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Transthoracic echocardiography guided percutaneous laser ablation of the interventricular septum: A successful sheep model for septal thickness reduction and one year follow-up

Jianli Fu [a,b,1], Chao Sun [a,c,1], Fang Liu [a,1], Rui Hu [a], David H. Hsi [d], Shengjun Tuo [a,e], Hong Shao [f], Hong Yin [g], Jianmin Zheng [g], Jianlei Zhang [e], Jian Yang [h], Jun Zhang [a], Xiangping Guan [b,*], Liwen Liu [a,**]

[a] Ultrasound Department, Xijing Hospital, Fourth Military Medical University, Xi'an, China
[b] Ultrasound Department, ShaanXi Province People's Hospital, Xi'an, China
[c] School of Life Sciences, Northwestern Polytechnical University, Xi'an, China
[d] Department of Cardiology, Stamford Hospital, Stamford, CT, USA
[e] Department of Ultrasound, Yan'an People's Hospital, Yan'an, China
[f] Department of Cardiology, Xijing Hospital, Fourth Military Medical University, Xi'an, China
[g] Department of Radiology, Xijing Hospital, Fourth Military Medical University, Xi'an, China
[h] Department of Cardiac Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an, China

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ABSTRACT

Background: Hypertrophic cardiomyopathy (HCM) is a common genetic cardiovascular disease, causing breathlessness, chest pain, syncope and sudden death. One-year outcome of echo-guided transthoracic percutaneous laser ablation (TPLA) of the sheep interventricular septum was studied as a novel treatment to reduce the septal thickness. It may partially address the limitations of surgical myectomy and alcohol septal ablation in terms of trauma, safety, and efficacy.

Methods: Twelve healthy adult sheep were randomly categorized into two groups: with and without the laser application of TPLA of the interventricular septum (IVS) at the energy level of 5 W for 3 min. Echocardiography, electrocardiography (ECG), cardiac magnetic resonance (CMR), serological and pathological examinations were performed over a 12-month follow-up.

Results: After the laser ablation all animals survived with normal cardiac function; No severe complications or bundle branch block were noted. The septal thickness (3.11 ± 1.14 vs. 8.40 ± 0.45 mm, $p < 0.05$), regional movement of ablated IVS and longitudinal strain significantly decreased when comparing the experimental and control groups. The Troponin I level was significantly elevated after the operation, which validated immediate cardiac coagulation necrosis. On cardiac magnetic resonance (CMR) imaging, the ablated myocardium showed significant fibrosis evidenced by late gadolinium enhancement. Pathological results revealed damaged ultra-structure of the ablated myocardium and development of fibrosis.

Conclusions: TPLA is a safe and effective minimally invasive method to reduce IVS thickness in the long term, making it a potential alternative for HOCM treatment.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is one of the most common genetic cardiovascular diseases that generally manifests as asymmetrical hypertrophy of the interventricular septum (IVS) [1]. It may lead to obstruction in the left ventricular outflow tract (LVOT), which may cause breathlessness, chest pain, syncope and even sudden death [2]. Surgical myectomy and alcohol septal ablation (ASA) are the two conventional treatments for patients with drug-refractory obstructive HCM (HOCM). Surgical myectomy has been proposed as the therapeutic gold standard to relieve the obstruction at LVOT, albeit it requires sternotomy and establishment of cardiopulmonary bypass [3]. In addition, after surgical myectomy, approximately 46% of the patients developed left bundle branch block (LBBB) [4] and 3.2% may present with complete atrioventricular block [3]. A meta-analysis showed that 4.4% of the 2791 patients after myectomy required permanent pacemaker [5]. ASA is a minimally invasive treatment by releasing alcohol in the coronary arteries that support the hypertrophic region causing

* Corresponding author.
** Correspondence to: L. Liu, Xijing Hospital, Fourth Military Medical University, 127#Changle West Road, Xi'an 710032, Shaanxi, China.
E-mail addresses: ggmmzyq1963@139.com (X. Guan), liuliwen@fmmu.edu.cn (L. Liu).
[1] Authors contributed equally to this work.

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LVOT obstruction via a catheter. However, ASA possesses the risk of damaging the unexpected myocardium due to the misplacement of alcohol through the collateral arteries [6]. About 10% of 2013 patients needed permanent pacemakers after ASA in a meta-analysis [5]. We sought to explore an alternative approach for HOCM treatment.

