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ORIGINAL ARTICLE

Non-invasive tragus stimulation improves cardiac post-ischemic remodeling by regulating cardiac parasympathetic activity

Mingxian Chen[1], Hui Chen[2], Zhen Wang[2], Yuchen Pan[2], Huihui Hu[2], Songyun Wang[2], Yuan Yuan[3], Zhuo Wang[2]* and Hong Jiang[2]*

[1]*Department of Cardiology, The Second Xiangya Hospital of Central South University, Changsha, China;* [2]*Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan, China; and* [3]*Cardiovascular Center, Liyuan Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*

Abstract

Aims Our previous study proved that low-level tragus nerve stimulation (LL-TS) could improve left ventricular remodelling by cardiac down-stream mechanisms. However, the cardiac up-stream mechanisms remain unknown.

Methods and results Twenty-eight adult beagle dogs were randomly divided into an MI group (myocardial infarction was induced by permanent ligation of the left coronary artery, *n* = 10), an LL-TS group (MI plus intermittent LL-TS treatment, *n* = 10), and a control group (sham ligation with the same stimulation as the LL-TS group, *n* = 8). Auricular tragus nerve was bilaterally delivered to the tragus via ear-clips connected to a custom-made stimulator. The voltage slowing sinus rate was used as the threshold to set the LL-TS 80% below this level. At the end of 4 weeks post-MI, LL-TS could significantly increase atrial ganglion plex (GP) activity, decreased left stellate ganglion (LSG) activity, reduced LV dilation, and improved ventricular functions. Chronic intermittent LL-TS treatment significantly attenuated left ventricular remodelling via the up-regulation of α7nAChR expression and the down-regulation of MMP-9 level in post-MI LV tissue. The elevated protein and mRNA of MMP-9 levels in remote areas were significantly ameliorated by LL-TS treatment.

Conclusions Chronic LL-TS increased GP neural activity and improved ventricular remodelling possibly via α7nAChR/MMP-9 axis.

Keywords α7 nicotinic acetylcholine receptor; Matrix metallopeptidase 9; Tragus nerve stimulation; Ventricular remodelling

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**Correspondence to: Zhuo Wang and Hong Jiang, Department of Cardiology, Renmin Hospital of Wuhan University, No. 238 Jiefang Road, Wuchang District, Wuhan, Hubei Province 430060, China. Email: whuwangzhuo@163.com; whujianghong@163.com*

Introduction

Left ventricular (LV) post-ischaemic remodelling is a process of infarct expansion followed by non-infarcted myocardial hypertrophy and progressive LV dilation,[1] which is associated with adverse clinical outcomes.[2] Despite reperfusion and medicinal therapies, patients who survived an acute MI still harboured a significant long-term risk of sudden cardiac death because of the LV remodelling promoted by enhanced sympathetic activity.[3] Autonomic dysfunction, which is characterized by sympathetic activation and vagal withdrawal, has traditionally been recognized as the promoter of LV remodelling after myocardial infarction (MI).[4,5] Vagus nerve stimulation (VNS) has previously been shown to be cardio-protective in MI and heart failure via the amelioration of LV remodelling.[6–8] Our previous research demonstrated that low-level tragus nerve stimulation (LL-TS) was a novel non-invasive vagus nerve stimulation without implantation of a neurostimulator system and protected heart against cardiovascular diseases.[9,10] And LL-TS was capable of suppressing left stellate ganglion (LSG) activity[11] and enhancing the neural activity of the anterior right ganglionated plexi (ARGP) in a canine model.[9] However, the autonomic mechanisms by which the LL-TS mediates LV remodelling remains unclear.

The α7 nicotinic acetylcholine receptor (α7nAChR) plays critical roles in the nervous system and in the cholinergic inflammatory pathway.[12] Matrix metalloproteinase 9 (MMP-9) plays key roles in the progression of post-MI remodelling.[13] Previous study demonstrated that ACh effectively inhibited MMP-9 via α7nAChR-JAK2/STAT3 pathway.[14] So, in this research, we hypothesized that the possible mechanisms LL-TS treatment is the regulation of the α7nAChR/MMP-9 axis.

Methods

Animals

The study protocol was approved by the Ethical Committee of Wuhan University. All animal handling was performed in accordance with the Wuhan Directive for Animal Research and the current Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85-23, revised 1996). All surgery was performed under sodium pentobarbital anaesthesia, and every effort was made to minimize suffering.

Experiment protocol

Twenty-eight adult male beagle dogs (body weight, 10 to 15 kg) were supplied by the experimental animal centre of the Medical College of Wuhan University. The animals were randomly divided into an MI group (MI was induced by permanent ligation of the left coronary artery, $n = 10$), an LL-TS group (MI plus intermittent LL-TS, $n = 10$), and a control group (sham ligation with the same stimulation as the LL-TS group, $n = 8$). The LL-TS treatment was initiated 3 h after the induction of MI and lasted 2 h. Starting the following day, 4 h of stimulation was administered from 7–9 AM and 4–6 PM daily for 4 weeks. No other background therapy was provided.

