Impact of Vagal Nerve Stimulation on Left Atrial Structure and Function in a Canine High-Rate Pacing Model

Kenya Kusunose, MD, PhD; Youhua Zhang, MD, PhD; Todor N. Mazgalev, PhD; David R. Van Wagoner, PhD; James D. Thomas, MD; Zoran B. Popović, MD, PhD

Background—Cervical vagal nerve stimulation (VNS) can improve left ventricular dysfunction in the setting of heart failure (HF). However, little is known about the impact of VNS on left atrial (LA) function. The aim of this study was to compare LA mechanics and histology between control and VNS-treated animals during HF development.

Methods and Results—Fifteen mongrel dogs were randomized into control (n=7) and VNS (n=8) groups. All dogs underwent 8 weeks of high-rate ventricular pacing (at 220 beats per minute for the first 4 weeks to develop HF and another 4 weeks at 180 beats per minute to maintain HF). LA contractile function (LA negative peak strain), conduit function (LA positive peak strain), and reservoir function (LA total strain) were measured from speckle tracking in 2 groups. At the end of the terminal study, the LA appendage was obtained. Baseline LA strains were comparable in the control and VNS-treated dogs. At 4 and 8 weeks of ventricular pacing, all LA strains were decreased and LA volumes were increased in the control group compared with the VNS group (P<0.05). Histological evaluation of the left atrium revealed that percent fibrosis was significantly lower in the VNS versus the control group (8±1% versus 13±1%; P<0.001). Finally, transmural flow showed decreased atrial contribution to left ventricular filling in the control group (P<0.05).

Conclusions—VNS improved LA function and volumes and suppressed LA fibrosis in the canine high-rate ventricular pacing model. VNS is a novel and potentially useful therapy for improving LA function during HF. (Circ Heart Fail. 2014;7:320-326.)

Key Words: atrial function, left vagus nerve stimulation

Autonomic nervous dysfunction is known to have an important role in the progression of heart failure (HF), with characteristic increase of sympathetic and loss of parasympathetic (vagal) tone. Vagal nerve stimulation (VNS), which aims to correct this imbalance, has emerged as a novel strategy for controlling chronic HF. Previous studies in experimental models of HF showed that the VNS provided beneficial effects on left ventricular (LV) function and on survival. Initial clinical studies have shown that VNS treatment in patients with HF is feasible and tolerable and leads to a subjective clinical improvement. However, the magnitude of VNS effects and its exact anatomic targets are still not well understood. Recently, we reported that VNS can improve cardiac autonomic control and significantly attenuate HF development in a canine model with tachycardia-induced dilated cardiomyopathy (TIC). In this model, VNS improved the LV function (LV volumes and ejection fraction [EF]) with TIC, and this benefit was associated with anti-inflammatory effects, although ventricular pacing eliminated the VNS impact on heart rate. Interestingly, there is little knowledge about the effects of VNS on left atrial (LA) mechanics. Recent work in humans has shown that the measurement of LA mechanics is feasible, and that measures of LA deformation using strain assessment by speckle tracking echocardiography are related to LA structural remodeling, exercise capacity, and prognosis in HF. The aims of this study were (1) to define changes in LA mechanics and volumes with the development of TIC and (2) to compare LA mechanics and LA histology between control and VNS-treated animals.

Clinical Perspective on p 326

Methods

Study Population
In brief, experiments were performed on 15 adult mongrel dogs (both sexes; body weight, 22–27 kg). All dogs were implanted with a right ventricular pacemaker and were randomized into control (n=7) and VNS (n=8) groups. A right cervical vagus nerve stimulator was implanted in the VNS group. The study was approved by the Institutional Animal Care and Use Committee and is in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Procedures
Details of the study protocol as well the impact of VNS on LV size function in this group of animals have already been published. In brief, all dogs were anesthetized with thiopental (20 mg/kg, intravenously), intubated, and ventilated, with anesthesia maintained by 1% to 2% isoflurane. A bipolar screw-in endocardial pacing lead (model...
Assessed mitral regurgitation (MR) because its presence may modulate the 4-chamber and late or atrial (A) wave velocities of the LV filling. We additionally used pulsed wave Doppler of the mitral inflow to assess early (E) and late (A) mitral inflow velocities. LV volumes, LA volumes (maximum, minimum, and precontraction volumes) were measured from 4-chamber and 2-chamber apical views by the Simpson equation. LA volumes (maximum, minimum, and precontraction volumes) were measured from 4-chamber and 2-chamber apical views by the Simpson equation. Data were digitized and stored in a proprietary format for further analysis. Data Collection and Analysis