Laser ablation may emit heat and lead to coagulation necrosis of the tissue showing significant high accuracy of ablation and convenience in manipulation [7]. For this reason, laser ablation is widely used to treat thyroid microcarcinoma [8], varicose vein [9], benign prostatic hyperplasia [10], and atrial fibrillation [11]. Dowling and colleagues first reported in 1993 performing septal myectomy with a carbon dioxide laser for hypertrophic cardiomyopathy with employing median sternotomy [12]. In a preliminary trial for HCM treatment [13], our previous research completed echocardiography guided transthoracic percutaneous laser ablation (TPLA) of canine IVS and proved its efficacy and safety in the short term. All experimental animals survived during and immediately after the ablation process. However, the long-term efficacy and safety of this method require further investigation to obtain solid evidence, supporting its application in clinical practice.

In the present study, a healthy sheep model was studied under the guidance of transthoracic echocardiography (TTE) over a long-term period (up to 12 months) to monitor the development of potential complications such as tamponade, ventricular fibrillation or bundle branch block; or other effects on the cardiac function and conduction system. Moreover, the long-term efficacy in terms of reduced thickness and regional movement of the ablated IVS region was studied. TTE, electrocardiography (ECG), cardiac magnetic resonance (CMR), serological and pathological examination were performed to fully evaluate the long-term outcomes of the laser applications.

2. Methods

2.1. Animal preparation

This study was approved by our Institutional Animal Ethics Committee for Research Animal Care and performed in accordance with the ethical standards of the Declaration of Helsinki. It included 12 sheep (age: 12–18 months; weight: 52.0 ± 4.1 kg). The animals were randomly divided into two equal groups: the experimental group and the control group (n = 6 each). The animals were fasted for 24 h before applying general anaesthesia through an intravascular injection of xylazine hydrochloride (0.05 mL/kg; Hua Mu Animal Care Inc., Ji Lin, China).

2.2. TPLA of IVS

The operational procedure has been described previously [13]. Briefly, neodymium-yttrium-aluminium-garnet (Nd-YAG) laser fibre (Echo Laser X4; Elesta S.R.L., Italy) of 800–1064 nm wavelength and a diameter of 300 μm was connected to an ultrasound scanner (MyLab 9.0; Esaote, Italy), which was used for laser ablation. Nd-YAG laser (a wavelength of 1064 nm) has a penetration depth of 10 mm and provides good homeostasis for blood vessels. YAG laser has been in clinical use in our center with successful results in efficacy and safety. As shown in Fig. 1A and B, under the guidance of an ultrasound scanner (iE Elite; Philips, Netherlands) equipped with a 3.5-MHz C5-1 transducer, a PTC-needle sheath (21-G; Ecochiba, Italy) was inserted into the IVS from the apical region on the right ventricular (RV) side until the tip of the needle reached basal segment of IVS, avoiding the membranous segment and maintaining the safe distance of 5–10 mm under the aortic valve. Next, the laser fibre was inserted in the PTC needle sheath. Then, the PTC needle was withdrawn by approximately 1 cm to expose the fibre in the IVS myocardium for ablation. For the experimental group, the basal IVS segment was ablated for 3 min with a 5 W laser. For the control group, the insertion procedure was completed without performing laser ablation. The ablation needle should be centered in the septum if possible in spite of the RV entry.

2.3. ECG and TTE examinations

ECG (MAC 2000; GE, US) was performed before, during and after the operations. Two-dimensional TTE (Vivid E9; GE, US) was used to guide the procedure and to evaluate the cardiac functions with a transducer M5S (1–5 MHz). The parameters included left ventricular ejection fraction (LVEF), amplitude of the motion of the regional IVS on the M-mode, the ratio of peak early mitral inflow velocity (E-wave) and peak late mitral inflow velocity (A-wave), E/A ratio on the pulsed-wave (PW) Doppler, Ea/Aa measured at the mitral valve (MV) annulus, the peak systolic (Sa) and diastolic waves (Ea and Aa) at the ablated region and its opposite wall using the velocity spectrum of Tissue Doppler Imaging (TDI). Each dataset was measured and averaged over three cardiac cycles. The EchoPAC workstation (EchoPAC; GE, US) was used to analyse the longitudinal strain (LS) of the ablated region.

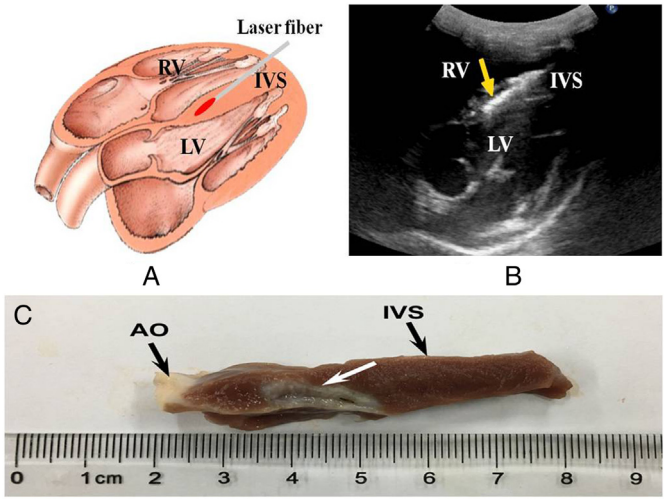

Fig. 1. (A) Illustration of the needle path from the apex of right ventricle (RV) into the interventricular septum (IVS) LV: left ventricle. (B) Echocardiographic image shows the needle path in the view of parasternal LV long-axis and the ablation site (yellow arrow). (C) The gross specimen of the IVS. The white arrow shows the laser-ablated region. AO: aorta and IVS: interventricular septum.