MI induction

All dogs were anaesthetised with 40 mg/kg of Na-pentobarbital and ventilated with room air using a positive pressure respirator (MAO01746, Harvard Apparatus Holliston, USA). Additional maintenance doses of 2 mg/kg of Na-pentobarbital were administered at the end of each hour during the procedure. Normal saline at 50 to 100 mL/h was infused to replace spontaneous fluid loss. A body surface electrocardiogram (ECG) was recorded with subcutaneous needle electrodes during the entire procedure using a computer-based Lab System (Lead 7000, Jingjiang, Inc., China). A heating pad was used to maintain the core body temperature of the dogs at 36.5 ± 1.5°C. A thoracotomy was performed through the left fifth intercostal space. A Harris two-stage occlusion[15] was performed on the left anterior descending coronary artery with 3-0 silk ligament above the first diagonal branch to produce MI; all major diagonal branches were ligated to decrease collateral flow to the infarct area. The vessel was partially occluded for 20 min and then completely tied off. LAD artery occlusion was displayed on the ECG as ST segment elevation. Following 3 h of observation, the chest was closed in layers, and the pneumothorax was reduced. Antibiotics were administered for 3 days after surgery.

Low-level tragus nerve stimulation

Tragus stimulation (auricular VNS) was delivered to the tragus bilaterally in the external auditory canal using ear-clips connected to a custom-made stimulator as previously described.[16] Namely, a 20-Hz frequency with a pulse width of 1 ms was applied with duty cycles of 5 s on and 5 s off. Initially, incremental voltages were applied to the tragus until a slowing of the sinus rate was observed (at least 50 ms prolongation of R-R interval). The voltage necessary to slow the sinus rate was used as the threshold, and the LL-TS was delivered at 80% below this level. The actual electrical voltage was in the range of 16 to 24 V, which did not cause changes in heart rate or result in serious adverse reactions.

Neural recording in left stellate ganglion and anterior right ganglion plexus

Neural activity from the LSG and anterior right ganglion plex (ARGP) was recorded for 5 min at baseline before ischaemic induction and 4 weeks post-MI canine model. And then the best 1 min segment was analysed at each time point. Tungsten-coated microelectrodes were inserted into the fascia of the LSG or ARGP, and a ground lead was connected to the chest wall. Electrical signals generated by the LSG or ARGP were recorded with a Power Lab data acquisition system (product 8/35, AD Instruments, Sydney, Australia) and amplified (product DP-304, Warner Instruments, Hamden, Connecticut) with band-pass filters set at 300 to 1 kHz and with an amplification range of 30 to 50 times. The neural activity, characterized by the recorded amplitude and frequency, was defined as deflections with a signal-to-noise ratio greater than 3:1 and manually determined as described in our previous studies. All data collections were performed by the same technician. They were measured and analysed blindly by another researcher.

Measurements of heart rate variability (HRV)

HRV was used to represent the cardiac autonomic activity. The autoregressive algorithm was used for frequency domain

analysis from the ECG recordings. LF (0.04–0.15 Hz, potentially correlated with sympathetic tone or autonomic balance) and HF (0.15–0.4 Hz, a marker of the parasympathetic tone) were expressed in normalized units, and the LF/HF ratio (index of the interaction between sympathetic and vagal activities) was calculated. Quantitative HRV analysis was performed with 5 min ECG recorded segments that were obtained at baseline and 4 weeks after MI according to the guidelines of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. The raw ECG data were extracted from the telemetric recording using custom software (BI9800 Biomedical Instruments, China) and edited to manually remove technical and/or physiological artefacts.[5]

Blood sampling

Blood samples collection was performed in a dedicated conscious testing room, where dogs were lying on a coach in a quiet, dim-lighted environment. After a stabilization period of 15 min, venous blood samples were collected in ice-chilled tubes coated with EDTA (BD Vacutainer K2E, BD Diagnostics, NJ, USA) on the day before the surgery and the 1st and 28th day after surgery. The plasma was separated by centrifugation at 3000 rpm for 15 min at 4°C and stored at −80°C until assayed. The creatine kinase myocardial bound isoenzyme (CK-MB) and high-sensitive cardiac troponin I (hs-cTnI) levels were determined using a canine-specific high-sensitivity enzyme-linked immunosorbent assay kit (Nanjing Jiancheng, Bioengineering Institute, Nanjing, China, and ELK Biotechnology CO., Ltd, Wuhan, China).