Animals were given a 2-week recovery period after device implantation. Daily standard postoperative care was performed until the incisions healed. After recovery, all dogs underwent 8 weeks of high-rate ventricular pacing. For the first 4 weeks, the pacing rate was set at 220 beats per minute to develop HF; the ventricular pacing rate was then reduced to 180 beats per minute for an additional 4 weeks. In the VNS group, VNS (frequency, 20 Hz; pulse width, 0.5 ms, duty cycle, 14 seconds ON/12 seconds OFF) was delivered continuously concomitant with ventricular pacing for the entire 8 weeks. VNS intensity (0.75–2.5 mA; average, 1.5±0.6 mA) was individually titrated to reduce the spontaneous sinus rate by 20 beats per minute. Data were acquired at 3 points in all dogs: baseline data before initiation of ventricular pacing, at 4 weeks of high-rate ventricular pacing, and at 8 weeks of high-rate pacing. Data were acquired with the animals in a sinus rate by temporarily turning off both the ventricular pacemaker and the vagus nerve stimulator (in the VNS group), so as to permit spontaneous sinus rhythm to return. After a stabilization period of ≥15 minutes, echocardiographic data were acquired.

Data Collection and Analysis

All echocardiographic data acquisition was planned and performed prospectively. Echocardiography was performed using Vivid 7 echocardiography machine (GE Medical, Milwaukee, WI). Dogs were trained to lie down calmly on their side and were imaged in left decubitus while awake. The minimal frame rate acquired during standard postoperative care was used to return. After a stabilization period of ≥15 minutes, echocardiographic data were acquired.

Histology Analysis

At the end of the terminal study, under anesthesia (isoflurane inhalation 1.5% to 2%), ventricular fibrillation was induced using a 9-V battery to euthanize the animals, and the heart was removed. LA appendage was obtained in 10 dogs (5 from control and 5 from VNS), immersion-fixed with 4% formalin, and then processed by paraffin embedding. Tissue was not taken in the remaining dogs because of technical issues. Serial histological sections (6.0-μm thick) were cut and stained with Masson trichrome stain. The stained sections were examined using an Olympus BX53 microscope, and high-resolution digital images were captured with an Olympus DP72 digital camera. Morphometric analyses of digitized images were performed in a blinded manner using Image-Pro Analyzer software (Media Cybernetics, Inc, Bethesda, MD). The extent of interstitial fibrosis was estimated from Masson-stained sections and was expressed as a percentage of the total area occupied by interstitial tissue that was stained blue. For each appendage, 4 to 5 optical fields were examined, and the data were averaged. The regions of endocardial, pericardial, and perivascular fibrosis as well as the empty areas of tissue separation, caused by histology artifact, were subtracted from the total area to standardize among the samples.

Biochemical Assay

Venous blood samples were collected in ice-chilled tubes coated with EDTA. For angiotensin (Ang)-II samples, the aminopeptidase inhibitor bestatin (80 μL; Alpco, Salem, NH) was preadded to the tubes. Plasma C-reactive protein (CRP) level was measured using a canine-specific, high-sensitivity CRP ELISA (KT-093; Kamiya Biomedical Company, Seattle, WA) per the manufacturer’s directions. Plasma NE concentrations were determined by a validated radioimmunoassay method (17-NORHU-R50; Alpco), and plasma was processed for radioimmunoassay method according to the manufacturer’s procedure. Plasma Ang-II contents were determined using a validated radioimmunoassay method (01-RK-A22; Alpco).

