Anti-arrhythmic and anti-heart failure effects of low-level electrical stimulation on aortic root ventricular ganglionated plexi

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September 2, 2020

Abstract

Background: It remains uncertain whether low-level electrical stimulation (LL-ES) of ventricular ganglionated plexi (GP) improves heart function. Aim: This study investigates the anti-arrhythmic, and heart function improving effects following LL-ES of aortic root ventricular ganglionated plexi (ARVGP). Methods: Thirty dogs were divided randomly into control, drug, and LL-ES groups after performing rapid right ventricular pacing to establish a heart failure (HF) model. The inducing rate of arrhythmia, bioactive factors of HF, including angiotensin II type I receptor (AT-1R), transforming growth factor (TGF-β), matrix metalloproteinase (MMP), and phosphorylated extracellular signal-regulated kinase (p-ERK1/2), left ventricular stroke volume(LVSV) and ejection fraction(LVEF) were measured at baseline, and after treatment with a placebo, drugs, and LL-ES, respectively. Results: The inducing rate of arrhythmia decreased from 80% in the control group to 60% in the drug group, and to 10% after 1 week(w)of LL-ES (P=0.009). The expression of AT-1R, TGF-β, and MMP was down-regulated, whilep-ERK1/2 increased significantly in the LL-ES group (P=0.001, all) compared with drug group. The ventricular effective refractory period (VERP) was prolonged from 139±8 ms in the drug group to 166±13 ms after 1w of LL-ES (P=0.001).Moreover, LVSV increased markedly from 13.16±0.22ml to 16.86±0.27ml after 1 w of LL-ES compared with the drug group (P=0.001), and LVEF increased significantly from 38.48±0.53% to 48.94±0.57% during the same timeframe (P=0.001). Conclusion: Short-term LL-ES of ARVGP had both anti-arrhythmic and anti-inflammatory effects and contributed to the treatment of tachycardia-induced HF and its associated arrhythmia.

Introduction

Heart failure (HF) and arrhythmia share a similar underlying pathogenesis, such as an autonomic imbalance, atrial or ventricular electrical remodeling, and inflammatory reactions [1]. In humans, tachycardia-induced cardiomyopathy often leads to HF,which includes atrial fibrillation (AF), incessant supraventricular tachycardia, frequent ventricular ectopy, and ventricular tachycardia[2]. Traditional pharmacologic therapy does not appear to be efficacious at treating this type of HF. Even the relatively new resynchronization therapy is not as effective in arrhythmic patients. Therefore, a new method eagerly awaited in clinical practice. As autonomic remodeling and inflammation are associated with the initiation and maintenance of HF and AF, suppressing the activity of both elements has been widely debated. Since the ventricle plays a more important role than the atrium when assessing heart function, the modulation of ventricular ganglionated plexi (GP) is thought to be better than atrial GP in promoting heart function. However, aortic root ventricular GP (ARVGP), influences the function of both the ventricle and coronary artery [3]. In addition, a previous study [4] demonstrated that low-level electrical stimulation (LL-ES) of ARVGP also affected the activity of the atrium. In this study, LL-ES attenuated and balanced the tone of the autonomic nervous system (ANS) and thus lessened the inducing rate of AF mediated by the ANS. If LL-ES of ARVGP also shows short-term

effects and has an anti-inflammatory effect on the ventricle, it is plausible to expect that it may benefit both HF and arrhythmia.

Therefore, the present study established a tachycardia-induced HF model by rapid pacing. One week (w) of short-term LL-ES of ARVGP was performed, which was followed by programmed/burst electrical stimulation, immunohistochemical assays, polymerase chain reaction, and Western blotting to investigate the following: (1) the inducing rates of both atrial and ventricular arrhythmia to determine if LL-ES of ARVGP reduces arrhythmic episodes; (2) bioactive factors of HF, such as angiotensin II, transforming growth factor-beta (TGF-β), mitogen-activated protein kinase (MAPK), and phosphorylated extracellular signal-regulated kinase (p-ERK1/2) to explore whether LL-ES of ARVGP suppresses the inflammatory reaction; and (3) the ventricular effective refractory period (VERP),the left ventricular end-diastolic volume(LVEDV) and end-systolic volume(LVESV),the stroke volume of the left ventricle (LVSV), and the left ventricular ejection fraction (LVEF) to demonstrate whether LL-ES of ARVGP contributes to improving heart function.