Echocardiographic evaluation of LV function

Transthoracic Doppler echocardiography was performed at baseline and 4 weeks after MI on conscious dogs that laid in lateral recumbency with the use of the high-resolution imaging system Vivid E9 (GE healthcare, USA). Doppler echocardiography was conducted under continuous ECG monitoring with a 3.5-MHz electronic probe. Images were obtained according to echocardiographic criteria and recorded in the computer for subsequent analysis, as previous used.[16] The LV length and endocardial borders were measured at end-diastole and end-systole using the bidimensional right long axis view, which best presents the LV cavity. The end-systolic LV volumes (ESV), end-diastolic LV volumes (EDV), and ejection fraction (LVEF) were calculated using a Simpson's biplane equation. The velocity waveforms of the peak mitral inflow velocity in early diastole (E) and the peak mitral inflow velocity during left atrial contraction (A) were measured. The ratio of E to A was calculated. All parameters were measured for at least three random end-expiratory beats and evaluated by the same experienced echocardiographer who was not involved in the actual echocardiographic recordings and was blinded to the intervention.

Western blotting

Transmural myocardial tissue samples, which were approximately 1 cm^2 and obtained from the non-infarcted zone, were homogenized in 200 μL of ice-cold lysis buffer and 2 μL of phenylmethylsulfonyl fluoride and centrifuged at 15 000 rpm for 10 min at 4°C. The supernatants were subsequently collected. The samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (10% polyacrylamide) and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). Tissue inhibitors of α7nAChR and MMP-9 were identified using a polyclonal rabbit anti-dog α7nAChR antibody (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and a polyclonal goat anti-dog MMP-9 antibody (1:500; Santa Cruz Biotechnology), respectively. A monoclonal antibody against β-actin (Santa Cruz Biotechnology) was used in each experiment as the internal control. The band densities were measured with Quantity One (Bio-Rad Laboratories, Inc., Shanghai, China). The experiments were performed in triplicate.

Real-time quantitative polymerase chain reaction (RT-qPCR) analysis

The mRNA expression levels of MMP-9 were analysed by RT-qPCR. The total RNA was isolated from homogenized LV non-infarcted myocardial tissue using a Trizol kit (15596-026, Invitrogen, Life Technologies). RT-qPCR was performed on an ABI PRISM 7000 sequence detection system (Biosystems, CA, USA) with SYBR Green PCR master mix (Biosystems). All procedures were performed strictly in accordance with the manufacturer's instructions. In brief, PCR was initiated by denaturation at 94°C for 30 min, and the complementary DNA products were amplified for 50 PCR cycles that comprised denaturation for 10 s at 95°C, annealing for 30 s and extension for 20 s at 72°C. The endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene was used as the internal standard to normalize the differences in total RNA levels. The threshold cycle (Ct) value of each marker was subtracted from the Ct value of glyceraldehyde 3-phosphate dehydrogenase to provide the difference in Ct (ΔCt). The normalized expression of each marker was calculated as $2^{-\Delta Ct}$. To minimize variability because of human error, all RT-qPCR analyses were performed by the same operator.

The primer sequences for each gene (Invitrogen Biotechnology, China) are listed as follows: the upstream primer for MMP-9 was 5′-CAGTTTGGTGTTAGGGAGCACG-3′, and the downstream primer was 5′-CCCAGAGTCCATAACTCCTCGTC-

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3′. For GAPDH, the forward primer was 5′-CACGGCAAATTCCACGGCACAGT-3′, and the reverse primer was 5′-GGGGGCATCAGCAGAAGGAGCAG-3′.

Data analysis

All data from each group are reported as the mean ± SD and were compared using one-way ANOVA, followed by a Dunnett's *t* test with GraphPad Prism Version 8 (GraphPad Software, Inc., La Jolla, CA). All statistical tests were two-sided, and a $P < 0.05$ was required for statistical significance.

Results

The average stimulation threshold, which was required to induce any slowing of the sinus rate was 9.7 ± 3.3 V. The heart rate was maintained at a stable level without significant change during 4 weeks of LL-TS. There was no significant change in voltage levels of tragus stimulation over 4 weeks, indicating that there was no injury of the tragus. There were also no significant differences in the sinus rate among the four groups at baseline and after 4 weeks follow-up.

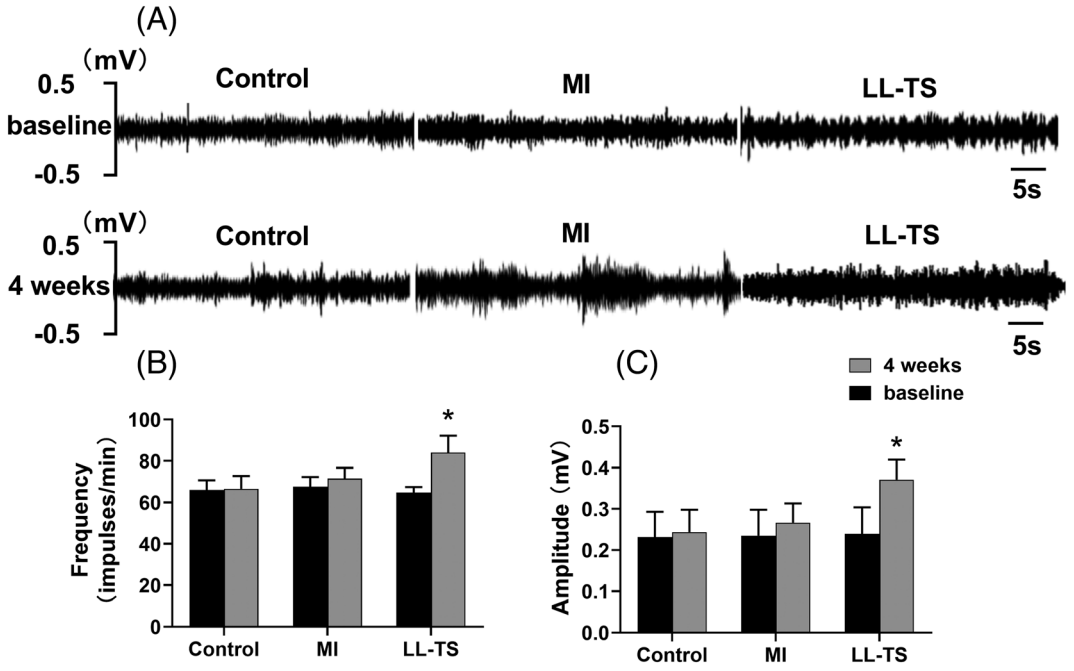
LL-TS increased the ARGP neural activity