Statistical Analysis

Continuous data are presented as means±SD unless otherwise specified. Statistical analysis was performed using a standard statistical software package (SPSS software 14.0; SPSS Inc.). A Mann–Whitney U test was used to evaluate the significance of differences between the 2 groups. To assess the overall impact of VNS on LA volumes, LA function, and LV diastolic function, we used a 2-way, repeated-measures analysis of covariance with group as a fixed factor, animal number as random factor, and time as a covariate. We tested the effects of groups as a factor, time after HF development as a covariate, as well as timexgroup interactions. Parameter estimates were used to assess the significance of parameter change over time in individual groups. To eliminate the impact of nonnormal distribution and the presence of outliers, we also correlated pericardial thickness and strain using Spearman ρ coefficient. To assess the intra- and interobserver variability of the 3 components of LA strain, 5 randomly selected data sets were exchanged by 2 independent observers, with each observer measuring the same data set twice. We then calculated the intra- and interobserver SEM using the method of Eliaszew et al.13

Statistical significance was defined by P<0.05.

Results

VNS Effects on LA Volumes and Function

One dog in the VNS group was excluded from the analysis of LA strain because the echo images were inadequate. Figure 1 shows examples of characteristic 2-dimensional echocardiographic images, individual LA strain profiles, and LV filling profiles obtained in 2 representative dogs belonging to the control and VNS groups, respectively. As shown in Figure 2A to 2D, LA volumes and LA EF were comparable in the control and VNS-treated dogs.
at baseline (LA minimum volume: 7±2 versus 6±3 mL; 
$P=0.46$; LA precontraction volume: 11±3 versus 10±4 mL; 
$P=0.74$; LA maximum volume: 18±5 versus 15±4 mL; 
$P=0.20$; LA EF: 62±8% versus 62±10%; $P=0.97$). In both 
groups, LA volumes showed an overall increase, whereas 
LA EF decreased ($P \leq 0.005$ for all comparisons). However, 
these changes were significantly less pronounced in the VNS 
group ($P \leq 0.006$ for group×time interaction for all compari-
sions). Furthermore, there was no significant change over 
time observed for minimal LA volume ($P=0.2$) and LA EF 
($P=0.2$) in the VNS-treated group.

Changes in LA strain parameters are shown in Figure 3A 
to 3C. Baseline LA strains were comparable in the control 
and VNS-treated dogs (LA positive peak strain: 20±3% versus 19±6%; $P=0.58$; LA negative peak strain: −10±2% versus 
−9±3%; $P=0.65$; LA total strain: 30±4% versus 28±7%; $P=0.52$).

In both groups, the positive LA strain showed an overall 
decrease ($P<0.001$). This decrease was slightly but significantly
less pronounced in the VNS group (Figure 3A; $P=0.04$ for group×time interaction). Similarly, the absolute values of negative LA strain showed an overall decrease over time (Figure 3B; $P=0.008$). However, this decrease was significantly less pronounced in the VNS group ($P<0.001$ for group×time interaction). Moreover, when only the VNS group was assessed in isolation, no changes in negative LA strain occurred ($P=0.8$).

Finally, in both groups, the total LA strain, which is an arithmetic sum of LA positive and negative strains, showed an overall decrease over time (Figure 3C; $P<0.001$). Again, the decrease was significantly less pronounced in the VNS group ($P=0.001$ for group×time interaction), and when only the VNS group was assessed in isolation, changes in total LA strain were borderline ($P=0.1$).

The LA systolic function follows the Frank Starling relationship; thus, the LA systolic output increases with the increase of LA preload. Figure 3D illustrates the Frank Starling relationship by plotting negative LA strain against LA precontraction volume (ie, LA preload) is shown on the x axis. Circles indicate VNS; and while triangles, the control group. The VNS group shows a small concomitant parallel increase of LA negative strain and volume, indicating preserved LA contractility. In contrast, the control group shows decreased LA strain with increased LA volumes, indicating loss of LA contractility. Error bars represent standard error.

VNS Effects on LV Function and Filling

In both groups, the global LV strain showed an overall decrease ($P<0.001$). This decrease was slightly but significantly less pronounced in the VNS group (Figure 4; $P=0.05$ for group×time interaction).