The difference of time to peak systolic velocity (TPSV) was calculated between the ablated region and the opposite wall. Myocardial contrast echocardiography (MCE) was performed using a contrast agent (SonoVue, Bracco, Italy) by using an ultrasound scanner (iE Elite; Philips, The Netherlands) and C5-1 transducer (3.5 MHz). During the follow-up observations, ECG and TTE were recorded immediately after and at 1 week, 1 month, 3 months, 6 months and 12 months of the ablation.

2.4. Serological examination

Blood samples (5 mL) were obtained from each animal before and 1 h after the operation to measure cardiac markers Troponin I.

2.5. Cardiac magnetic resonance imaging

CMR imaging scan was obtained before the operation, and 12 months after the ablation by using an MR imaging system (Area 1.5 T Siemens Medical Solutions; Germany). A bolus of gadolinium-DTPA (0.2 mmol/kg; Beijing Beilu Pharmaceutical Co. Ltd., China) was administered intravenously to acquire 8–15-min-delayed contrast-enhanced images. The cine images were ECG-gated and consisted of steady-state phase-sensitive inversion recovery (PSIR) sequences with the following parameters: repetition time/echo time of 587 ms/1.19 ms, flip angle of 40°, matrix of 144 × 256, the field view of 360 mm with a slice thickness of 6 mm and an inter-slice gap of 0.6 mm through the LV four-chamber and short-axis views. The inversion delay (260 and 320 ms) was determined using an inversion-recovery scout sequence and was lengthened during scanning to maintain an optimal nulling of the normal myocardium.

2.6. Pathological examinations

One year after the operation, all sheep hearts were removed for pathological examinations. The specimens were sectioned along the longitudinal myocardial fibres and stained with haematoxylin and eosin (HE) and Masson trichrome staining.

For electron microscopic examination, a portion of the IVS was cut and fixed. After dehydration and embedment, the specimens were sectioned at 1-μm thickness and stained. Ultrathin sections were cut and studied under a transmission electron microscope (TEM) (JEM-2000; Jeol Limited, Tokyo, Japan).

2.7. Statistical analysis

Quantitative variables were presented as mean ± standard deviation (SD). A repeated measurement analysis of variance (ANOVA) was used to compare the variation in the repeated measurement over time between the experimental and control groups. Multivariate ANOVA (MANOVA) test was performed to investigate the differences between the variables of the two groups at the same time point. The significance level was set at 0.05, and $p < 0.05$ indicated a statistically significant difference. SPSS (IBM SPSS Statistics, Version 21; IBM, USA) was used for the statistical analysis.

3. Results

Each experiment involved 2–3 needle passes. All sheep survived during the ablation process and through the 12-month follow-up. No major complications such as pericardial tamponade or ventricular fibrillation were recorded. In one sheep, small pericardial effusion (PE) was noted at the RV anterior wall, which was absorbed 1 week after the ablation.

3.1. Electrocardiography

Ventricular premature beat (VPB) and atrial premature beat (APB) were found during the process of needle insertion. During laser ablation, ventricular tachycardia (VT) occurred and disappeared immediately when the ablation stopped. Occasional APB was noted immediately after the operation. No bundle branch block or other abnormalities were recorded 12 months after the operation. The heart rate of the sheep was faster during the operation than that before the procedure (147 ± 16 vs. 75 ± 7, $p < 0.05$).

3.2. Echocardiographic and contrast echocardiographic evaluation of the thickness of ablated IVS

Fig. 2 shows the variation in the IVS on 2D TTE (A, C, E) and MCE (B, D, F) before the operation, during laser ablation and 12 months after the ablation. The variation in the thickness of IVS is marked by white arrows in 2D TTE and MCE in Fig. 2A–F. Immediately after the operation, the thickness of the ablated IVS significantly increased due to the edema from the ablation process (Fig. 2C–D). The ablated region appeared as hyper-echogenic area (indicated by the arrow). In addition, no myocardial perfusion was visualized in the ablated IVS on MCE (Fig. 2D). After 12 months, the thickness of the ablated IVS became significantly thinner (Fig. 2E–F), and the MCE showed enhancement as compared with that in the surrounding myocardium (Fig. 2F).