The frequency and amplitude of the neural activity directly recorded from the ARGP were significantly increased after 4 weeks of post-MI by LL-TS treatment in contrast to the baseline state (*Figure 1*). *Figure 1A* shows a typical example of neural recordings from the ARGP before and after 4 weeks of post-MI among three groups. There are not significant differences in the ARGP neural activities between baseline and 4 weeks of post MI in control and MI groups. However, in the LL-TS group, the mean values of frequency and amplitude of ARGP neural activity at baseline were 64.8 ± 2.7 impulses per minute and 0.24 ± 0.06 mV, which were significantly increased to 84.0 ± 8.2 impulses per minute and 0.37 ± 0.06 mV (all $P < 0.05$), after 4 weeks of post-MI (*Figure 1B,C*). In contrast, the mean values of frequency and amplitude of ARGP neural activity were not altered by 1 hour of sham LL-TS.

LL-TS attenuated the activation of LSG neural activity

Figure 2 shows the typical examples of LSG neural activity record at baseline and at 4 weeks follow-up among three groups. No significant differences were presented among

Figure 1 Neural recording from ARGP. (A) Representative examples of ARGP neural activity in the control group, MI group and LL-TS group. (B) The average frequency of ARGP activity were significantly increased in LL-TS group. (C) The average amplitude of ARGP activity were significantly increased in LL-TS group. *$P < 0.05$ compared with the baseline. ARGP, anterior right ganglion plexus; LL-TS, low-level tragus nerve stimulation; MI, myocardial infarction.



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groups at baseline. There is no significant changes at 4 weeks in the control group (frequency: 68.4 ± 3.2 impulses/min vs. 70.0 ± 4.5 impulses/min; amplitude: 0.91 ± 0.05 mV vs. 0.94 ± 0.10 mV; both: $P > 0.05$), whereas a significant increase was shown in MI group (frequency: 67.8 ± 5.5 impulses/min vs. 91.1 ± 7.7 impulses/min; amplitude: 0.95 ± 0.08 mV vs. 1.36 ± 0.08 mV; both: $P < 0.05$), and a slight but not significant change was shown in the LL-TS group (frequency: 67.5 ± 4.47 impulses/min vs. 471.4 ± 5.77 impulses/min; amplitude: 0.94 ± 0.09 mV vs. 1.04 ± 0.07 mV, both $P > 0.05$).

Effect of LL-TS on HRV

The HRV has been shown to indirectly estimate cardiac autonomic activity. LF is a marker that indicates the sympathetic tone, HF is a marker that represents parasympathetic nervous activity, and LF/HF ratio is a marker that indicates the sympatho-vagal balance. All dogs completed the entire four-week experimental protocol. The HRV analysis is shown in *Figure 3*. There were no differences in the changes of LF, HF or the LF/HF ratio in the control group. Compared with the control group, the MI group significantly increased the LF power and LF/HF ratio but slightly reduced HF power. However, LL-TS treatment significantly reduced the increasing trends in the LF/HF ratio but increased HF. It indicated that MI significantly increased the cardiac sympathetic activity and inhibited parasympathetic nervous activity; however, LL-TS significantly increased parasympathetic nervous activity and attenuated the activation of cardiac sympathetic activity.

Effects of LL-TS on plasma CK-MB and hs-cTnI

Plasma levels of CK-MB and hs-cTnI increased significantly on the first day after MI in both MI and LL-TS group; LL-TS therapy significantly reduced the CK-MB and hs-cTnI level at the first day and markedly attenuated the increase trend on the 28th day compared with MI group. (*Figure 4*, both $P < 0.05$).