Material and Methods

Creation of an HF model

This study conforms to the Guide for the Care and Use of Laboratory Animals. All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Jiao Tong University (Xi'an, China).

HF was induced by rapid ventricular pacing to simulate tachycardia-induced cardiomyopathy, as described by Armstrong et al. [5]. Thirty dogs were anesthetized with an IV injection of 3% sodium pentobarbital (30 mg/kg). An extradose was given to maintain anesthesia during this study, if necessary.Five percent glucose in normal saline (500 mL) with penicillin was administered intravenously. An endocardial pacemaker electrode (St. Jude Medical, MN, USA) was inserted using fluoroscopy into the right ventricular apex via the left external jugular vein. A pacemaker generator was implanted into a small subcutaneous pocket created between the scapulas, and the pacemaker lead was connected to the generator through a subcutaneous canal. The pacing threshold was 0.3-1.5 V, the amplitude of the R-wave was 4-10 mV, and the impedance was 0.3-1.0 K. The pacemaker frequency was set at 240 beats per minute with an output voltage of 5.0 V and a pulse width of 0.5 ms one week (w) after the initiation of rapid pacing. An echocardiography and cardiac ultrasound were performed twice with an interval of 24 h to confirm the presence of stable congestive HF. Then, the pacing electrode was extracted.

Drug administration and LL-ES of ARVGP

The HF dogs were anesthetized with an intraperitoneal injection of 3% sodium pentobarbital (30 mg/kg) and then ventilated with room air (DDH-1, NO. 3529 PLA, Henan, China). The right femoral vein was cannulated to infuse normal saline at 100-200 mL/h to replace spontaneous fluid loss. An electrocardiogram lead II was monitored throughout the study. After the chest was opened through a left fourth intercostal thoracotomy, the pericardium was opened and sewn to the chest wall to cradle the heart. A custom electrode with eight metal electrode heads (Henan Huanan Medical Science & Technology Co., Zhengzhou, China) was sewed tightly on the surface of ARVGP to stimulate the neurons. The thirty HF dogs were subsequently divided randomly into control, drug administration, and LL-ES groups, and then the chest was closed. Dogs underwent no treatment in the baseline open chest status. Then different modifications were performed as follows: Dogs in the control group received a placebo (starch,1g,qd) for 1 w,while dogs in the drug group were administered a mixed powder of drugs including metoprolol(6.25mg,bid), perindopril(2mg,qd), furosemide(20mg,bid),spironolactone(20mg,bid), and digoxin(0.125g,qd) for 1w. Simultaneously, dogs in the LL-ES group underwent 12h (immediate) and 1 w (short-term) of LL-ES ARVGP, which was embedded in the adipose tissues surrounding the root of the aorta and connected to the aorta by the mesangial ligament, as described in our previous study [6]. The lowest voltage level that induced any slowing of the sinus rate or atrial ventricular conduction (measured by the A-V interval) was considered as the threshold. Approximately10% below the threshold was then chosen as the voltage for LL-ES. During LL-ES, the sinus rate and A-V interval

were monitored to ensure that the stimulation voltage was below the threshold [7]. The study protocol can be seen in the flow chart (Figure 1).