3.3. Cardiac magnetic resonance imaging

As compared with the control group (Fig. 2G and H), CMR of the experimental group showed significant late gadolinium enhancement (LGE) in the ablated IVS area in the LV four-chamber view (Fig. 2I) and short-axis view (Fig. 2J) (black arrow).

3.4. Echocardiographic evaluation of the global and regional cardiac function

For detailed analysis of the variation in the thickness and cardiac function during the 12-month follow-up, TTE was performed to quantify the global and regional cardiac functions (Fig. 3). Regarding the global cardiac function (Fig. 3A–C), EF for LV systolic function and E/A and Ea/Aa for diastolic function were found to reduce immediately after the operation in the experimental group, after which these gradually returned to the pre-operative level and remained stable during the 12-month period. The heart rate accelerated after the operation and was then restored gradually during the 12 months (Fig. 3D). As shown in Fig. 3E–F, no significant variation was noted in the wall thickness and myocardial movement of the ablated region in the control group. In the experimental group, the thickness of the ablated IVS initially increased immediately after the ablation due to edema. After 1 week, the value returned to the pre-operative level. The IVS thickness then decreased gradually until reaching a plateau after 3 months, following which it remained stable throughout the remaining follow-up period. The average reduction in the thickness of the ablated IVS was

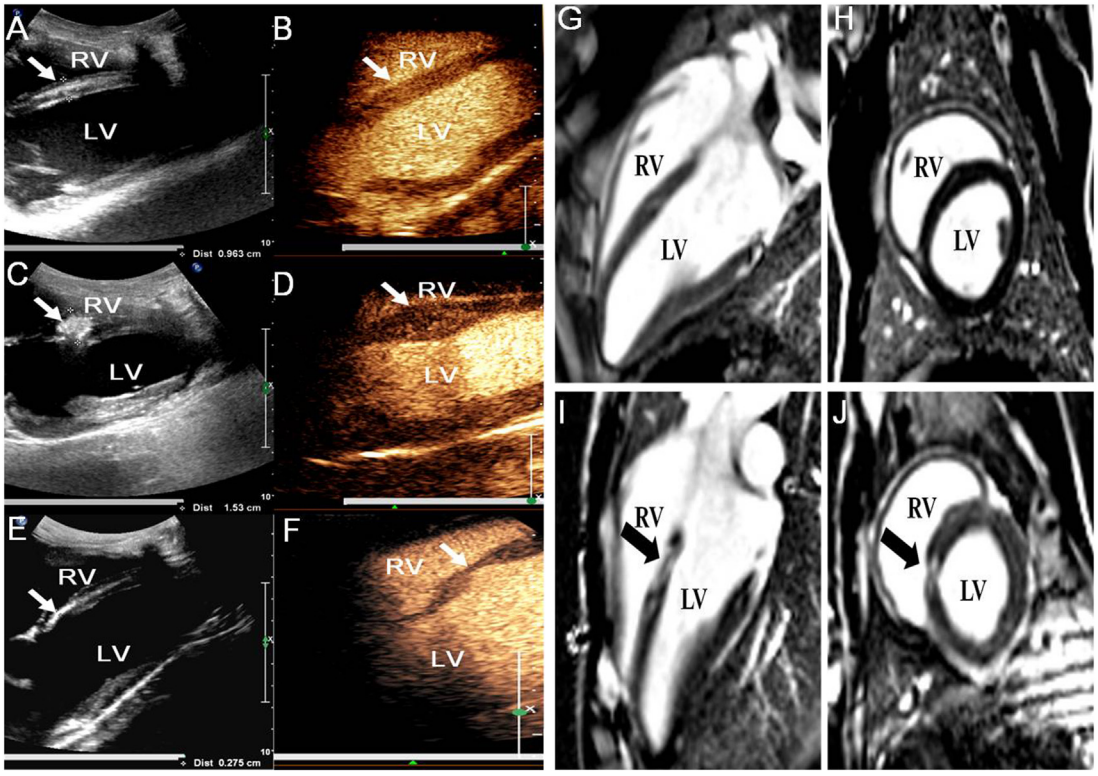

Fig. 2. Serial images in the ablated interventricular septum (IVS) by 2D echocardiography (A, C, E), contrast echocardiography (B, D, F) and late enhancement of gadolinium (LGE) on cardiac magnetic resonance (G–J). (A, B) before the operation, (C, D) immediately after laser ablation and (E, F) 12 months after the ablation. The white arrow represents the location of the ablated IVS in three phases. The measured thickness of IVS changed from (A) 0.963 cm to (C) 1.53 cm and finally to (E) 0.275 cm. The cardiac chambers look larger in Fig. 1E than in Fig. 1A and B because the animals grew over the 12-month experimental period. The LGE of IVS on CMR after 12 months between the control group (G, H) and the experimental group (I, J). In the control group, no fibrosis of IVS in the apical four-chamber (G) and LV short-axis view (H). In the experimental group, black arrows indicate obvious fibrosis in the apical four-chamber (I) and LV short-axis view (J).