LL-TS improved cardiac function

Echocardiographic outcomes obtained at baseline and 4 weeks after MI are presented in *Figure 5*. The MI group demonstrated a significant increase in the ESV and EDV compared with the control group (both $P < 0.01$). The MI group experienced deterioration in LV systolic and diastolic functions as evidenced by a decrease in the LVEF and the ratio of the peak mitral inflow velocity in early diastole (E)/peak

Figure 2 Neural recording from LSG. (A) Representative examples of LSG neural activity in the control group, MI group and LL-TS group. (B) The average frequency of LSG activity were significantly increased in MI group, while no significant change was observed in LL-TS group. (C) The average amplitude of LSG activity were significantly increased in AMI group, while no significant change was observed in LL-TS group. *$P < 0.05$ compared with the baseline. LL-TS, low-level tragus nerve stimulation; LSG, left stellate ganglion; MI, myocardial infarction.

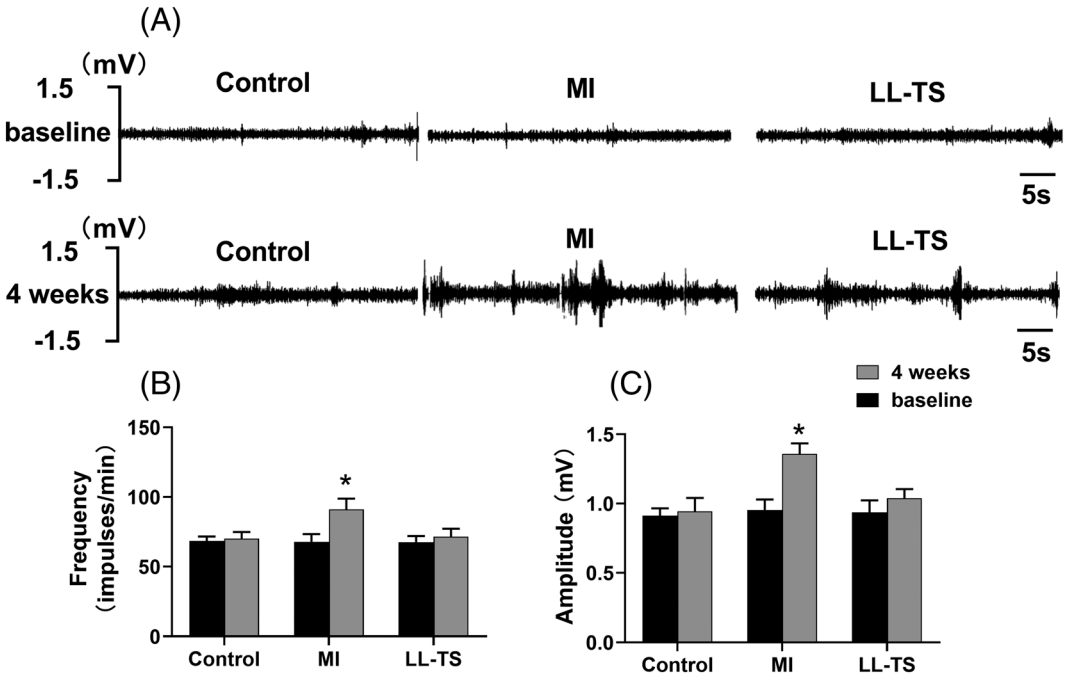

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Figure 3 Indices of HRV analysis at the end of 4 weeks. Compared with the control group, the MI group significantly increased the LF power and LF/HF ratio but slightly reduced HF power. However, LL-TS treatment significantly reduced the increasing trends in the LF/HF ratio but increased HF. *$P < 0.05$ vs. group baseline. LF, low frequency; HF, high frequency.

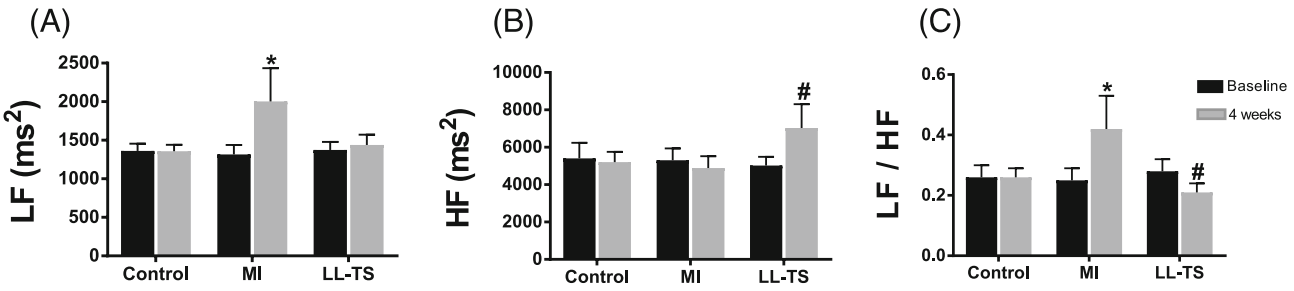
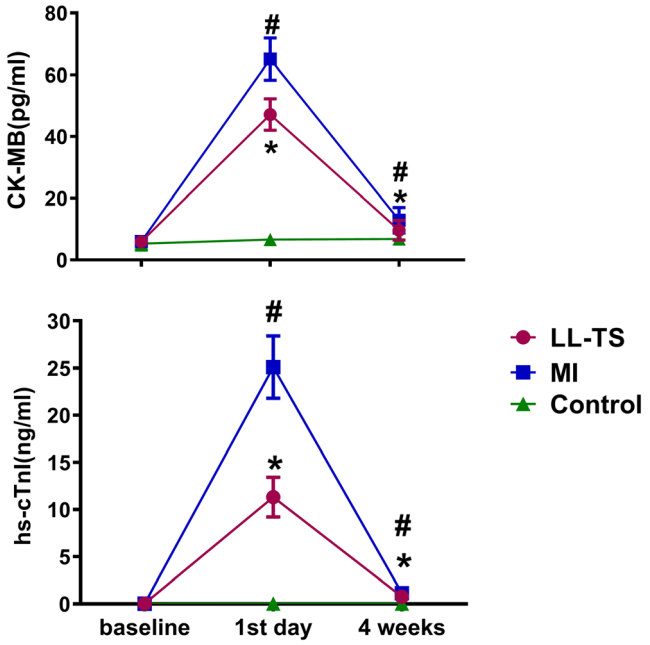