Measurement of the inducing rate of arrhythmia

Programmed electrical stimulation ($S_1S_2S_3$) and burst pacing (S_1S_1) were used sequentially to induce arrhythmia at baseline, after drug administration, or during LL-ES of ARVGP. Atrial arrhythmia was provoked first by $S_1S_2S_3$at the base of the left atrial appendage, and ventricular arrhythmia was induced at the left ventricular via bipolar screw-in pacing with leads fixed epicardially[8]. Each two-burst pacing protocol was performed with a 10 min interval in-between to allow for recovery from the rapid pacing to occur via atrial remodeling. The programmed stimulation protocol was set at basic cycle lengths of 400 and 300 ms with up to two extra stimuli (S_3). All stimuli were monitored on a 64-channel electrophysiological recorder (Henan Huanan Medical Science & Technology Co., Zhengzhou, China). The first extra stimulus (S_2) was introduced with an S_1-S_2 interval 30 ms longer than the atrial effective refractory period, and the coupling interval was shortened in 10 ms decrements. If the S_1-S_2 extra stimuli failed to induce arrhythmia, a second extra stimulus (S_3) was introduced at 10 ms scanning decrements during an S_1-S_2 interval set at 80% of the basic cycle length. If programmed stimulation failed to induce arrhythmia, burst pacing at a cycle length of 200 ms for 30 seconds was applied to provoke arrhythmia, and the cycle length was subsequently decreased to 160 and 120 ms if 200 ms was ineffective. The above electrical stimulation procedure was repeated twice. A successful endpoint was defined as arrhythmia started either by $S_1S_2S_3$, by a subsequent S_1S_1, or both if either procedure failed. Arrhythmiais defined as atrial or ventricular tachycardia (sustained>10sec), atrial fibrillation (sustained>30sec), or ventricular flutter or fibrillation. If malignant arrhythmia occurred (persistent ventricular tachycardia over 5 min or ventricular flutter or fibrillation), epicardial electrical conversion (50J) was performed immediately to recover a stable internal environment.

Measurement of VERP, stroke volume of the LV, and ejection fraction of the LV

VERP was measured at baseline and after treatment with a placebo, drugs, and LL-ES, respectively. It was recorded using the extra stimulus technique (basic cycle length of 400 ms and final extra stimulus steps of 5 ms; Electrophysiological Recorder, 64 channels, Henan Huanan Medical Science & Technology Co., Zhengzhou, China). Furthermore,transthoracic echocardiography was performed with dogs in the left lateral decubitus position, breathing slowly, using aphased-array probe(Vivid E9, GE, USA). The LV endocardial surface was detected using the Simpson's method from the apical four-chamber view and the apical right heart two-chamber view to measure LVEDV and LVESV. Then, LVSV and LVEF were obtained at baseline and after 1 week of treatment with a placebo, drug, and LL-ES, respectively.

Western blotting

The dogs in the three groups were euthanized at the end of treatment. Their hearts were excised, and the LV was collected. The LV was quickly frozen in liquid nitrogen and stored at -80 until further use. LV tissue was lysed in lysis buffer [Cell Signaling Technologies (CST), MA, USA] containing a protease inhibitor and phosphatase inhibitor cocktail (Thermo Scientific, IL, USA) and was homogenized with beads in a Bullet Blender (Next Advance, NY, USA). After centrifugation at 13,000 x g for 5 min at 4°C, the supernatant was collected, and protein concentrations were determined by a Bradford assay (Cat. 500-0113, Bio-Rad, PA, USA). Equal amounts of protein (90 μL) were mixed with 30 μL of 4X NuPAGE LDS sample buffer (Cat. NP0008, Thermo Fisher, CA, USA) and 15 μL of 10X NuPAGE reducing agent (Cat.NP0009, ThermoFisher, CA, USA), boiled, separated on NuPAGENovex 4%–12% Bis-Tris Protein Gels (Cat. WG1402B, CA, USA), and transferred to nitrocellulose membranes (LiCor, NE, USA) using the NuPAGE electrophoresis system (ThermoFisher, CA, USA). Membranes were blocked using Odyssey blocking buffer (LiCor, NE, USA) for 1 h at room temperature before incubation with primary antibodies overnight. The membranes were then washed with 1X PBST (0.1% Tween 20 in Tris-buffered saline) and incubated with secondary antibody for 1 h at room temperature. The signal was detected using an Odyssey scanner (LiCor, NE, USA). The primary antibodies used were TGF-β, p-ERK1/2), ERK1/2, matrix metalloproteinase-9 (MMP-9), angiotensin II type I receptor

(AT-1R), and glyceraldehyde 3-phosphate dehydrogenase (CST, MA, USA). The secondary antibodies used were goat anti-mouse IRDye 800 (LiCor, NE, USA) and IRDye 680 goat anti-rabbit (Rockland, PA, USA).

Statistical analysis

All data are expressed as a mean±SD. The inducing rate of arrhythmia was compared using the chi-square test. The comparison of TGF-β, p-ERK1/2, MMP-9, and AT-1R as well as VERP, LVEDV,LVESV,LVSV, and LVEF values before and after treatment were evaluated using a repeated measure variance analysis. A P- value of [?] 0.05 was considered statistically significant.