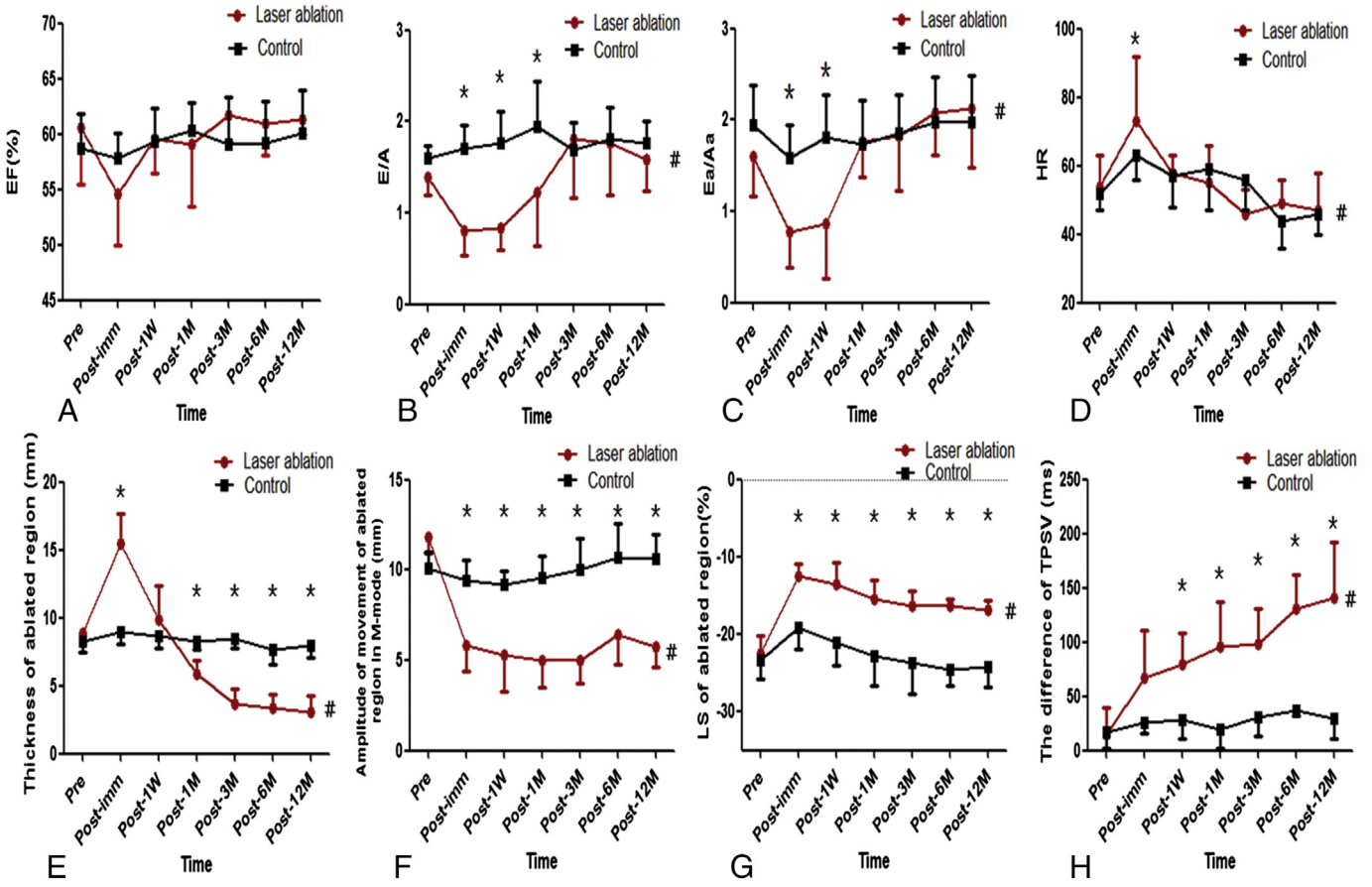

Fig. 3. The variation in the global and regional cardiac function of the experimental and control groups before the ablation and over the 12-month follow-ups. (A) LVEF, (B) E/A, (C) Ea/Aa at the mitral valve annulus, (D) Heart rate (HR), (E) Thickness of the ablated region, (F) Amplitude of the movement of the ablated region in the M-mode, (G) The longitudinal strain (LS) of the ablated region; (H) the difference of the time to peak of the systolic velocity (TPSV) between the ablated IVS and opposite wall. # Indicates significant difference between the two groups ($p < 0.05$). * Indicates significant difference in the value at the same time point between the two groups ($p < 0.05$). Pre-represents the value before the ablation. Post-immediate, post-1W, post-1M, post-3M, post-6M and post-12M represent the post-operative value acquired immediately after and 1 week, 1 month, 3 months, 6 months and 12 months after the ablation respectively.