Figure 4 Plasma levels of CK-MB and hs-cTnI on the day before the surgery and 1st and 28th day after surgery. CK-MB, creatine kinase myocardial bound isoenzyme; hs-cTnI, High-sensitive cardiac troponin I. *$P < 0.05$ vs. control group; #$P < 0.05$ vs. LL-TS group.



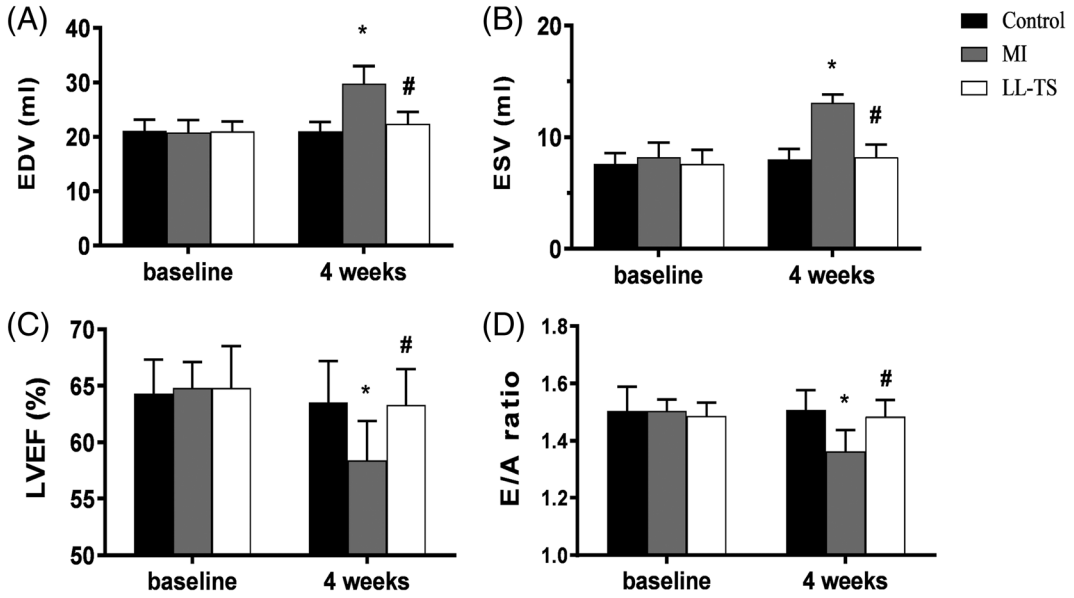
mitral inflow velocity during left atrial contraction (A) (both $P < 0.01$). Intermittent long-term LL-TS treatment attenuated the increase in the ESV and EDV. Moreover, LL-TS significantly prevented improvement in the LVEF and E/A ratio (all $P < 0.01$) as shown in *Figure 5*.

Expression of MMP-9 and α7nAChR in heart tissues

The MMP-9 and α7nAChR protein levels in the LV non-infarcted tissue are shown in *Figure 6*. The protein and mRNA expression levels of MMP-9 were significantly increased in the MI group compared with the control group ($P < 0.01$). However, LL-TS significantly reduced the increase in the MMP-9 protein and mRNA levels ($P < 0.01$).

As also shown in *Figure 6*, α7nAChR expression was investigated in the non-infarcted zone. The α7nAChR protein expression was significantly lower in the MI group compared with control group ($P < 0.01$), but LL-TS significantly increased α7nAChR protein expression compared with the control group ($P < 0.01$).