Results

As 12h of LL-ES generally showed negative results, the data is not presented below.

Effect of LL-ES of ARVGP on the inducing rate of arrhythmia

At baseline, there are no significant differences among the three groups (P =1.0).After 1 w of treatment, atrial and ventricular arrhythmic episodes were initiated a total of 8 times in the control group including 4 events of atrial tachycardia, 2 of atrial fibrillation, 1of non-sustained ventricular tachycardia lasting 30-60 sec, and 1of persistent ventricular tachycardia over 5min that caused hemodynamic instability and received electrical conversion. The inducing rate was calculated as 80 %(P =0.71 n=10, Figure 2) compared with baseline. After drug administration for 1w, arrhythmic episodes decreased to 6 events (3 of atrial tachycardia, 2 of atrial fibrillation, and 1 of non-sustained ventricular tachycardia about 7 sec). The inducing rate was 60% (P =0.15, n=10, Figure 2) compared with baseline. However, after 1 w of LL-ES of ARVGP, the number of arrhythmic episodes dropped significantly to only 1 event of atrial tachycardia. The inducing rate decreased to 10% (P =0.003 vs. baseline, P =0.009 vs. drug group, n=10, Figure 2).

Effect of LL-ES of ARVGP on protein expression in the LV

After 1w of drug administration, the TGF-β expression down-regulated from 1.2±0.12 in the control group to 0.99±0.06 (P =0.001, n=10, Figure 3), levels of MMP-9 decreased from 1.37±0.13 to 1.13±0.12(P =0.001, n=10, Figure 3), and AT-1R reduced from1.20±0.73to 0.99±0.15(P =0.001, n=10, Figure 2).While levels of p-ERK1/2were not significantly different compared to the control group(1.14±0.15vs.1.12±0.13; P =0.79, n=10, Figure 3). However, TGF-β protein levels in the LL-ES group decreased significantly from 0.99±0.06 to 0.44±0.07 compared to the drug group, MMP-9 down-regulated significantly from 1.13±0.13 to 0.24±0.07(P =0.001, n=10, Figure 3), and AT-1R reduced significantly from 0.99±0.15 to 0.67±0.10 (P =0.001, n=10, Figure 3). To explore potential signaling pathways by which LL-ES mediated cardiomyocyte protection, p-ERK1/2 was measured. p-ERK1/2 is a well-known stress-activated MAPK that plays a protective role in the cell death signaling pathway. As shown in Figure 2, p-ERK1/2 increased significantly from 1.14±0.15 in the drug group to 2.09±0.13 after LL-ES (P =0.001, n=10, Figure 3). In this figure, GAPDH is being used as a loading control, and the results are from three independent experiments. Additional western blotting data are presented in Figure 4.

Effect of LL-ES of ARVGP on LV function

At baseline, there are no significant differences among the three groups (VERP:P =0.99;LVEDV:P =0.98;LVESV:P =0.95;LVSV:P =0.90;LVEF:P =0.98).VERP increased slightly from 138±6ms in the control group to 139±8ms in the drug group (P =0.85, n=10, Table 1). However, after 1w of LL-ES, VERP significantly increased to 166±13ms compared to the drug group (P =0.001, n=10, Table 1). The LVEDV decreased significantly from 35.39±0.68ml in the control group to 34.20±0.68ml after drug administration (P =0.01, n=10, Table 1),while changed slightly to 34.43±0.66ml after 1 w of LL-ES of ARVGP compared with drug group(P =0.45, n=10, Table 1).Likewise, the LVESV decreased markedly from 22.46±0.51mlin the control group to 21.04±0.54ml after drug administration(P =0.001, n=10, Table 1),and also decreased significantly to 17.57±0.47ml after 1 w of LL-ES of ARVGP compared with drug group(P =0.001, n=10, Table 1).Therefore, the LVSV was calculated as 13.03±0.30ml in the control group and remain nearly constant to 13.16±0.22ml after drug administration(P =0.28, n=10, Table 1) but increased significantly to 16.86±0.27ml

after 1 w of LL-ES of ARVGP compared with drug group(P =0.001, n=10, Table 1).As a result, LVEF rising from 36.81±0.66% in the control group to 38.48±0.53%after drug administration (P =0.001, n=10, Table 1). However, after 1 w of LL-ES of ARVGP, the LVEF increased significantly to 48.94±0.57% compared with the drug group (P =0.001, n=10, Table 1).