approximately 5.15 ± 1.07 mm (Fig. 3E). The amplitude of the motion of IVS in the experimental group decreased significantly on the M-mode (Fig. 3F) as compared with that in the control group. Compared to the control group (Fig. 3G), the longitudinal strain of the ablated IVS region in the experimental group reduced immediately after the operation, then gradually recovered but maintained at lower levels in the follow-up period. The difference of TPSV between the ablated area and the opposite wall was larger in the experimental group (Fig. 3H), reflecting a scarring process. It was not found in the control group.

3.5. Serological examination

After the operation, the Troponin I levels were significantly higher in the experiment group than the control group (5.63 ± 1.94 vs. 0.05 ± 0.02 ng/mL, $p < 0.05$).

3.6. Pathological examination

No obvious pericarditis was found at autopsy. The heat generation by the YAG laser only caused precisely controlled coagulation necrosis in the interventricular septum. At necropsy, the thickness of the ablated IVS (white arrow) was noted to be significantly pale and thin as compared to the IVS in the experimental group (Fig. 1C). The experimental group histology example of the septum after the ablation and the small artery and vein are away from the ablation zone on the right side. Actually, some of the septal perforators would have been safely occluded, which is part of the septal ablation mechanisms. For the control group in Fig. 4A and C, the cells were neatly arranged and the nuclei were clearly visible. In the experimental group with HE (Fig. 4B), the cells were unevenly distributed; the partial nucleolus had disappeared; and the myocardial tissue had become necrotic. The ablated IVS in the experimental group was replaced by a large amount of blue collagen fibres in the Masson trichrome staining (Fig. 4D).

The electron microscopic examination showed details after the ablation under a magnification of ×2000. In Fig. 4E, the control group showed uniform distribution of the mitochondria (hollow arrow) and myofibril (white arrow), clear sarcomere and circular nuclei (grey arrow). In the experimental group (Fig. 4F), a large number of collagen fibres proliferated and replaced the normal tissue. The myofibrils (white arrow) were completely damaged and disordered. The nucleus (grey arrow) was deformed and the mitochondria had disappeared.

4. Discussion

Our study showed that TPLA may reduce the IVS thickness safely and efficaciously in the long term. All animals survived without any severe complications. Both the thickness and the regional movement of the ablated IVS decreased significantly during the 1-year follow-up. The blood test performed validated the immediate cardiac coagulation necrosis. The pathological results revealed long-term changes in the ablated myocardium. Similar to the outcome of surgical myectomy,