The early (E) component of LV filling is determined by the opposing effects of increased LV filling pressure and...
worsened LV relaxation, whereas the LA (A) component of LV filling is strongly affected by LA systolic function, and these 2 components showed different patterns of change in our study. Again, baseline transmitral flows were comparable in the control and VNS groups (E-wave: 70±10 versus 87±11 cm/s; \( P = 0.21 \); A-wave: 47±16 versus 49±19 cm/s; \( P = 0.81 \); Figure 5). At both 4 and 8 weeks of ventricular pacing, E-wave velocity remained unchanged (\( P = 0.52 \) for the difference over time). In contrast, A-wave velocity decreased in the control group but was unchanged in the VNS group (\( P = 0.02 \) for group×time interaction). As a result, the E/A ratio increased in the control group, whereas it was unchanged in the VNS group (\( P = 0.01 \)). MR degrees in the VNS group were trivial in 6 and mild in 2 animals, whereas MR degrees in the control group were trivial in 2, mild in 3, and moderate in 2 animals, with no significant difference between the groups (\( P = 0.1 \)). As previously published, there was no significant difference of systemic blood pressure and heart rate at baseline, 4 weeks, and 8 weeks of pacing. VNS did not affect systemic blood pressure level and heart rate.7

VNS Effects on LA Histology and Markers of Inflammation and Sympathetic Activation

As shown in Figure 6, histological evaluation of the left atrium in 10 dogs (5 control and 5 VNS) revealed that interstitial fibrosis was significantly suppressed in the VNS group. Significant fibrosis was notably more common among dogs in the control group (13±1% versus 8±1%; \( P < 0.001 \)). The Table shows that there were significant correlations between LA fibrosis and CRP, Ang-II, and norepinephrine. There were also significant correlations between LA strain and CRP, Ang-II, and norepinephrine. In addition, there were weak correlations between LV strain and CRP, Ang-II, and norepinephrine. In summary, VNS improves inflammatory markers in the blood sample, and the correlation between inflammatory markers and LA function (LA strain) is stronger than the correlation between markers and LV function (LV strain; \( P < 0.05 \)). This result may support the hypothesis that the VNS effects are based on the downregulation of the anti-inflammatory pathway and renin–angiotensin system in LA fibrosis.

Observer Variability

The intra- and interobserver SEM variability of LA total strain was 1.8% and 1.9%; LA negative peak strain was 1.5% and 1.6%; and LA positive peak strain was 0.9% and 1.1%, respectively.

Discussion

In this article, we evaluated the effects of chronic cervical VNS treatment on LA function and structure in a canine tachycardia-induced cardiomyopathy model using a combination of standard and novel (LA strain) methods. Our findings show that although LA contractility deteriorates during the development of TIC,7 VNS treatment significantly decreased this worsening of LA contractility. These findings were associated with less LA fibrosis in the VNS group, implicating a possible mechanism that may contribute to a better preservation of LA structure and function.
Left Atrial Strain in the Assessment of Atrial Function and Structure

The assessment of atrial function with conventional approaches remains challenging, prone to error, and is operator-dependent. Speckle tracking strain assessment addresses some of these issues by making the evaluation of atrial function semiautomatic, with feasibility studies showing acceptable measurement error,\(^\text{13,15}\) which we reaffirmed in this study. The measurement of LA strain has been clinically validated. For example, LA strain is sensitive to known age-induced changes of LA function.\(^\text{11}\) More importantly, LA strain correlates with LA wall fibrosis in various disease states.\(^\text{8,19}\) The results of this study are consistent with these previous studies linking LA function (strain) with LA structure (fibrosis), suggesting that earlier detection of LA dysfunction could be helpful for the detection of LA remodeling. In addition, HF may affect the left atrium both immediately and long term. The immediate increase of LV preload impedes LA emptying and decreases atrial systolic contribution to cardiac output. In the long term, HF leads to increased sympathetic tone and activation of various paracrine and inflammatory pathways leading ultimately to LA dilatation and fibrosis. This disease progression can be assessed using LA strain.