Discussion

The main findings of this study were that short-term LL-ES of ARVGP provided both anti-arrhythmia and anti-inflammation benefits, and therefore improved cardiac function in the short-term. In addition, compared to the drug group, TGF-β, MMP-9, and AT-1R protein levels in the LV decreased significantly after LL-ES, whereas p-ERK1/2 levels significantly increased. It is well known that the p-ERK signaling pathway is a protein kinase subfamily in the heart that provides a protective effect. Other subfamilies include the p38 and jun N-terminal kinases, which may play an opposite role in the heart. For this reason, they were not chosen for examination in this study. This study demonstrated that p-ERK could be activated by LL-ES, which suggests that LL-ES of ARVGP may play a protective role for the heart through the p-ERK signaling pathway.

Although TGF-β, MMP-9, and AT-1R protein levels reduced significantly after 1w of traditional drug administration compared with the control group, it did not improve heart function as well as expected. This conformed to the present clinical status that traditional drugs showed an obvious limitation in the treatment of tachycardia-induced HF. However, 12 h of immediate LL-ES did not promote heart function well because 1w of tachycardia-induced HF resulted in relatively persistent electrical remodeling, which was hard to reverse in such a short time.

Increased sympathetic nerve activity and reduced vagal cardiac tone have been demonstrated to be pathogenic in HF or AF [9]. With the shortening of VERP following an imbalanced ANS tone, premature ventricular beats or tachycardia can occur easily and thus facilitate HF[10].However, our results demonstrated a prolongation of VERP and rebalancing of ANS tone. As a result, episodes of ventricular arrhythmia decreased after LL-ES of ARVGP. In addition, an inflammatory reaction is involved in both the initiation and maintenance of HF and AF [11]. Therefore, current practices are expected to improve both HF and arrhythmia by reversing the imbalanced tone of the ANS and inhibiting the activity of inflammatory factors.

Vagal nerve electrical stimulation has been widely used to control HF and AF [12, 13]. Side effects commonly include neck pain, coughing, difficulty in swallowing, voice alteration, nausea, and indigestion, which limit the application of vagal nerve modification [14]. Recently, several studies demonstrated that LL-ES of local atrial GP was effective in suppressing AF and inflammatory reactions [15, 16]. However, our study demonstrated that LL-ES of ARVGP also resulted in anti-arrhythmia and anti-inflammation effects that were restricted to the heart, thus avoiding the side effects of vagal nerve stimulation. Moreover, 1 w of LL-ES improved the LVEF, reduced LV size, and reversed the acute structural and electrical remodeling of the heart. As a result, the electrical remodeling caused by rapid pacing was reversed. A potential mechanism for these effects lies in the significant suppression of stellate ganglion nerve activity and sympathetic nerve density [17]. In conclusion, short-term LL-ES of ARVGP may be a better choice than traditional drugs for treating tachycardia-induced HF and associated arrhythmia.

Clinical perspectives

As described in Yu et al. [18], non-invasive approaches that were applied clinically to treat HF or AF claimed that the incidence of reperfusion-related ventricular arrhythmia was significantly attenuated by LL-ES of the right tragus. Their study [19] also reported that a stimulator could be implanted through minimally invasive surgery to modulate cardiac sympathetic ganglia and monitored using a mobile phone with Bluetooth to achieve non-invasive and reversible regulation of the cardiac sympathetic nerves. Therefore, ARVGP could potentially be modified via this implantable stimulator. Any clinical application considerations should be based on true long-term data. Though long-term data can be challenging to collect in an open chest canine model, long-term LL-ES may be applied in humans, since the thoracoscopic approach is an easy way to perform LL-ES of ARVGP. Therefore, patients suffering from HF and associated arrhythmias should expect

to have better treatment in the future.