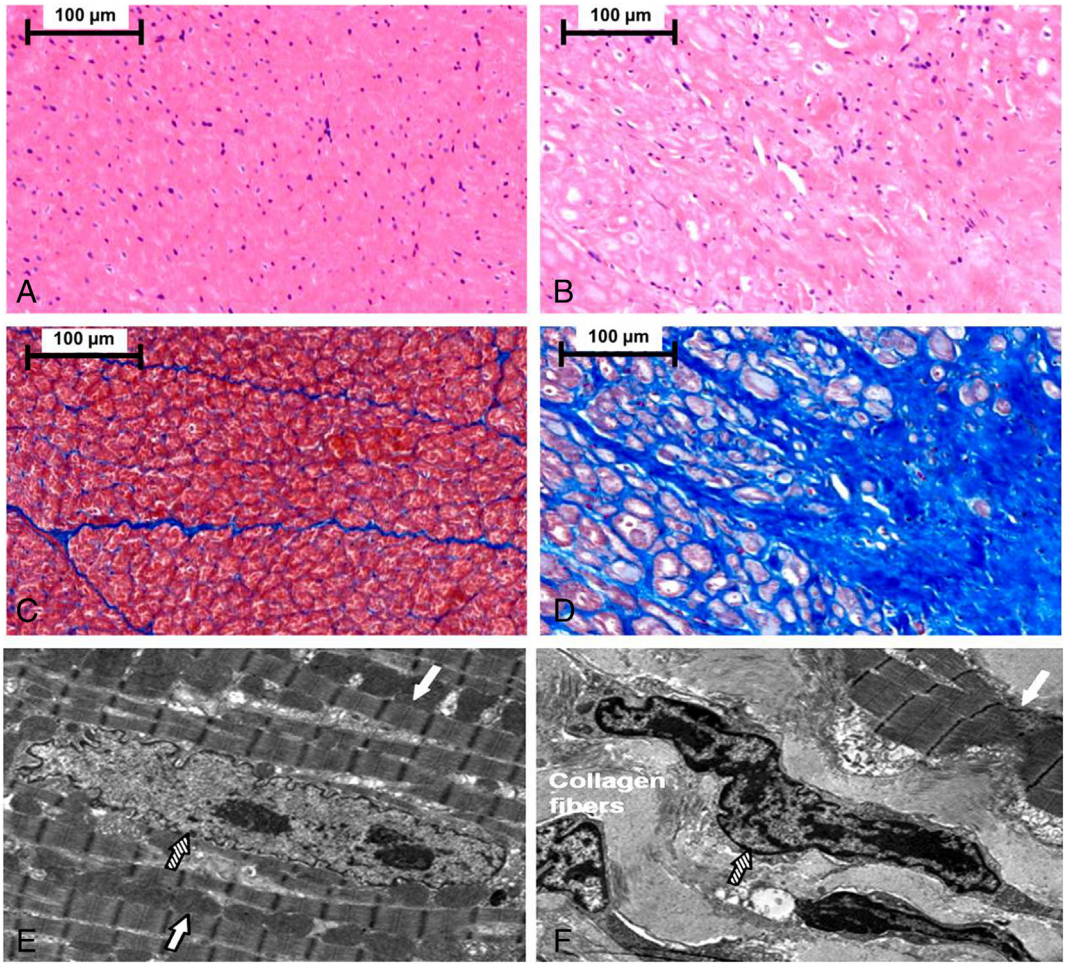

Fig. 4. The pathological (A–D) and electron microscopic (EM) observation (×2000) (E–F) of the ablated IVS between the control and the experimental groups after 12 months. (A) HE staining of the ablated IVS in the control group and (B) the experimental group. (C) Masson staining of the ablated IVS in the control group and (D) the experimental group. (E) EM observation in the control group and (F) the experimental group. Mitochondria (hollow arrow), myofibril (white arrow), and circular nuclei (grey arrow).

volume reduction of IVS and the weakened movement of regional IVS may widen the LVOT width and finally decrease the LVOT gradient effectively [2,3]. In this study, thinning of the ablated IVS (Fig. 2E–F) may broaden the LVOT in the long term. The amplitude of movement of the ablated IVS decreased significantly on the M-mode as compared to that of the opposing free wall (Fig. 3F). In addition, the reduced LS of the ablated IVS indicated decreased contraction of the regional ventricular wall. The TPSV of the ablated region was significantly longer than that of the control group, which produced desynchronization and may potentially alleviate the obstruction of LVOT. This mechanism is similar to the treatment of HOCM using a two-chamber pacemaker [14]. Thus, TPLA may decrease the multiple facets of the ablated IVS, including the thickness, amplitude of movement, LS and movement phase delay in the long term. We may therefore reasonably estimate that most of the factors that may contribute to the reduction in the obstruction at LVOT in HCM were addressed in the laser ablation. The eventual thinning of the myocardium after the ablation should be as effective as the septal myectomy and possibly more effective than the ASA due to its larger area of ablation. The ablation needle should be centered in the septum if possible in spite of the RV entry.

Troponin I is a myocardial regulatory protein showing high sensitivity and specificity in myocardial injury or necrosis [15], the Troponin I level confirmed immediate myocardial coagulation necrosis caused by laser ablation in the experimental group. Pathological results revealed development of fibrosis in the long term in LGE of CMR [13].

4.1. Safety of TPLA

TPLA was found to be safe in the long term without any severe complications. The global systolic cardiac function such as LVEF and diastolic cardiac function such as E/A and Ea/Aa decreased immediately after the ablation, but gradually recovered to the pre-operative level. TTE demonstrated the real-time imaging of the right ventricular apex to guide the needle entry and laser ablation. One sheep had a small pericardial effusion which resolved spontaneously. None of the sheep developed right ventricular tear or tamponade. Right ventricle is a low-pressure chamber in the absence of pulmonary hypertension. The contraction of the RV myocardium may occlude the small puncture site after withdrawing the needle as the outer diameter of the 21G PTC-needle sheath is 0.82 mm. Thus, the fine needle entry through the RV apex might be a reasonably safe approach. Some of the HCM patients have pulmonary hypertension. In future clinical application of HOCM patients, potential complication of bleeding into the pericardial space might be poorly tolerated due to the underlying ventricular diastolic hypertension. The patients with pulmonary (or RV) hypertension and ventricular diastolic hypertension might require more careful consideration due to possibly higher risk of bleeding from the RV puncture site.