Figure 5 Transthoracic echocardiographic evaluation at baseline and 4 weeks after the induction of MI. All results are presented as the mean ± SD. The upper part shows echocardiographic photos. The EDV and ESV were significantly higher, and the LVEF and E/A were significant lower in the MI group compared with the control group. LL-TS treatment significantly reduced the EDV and ESV dilation and increased the LVEF and E/A level compared with the MI group. EDV, left ventricular end-diastolic volume; ESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; E/A, peak mitral inflow velocity in early diastole (E)/peak mitral inflow velocity during left atrial contraction (A). *$P < 0.01$ vs. control group; #$P < 0.01$ vs. MI group.



Discussion

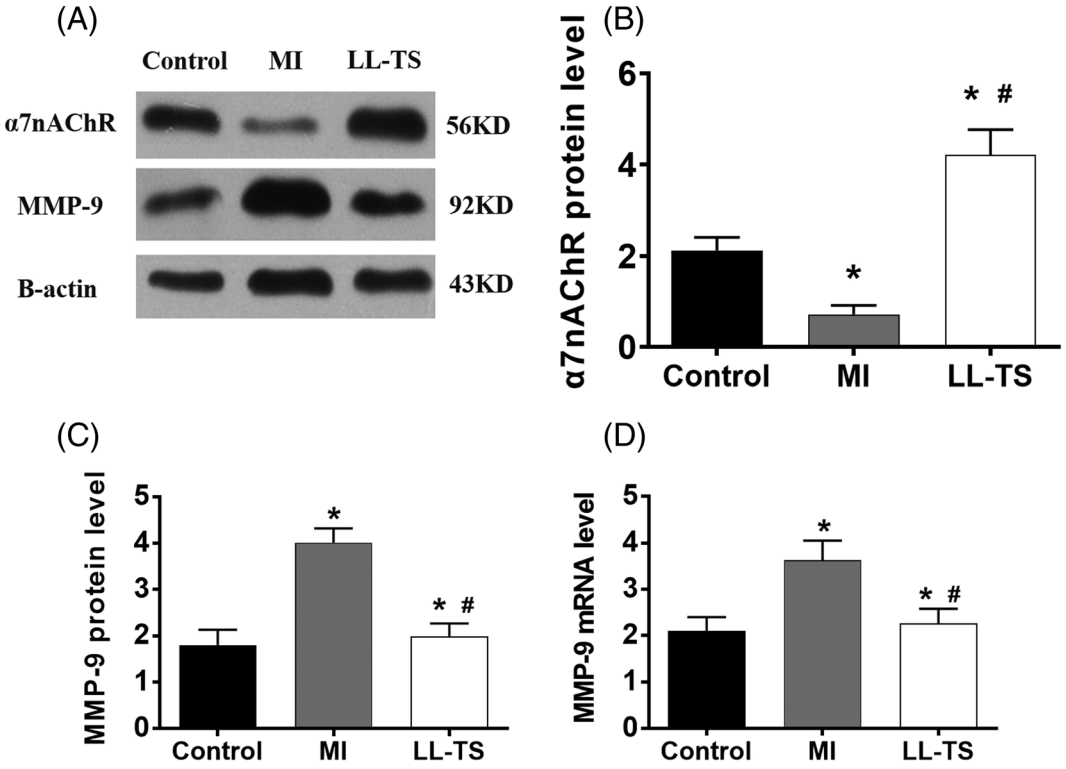
Major findings

The aim of the present study was to investigate the possible mechanisms of LL-TS treatment. The significant findings were as follows: (1) long-term intermittent LL-TS treatment attenuated LV dilation and improved both systolic and diastolic cardiac functions; (2) LL-TS attenuated the over-activation of LSG neural activity but increased the atrial GP neural activity; (3) LL-TS increased expression of α7nAChR and reduced expression of MMP-9 attenuated cardiac fibrosis and reversed LV remodelling.

LL-TS regulates the cardiac autonomic nervous dysfunction

The auricular branch of the vagus nerve (ABVN) is the only peripheral branch of the vagus nerve distributed to the skin and primarily supplies the auricular concha and most of the area around the auditory meatus.[17] Nomura and Mizuno[18] demonstrated that the afferent fibres of the ABVN predominately terminate in the nucleus tractus solitarius (NTS). The afferent vagal nerve fibres of ABVN enter the main vagal trunk through the jugular ganglions and project to NTS,[19] and then activates caudal ventrolateral medulla (CVM) and dorsal motor nucleus (DMN) to affect autonomic central neurons activity.[20] The hyper-activity of DMN delivered electrochemical signals through bilateral cervical vagal nerve to epicardial ganglion plexus and enhanced cardiac vagal tone. A large number of autonomic nerve fibres, including the fibres from the heart, project to the NTS. Gao et al.[21] demonstrated that acupuncture-like stimulation of the ABVN evoked cardiovascular inhibition via the activation of cardiac-related neurons in the NTS. Other researchers have demonstrated that stimulation of the distribution area of the auricular vagus nerve activated cardiac-related neurons in the NTS and evoked cardiovascular inhibition, whereas inactivation of the NTS with local anaesthetics decreased the cardiovascular inhibitory responses evoked by auricular stimulation.[22] In our previous research, we noted that LL-TS could substitute for VNS in the stimulation of vagal efferent fibres, which are part of the final pathway responsible for autonomic nervous system regulation.[10] A significant decrease in cardiac enzyme at the 1st and 28th day indicate LL-TS treatment could attenuate myocardial ischaemia/reperfusion injury. LL-TS could potentially attenuate the over-activation of cardiac sympathetic nervous activity and increase the GP activity. Regulation of cardiac autonomic nervous system reduced the catecholamine concentrations released by sympathetic activation induced by myocardial infarction, which thereby exerts a cardio-protective effect.