Study limitation

(1) The degree of myocardial fibrosis was not measured when examining the deterioration of both HF and arrhythmia. (2)Heart rate-based heart failure induction differs in some respects from the pathophysiologic factors underlying HF in humans. (3)The ischemic model of HF is more prevalent clinically and requires further review.

Conflict of interest

None

Ethics

All animals received humane care, and study protocols complied with the institution's guidelines.

REFERENCES

1. Chen Z, Purdon PL, Pierce ET, Harrell G, Walsh J, Salazar AF.Linear and nonlinear quantification of respiratory sinus arrhythmia during propofol general anesthesia. Conf Proc IEEE Eng. Med Biol Soc.2009; 2009:5336-5339.
2. Gopinathannair R, Etheridge SP, Marchlinski FE, Spinale FG, Lakkireddy D, Olshansky B. Arrhythmia-Induced Cardiomyopathies: Mechanisms, Recognition, and Management. J Am Coll Cardiol. 2015; 13; 66:1714-28.
3. Yuan BX, Ardell JL, Hopkins DA, Losier AM, Armour JA. Gross and microscopic anatomy of the canine intrinsic cardiac nervous system. Anat Rec. 1994; 239: 75-87.
4. Hong-Tao Wang, Ming Xu, Xiong-Tao Liu, Fei-Fei Su, Di Zeng,Qiang-Sun Zheng. Low-level electrical stimulation of aortic root ventricular ganglionated plexi attenuates autonomic nervous system-mediated atrial fibrillation. JACC Clin Electrophysiol. 2015; 1:390-397.
5. Armstrong PW, Stopps TP, Ford SE, de Bold AJ. Rapid ventricular pacing in the dog: pathophysiologic studies of heart failure.Circulation. 1986; 74:1075-1084.
6. Wang HT, Li ZL, Fan BY, Fei-Fei Su, Jin-Bo Zhao, Jun Ren,Qiang-Sun Zheng. The independent role of the aortic root ganglionated plexi in the initiation of atrial fibrillation: An experimental study. J Thorac Cardiovasc Surg. 2014; 148:73-76.
7. Chen M, Zhou X, Liu Q, Sheng X, Yu L, Wang Z, Zhou S. Left-sided Noninvasive Vagus Nerve Stimulation Suppresses Atrial Fibrillation by Up-regulating Atrial Gap Junctions in Canines. J Cardiovasc Pharmacol. 2015; 66:593-599.
8. Zhang Z, Zhang C, Wang HT, Zhao JB, Liu L, Lee J, et al. N-3 polyunsaturated fatty acidsprevents atrial fibrillation by inhibiting inflammation in a caninesterile pericarditis model. Int J Cardiol. 2011;153:14-20.
9. Linz D, Ukena C, Mahfoud F, Neuberger HR,Bohm M. Atrial autonomic innervation: a target for interventional anti-arrhythmic therapy? J Am Coll Cardiol.2014; 63:215-224.
10. Bradfield JS, Ajijola OA, Vaseghi M, Shivkumar K. Mechanisms and management of refractory ventricular arrhythmias in the age of autonomic modulation. Heart Rhythm. 2018; 15:1252-1260.
11. Fujiu K, Wang J, Nagai R. Cardioprotective function of cardiac macrophages.Cardiovasc Res. 2014; 102:232-239.
12. Schwartz PJ, De Ferrari GM. Sympatheticparasympathetic interaction in health and disease: abnormalities and relevance in heart failure. Heart Fail Rev. 2011; 16:101-107.
13. Floras JS. Sympathetic nervous system activation in human heart failure: clinical implications of an updated model. J Am Coll Cardiol.2009; 54:375-385.
14. Milby AH, Halpern CH, Baltuch GH. Vagus nerve stimulation for epilepsy and depression.Neurotherapeutics. 2008; 5:75-85.
15. He B, Lu Z, He W, Huang B, Jiang H.Low-intensity atrial ganglionated plexi stimulation decreases the serum level of inflammatory factors in canine. Heart Lung Circ. 2015; 24:407-410.