TPLA may also be an alternative to HCM patient with poor indication for surgery. No arrhythmia, LBBB or RBBB were recorded in the 12-month follow-up. Based on our experience, we found that the key to reduce damage to the conduction system is to control the angle and depth of needle insertion during ablation. It is very important to have

a 10 mm safety distance away from the aortic valve cusps to avoid the membranous septum and high-grade conduction delay. Our ablation lesions are at intra-myocardial region, without endocardial necrosis and damage to the intra-ventricular conduction system (located under the endocardium). The laser energy generally creates a restrained lesion with fine borders, and it is relatively easy to estimate and control the ablated area. The bundle of His lies immediately beneath the membranous septum at the crest of the IVS, extending by 4–6 mm beyond the central fibrous body before it bifurcates. The left and right bundle branches are located under the endothelium of the IVS [16]. In this study, it was feasible to create a precise ablation lesion in a healthy animal within an 8–10 mm thickness of the IVS. For patients with HOCM, the average thickness of IVS was generally >20 mm [3], which may leave sufficient space for safe ablation and avoiding damage to the conduction system. The ablation needle should be centered in the septum if possible in spite of the RV entry. Even though the pathology slide showed the scar tissue was more toward the right septum. We believe that in the truly hypertrophied septum, the ablation zone can be centered in the middle of the septum. The avoidance of the sub-aortic region of at least 10 mm is of more importance to protect the conduction system than the centricity of the ablation. The transient ventricular tachycardia in the laser treated sheep was well tolerated and always resolved spontaneously when the laser energy was turned off. There was no ventricular arrhythmia in the control group with needle insertion.

4.2. Comparison of TPLA with the conventional methods

TPLA is a minimally invasive method in which the laser fibre and PTC needle were inserted into the IVS via a transthoracic route without sternotomy. TTE guidance allowed precise identification of the target area. The ablated myocardium is not limited by the vascular anatomy. However, the anatomy of septal perforator artery determines whether ASA is feasible for patients with HOCM. Considering the relatively high occurrence of LBBB after surgical myectomy and RBBB after ASA, TPLA demonstrated safety in avoiding injury to the conduction system. No LBBB or RBBB was found in any animals in the long-term observations in this study. This can be attributed to TTE and ECG monitoring through the entire procedure. Once the ECG showed change in cardiac rhythm, the ablation would pause immediately.

In this study, a minimum distance of approximate 5 mm (ideally 10 mm) under the right aortic valve cusp is mandatory for the laser tip, which is similar to the safe margin in surgical myectomy [18–20]. The eventual thinning of the myocardium after the ablation should be as effective as the septal myectomy and possibly more effective than the ASA due to its larger area of ablation. This is in contrast to the ASA, which aims to ablate the very basal portion of the septum for maximal relief of the LVOT obstruction [21]. Clinicians may need to balance the risk of high grade A-V block and permanent pacemaker implantation vs. not ablating the very basal portion of the hypertrophic septum.

The traditional myectomy and ASA face the technical limitations for midventricular obstruction due to the anatomic considerations. Our method may potentially approach the area of midventricular obstruction in HOCM in the future.

4.3. Limitations

In this study, LVOT peak gradient was at <5 mm Hg before and after the operation, considering the use of healthy animals. At present, mature HCM animal model is limited to small animals such as mice, cat or rabbit [22]. The hearts of these HCM animals were too small for needle puncture and ablation through the current ablation system. The ablative dose for these animals would have been far from a meaningful parameter for the clinical requirements [23]. Additionally, it is difficult to insert the ablation needle into the interventricular septum in some other animal models. The average sized dog we used in the past weighted 15 kg. The sheep we used weighted 50 kg, which was close to the human weights. Sheep hearts are also nearly the size of humans. The intercostal space of sheep is wider than that of pigs and dogs. Its chest wall is relatively thin for ease of the procedure. Occurrence of rhythm disturbance requires prolonged ECG monitoring (Holter ECG), and cannot be assessed based on snapshot ECGs. Implantable loop recorders can also be potentially considered for long term rhythm monitoring. The sample size in our study is small, necessitating further study to optimise the laser dose and long-term reduction in the ablated IVS volume in animals or humans with septal hypertrophy.

5. Conclusion

TPLA sheep model is safe in the long term without causing severe complications or damages to the cardiac function and conduction system. It is efficacious in reducing the IVS thickness and regional wall motion and may therefore potentially reduce the LVOT gradient. This study highlights a new approach to eliminate excess left ventricular septal tissue and provide supporting evidence for laser application in the future clinical trials of HOCM treatment.

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Conflict of interest statement

The authors have no potential conflicts of interest to declare.

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