

**STUDY LIMITATIONS.** There was a lack of histological evidence demonstrating the direct connections between the LSG and the ventricles. However, a previous study found that the ventrolateral cardiac nerve may travel along the LOM to innervate the lateral and posterior walls of the ventricle (23). Also, the CsCl-induced long QT model does not carry the genetic fingerprints of human congenital LQTS. The antiarrhythmic effects of LOM ablation must be determined by future studies.
CONCLUSIONS

LOM_LSPV ablation may significantly attenuate the VAs induced by CsCl in combination with LSG stimulation by blocking the sympathetic neural connection between the LSG and the ventricles. Further studies are needed to verify the antiarrhythmic effects of LOM ablation in the congenital LQTS model.

REFERENCES


KEY WORDS autonomic nervous system, left stellate ganglion, ligament of Marshall, ventricular arrhythmias

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: LSG denervation or blockade has been indicated for the treatment of LQTS for several decades. However, Horner’s syndrome and several potentially serious complications described in previous studies have limited its usefulness in the clinical setting.

TRANSLATIONAL OUTLOOK: Our results showed that the LOM may be a conduit between the LSG and the ventricles and that selective ablation of the LOM might represent an alternative approach to LSG denervation or blockade in treating LQTS.