Figure 6 (A) Representative picture of western blots from LV non-infarcted tissues that demonstrate the effects of LL-TS treatment on α7nAChR and MMP-9 protein levels. The protein analyses indicate the relative protein levels of α7nAChR (B) and MMP-9 (C). The relative mRNA levels for (D) MMP-9 in each group are shown by real-time quantitative polymerase chain reaction analysis. The results are expressed as a ratio of the mRNA levels to the reference gene GAPDH. *$P < 0.01$ vs. control group; #$P < 0.01$ vs. MI group.



Cardiac autonomic activity improved LV remodelling by the regulation of the α7nAChR/MMP-9 axis

Cardiac dysfunction caused by interstitial fibrosis after MI is referred to as LV post-MI remodelling. The potential mechanism of post-MI remodelling had been discussed for decades. This study mainly focused on the mechanism by which LL-TS mediates LV remodelling post-MI via the regulation of the MMP-9. MMP-9 influences the outcome of various physiological and pathological processes, including MI, atherosclerosis, and congestive heart failure.[23–26] Ramirez et al. demonstrated that MMP-9 degrades collagen and contributes to ventricular remodelling in remote infarction areas after MI.[25] Furthermore, plasma MMP-9 correlates with increased end diastolic volumes, which implicates MMP-9 as a clinical biomarker of LV remodelling and adverse prognosis.[27]

It is known that early use of beta-blockers has been associated with reduction in mortality rates after acute myocardial infarction.[28] The protection effects from beta-blocker were reduction in oxygen consumption and metabolic demand, reversal of the catecholamine-mediated reduction in left ventricular compliance and attenuation of the direct toxic effects of norepinephrine on myocytes.[29] Vagal nerve stimulation (VNS) could slow the heart rate and attenuate sympathetic-induced ventricular remodelling. Previous study demonstrated that VNS exerted cardioprotective effects without heart rate alteration and the benefits could be independent of heart rate changes.[30] VNS exerts its cardioprotective effects on the failing heart independently of its anti-beta-adrenergic mechanism.[31] VNS has been proven to not only exert anti-inflammatory effects as well as other protective effects by cholinergic pathway. Acetylcholine (ACh), an important vagal neurotransmitter, reduced the production of pro-inflammatory cytokines via activation of α7nAChR in macrophages, finally leading to beneficial effects. A study previously demonstrated that ACh increased the expression of tissue inhibitor of metalloproteinase 1 (TIMP-1) in myocardium.[32] Another study also reported that ACh effectively inhibited MMP-9 via α7nAChR-JAK2/STAT3 pathway.[14] The present study found that LL-TS, a kind of non-invasive VNS, could obviously increase vagal nervous activity (GP activity), up-regulate α7nAChR expression, and effectively inhibit MMP-9 expression. It indicated that LL-TS attenuated

cardiac remodelling induced by MI possibly via α7nAChR/MMP-9 axis.

Clinical implications

Recently, both basic and clinical evidence have been proven that cardiac dysfunction played a critical role in heart failure. And numerous autonomic modulations strategies, including vagal nerve stimulation, spinal cord stimulation, LSG ablation, and renal sympathetic nerve ablation, can improve the ventricular remodelling and reduce the incidence of heart failure. However, all these autonomic modulations are invasive. Therefore, it is imperative to find a novel and non-invasive therapy to improve LV remodelling. Beside, transcutaneous electrical stimulation of the auricular branch of the vagus nerve has previously been used to treat epilepsy and depression,[33] to reduce the amount of anaesthetic used during operative procedures,[34] and to suppress sepsis in a murine model of endotoxemia.[35] We therefore propose a safe and convenient approach to optimize cardiac autonomic tone that may translate into clinical benefits in the future.

Limitations of the study

We did not measure the associated signal transduction pathways of MMP-9 or α7nAChR, which may elucidate the mechanism that underlies the treatment effects. Although we did assess the intracardiac electrophysiology and cardiac function index in the present study, additional research is required. The absence of medications limits extrapolation of these results to patients who would receive established post-ischaemic therapy for heart disease.

Conclusions

LL-TS treatment can improve the LV remodelling in a canine model of post MI. Inhibition of the neural activity of LSG and increase the neural activity of GPs by LL-TS may be responsible for these salutary results. The α7nAChR/MMP-9 axis may be the downstream pathway of LV remodelling improving by cardiac autonomic nervous system.

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Conflict of interest

None declared.

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