16. Stavrakis S, Nakagawa H, Po SS, Scherlag BJ, Lazzara R, Jackman WM. The role of the autonomic ganglia in atrial fibrillation. JACC Clin Electrophysiol. 2015; 1:1-13.
17. Shen MJ, Shinohara T, Park HW, Frick K, Ice DS, Choi EK, Han S, Maruyama M, Sharma R, Shen C, Fishbein MC, Chen LS, Lopshire JC, Zipes DP, Lin SF, Chen PS. Continuous low-level vagus nerve stimulation reduces stellate ganglion nerve activity and paroxysmal atrial tachyarrhythmias in ambulatory canines.Circulation. 2011; 123:2204-12.
18. Yu L, Huang B, Po SS,Tan T, Wang M, Zhou L, Meng G, Yuan S, Zhou X, Li X, Wang Z, Wang S, Jiang H.Low-Level Tragus Stimulation for the Treatment of Ischemia and Reperfusion Injury in Patients with ST-Segment Elevation Myocardial Infarction: A Proof-of-Concept Study. JACC Cardiovasc Interv. 2017; 10: 1511-1520.
19. Yu L, Zhou L, Cao G, Posnack NG, Mendelowitz D, Kay MW.Optogenetic Modulation of Cardiac Sympathetic Nerve Activity to Prevent Ventricular Arrhythmias.J Am Coll Cardiol. 2017; 70:2778-2790.

Table 1: Change of LV function after LL-ES ARVGP(mean±SD)

	Control	Drug	LL-ES	*P*
VERP(ms)	138±6	139±8	$166\pm13^{+++}$	0.001;0.001
LVEDV(ml)	35.39±0.68	$34.20\pm0.68^{+}$	$34.43\pm0.66^{+}$	0.004;0.453
LVESV(ml)	22.46±0.51	$21.04\pm0.54^{+}$	$17.57\pm0.47^{+++}$	0.001;0.001
LVSV(ml)	13.03±0.30	13.16±0.22	$16.86\pm0.27^{+++}$	0.001;0.001
LVEF (%)	36.81±0.66	$38.48\pm0.53^{+}$	$48.94\pm0.57^{+++}$	0.001;0.001

^{+}P <0.05 vs. Control.^{++}P <0.05 vs. Drug. The first *P*value indicates LL-ES vs.control, and the second *P* value indicates LL-ES vs. Drug. VERP=ventricular effective refractory period; LVEDV=left ventricular end-diastolic volume; LVESV=left ventricular end-systolic volume; LVSV=left ventricular stroke volume; LVEF=left ventricular ejection fraction.

Figure legends:

Figure 1: Flow chart of the study protocol

HF=Heart failure; LL-ES=Low-level electrical stimulation; ARVGP=aortic root ventricular ganglionated plexi; TG-β=transforming growth factor-beta; MMP-9=matrix metalloproteinase-9; AT-1R=angiotensin II type I receptor; p-ERK1/2=phosphorylated extracellular signal-regulated kinase; VERP=ventricular effective refractory period; LVEDV=left ventricular end-diastolic volume; LVESV=left ventricular end-systolic volume; LVSV=left ventricular stroke volume; LVEF=left ventricular ejection fraction.

Figure 2: Comparison of arrhythmic incidences before and after LL-ES ARVGP

At baseline HF status, the inducing rate of arrhythmia presented almost no difference among the control, drug, and LL-ES groups. However, after 1 week of treatment, the inducing rate of arrhythmia remained at 80% in the control group but dropped to 60 % in the drug group and to 10 % in the LL-ES group.

Abbreviations see figure 1.

Figure 3: Change of protein levels in HF factors after LL-ES ARVGP

A: Compared with the control group, TGF-β, MMP-9, and AT-1R protein levels decreased significantly while the expression of p-ERK1/2 was not significantly different after 1 week of drug administration. However, compared with the drug group, TGF-β, MMP-9, and AT-1R protein levels reduced significantly, and the expression of p-ERK1/2 increased remarkably after 1 week of LL-ES ARVGP.

Abbreviations see figure 1.

Figure 4: Effect of LL-ES of ARVGP on protein expression in the LV

Compared with drug group, TGF-β, MMP-9, and AT-1R protein expression levelsdown-regulated significantly whilethe level of p-ERK1/2 increased remarkedly after 1 week of LL-ES ARVGP. GAPDH was used as a loading control.

Abbreviations see figure 1.