Impact of Vagal Stimulation on Left Atrium During HF

Our study has shown that during the development of TIC, VNS-treated dogs had a preserved LA function with less atrial dilatation and fibrosis. Although previous studies have shown that VNS decreases ventricular fibrosis in dogs with infarct-induced HF,\(^\text{20}\) this is the first study of the same phenomenon also occurring in atrial myocardium. Although worsened LA function and increased LA size can be expected due to worsening of LV function,\(^\text{21}\) the impact of VNS was more pronounced on contractile rather than diastolic LA function. Moreover, our findings suggest that VNS affects negative LA strain (LA pump function) more than it does LV strain (LV systolic function). This, along with less LA fibrosis in the VNS-treated group, suggests that VNS may directly affect LA function rather than acting solely through improved LV function.\(^\text{17}\)

Although we show that dogs subjected to rapid ventricular pacing have preserved atrial function and less fibrosis if treated with VNS, the possible molecular pathways responsible for these findings were not assessed in this study. We have shown that animals treated with VNS had decreased levels of CRP, an inflammatory marker, beside decreased plasma levels of norepinephrine and Ang-II. Vagus nerve stimulation is a potent anti-inflammatory agent.\(^\text{22}\) It attenuates the production of tumor necrosis factor and interleukins in endotoxic shock.\(^\text{23}\) It decreases oxidative stress after experimental myocardial infarction.\(^\text{24}\) Furthermore, in an ischemic HF model, the VNS attenuates inducible nitric oxide synthase synthesis, an enzyme associated with both fibrosis and inflammation.\(^\text{25}\)

Limitations

Our main limitation was that we were unable to perform histological inflammation analysis in the left atrium to enable the mechanistic insight into VNS effects. We initiated VNS simultaneously with rapid ventricular pacing and, therefore, we can only speculate what the benefits of VNS would be in the setting of established cardiac dysfunction. In addition, the effect of VNS treatment was tested using a ventricular rapid pacing model, and it remains to be determined whether a similar approach influences HF development when rhythm is not accelerated. However, a previous study showed a similar magnitude of VNS effect on LV function in dogs with ischemic HF.\(^\text{20}\) We did not assess LV histology, but Hanna et al\(^\text{26}\) have shown that TIC results in increased LA fibrosis from \(1\%\) to \(10\%\), whereas LV fibrosis is minimal (\(<1\%\)). There remains a possibility that at least a part of the changes seen with VNS is mediated by, or occurs in parallel to, changes in the LV structure. We cannot exclude that the effects of VNS on left atrium in TIC are solely mediated by improved LV function. However, experimental uncoupling of LA effects of VNS from its LV effects would be difficult to obtain. Finally, we did not discern between relative contributions of direct effect of VNS on the left atrium versus its indirect effect induced by better preserved LV function.

Conclusions

Our results show that chronic VNS improves LA function and structure in a canine TIC model of HF. Chronic VNS, by improving vagal control, may affect LA function by protecting LA myocardium. This insight may be useful in further therapeutic studies of VNS in human HF.

Sources of Funding

This study was supported by a grant from the American Heart Association (the Great Rivers Affiliate, 075404B) and by the Atrial Fibrillation Innovation Center, a State of Ohio Wright Center of Innovation, and a Biomedical Research and Technology Transfer Partnership Award (Ohio’s Third Frontier Project, and by the Foundation Leducq (European North American Atrial Fibrillation Research Alliance).

Disclosures

None.

References

Autonomic nervous dysfunction is known to have an important role in the progression of heart failure (HF), with characteristic increase of sympathetic and loss of parasympathetic (vagal) tone. Vagal nerve stimulation (VNS), which aims to link ventricular dilatation to functional mitral regurgitation.


Impact of Vagal Nerve Stimulation on Left Atrial Structure and Function in a Canine High-Rate Pacing Model
Kenya Kusunose, Youhua Zhang, Todor N. Mazgalev, David R. Van Wagoner, James D. Thomas and Zoran B. Popovic

Circ Heart Fail. 2014;7:320-326; originally published online January 7, 2014; doi: 10.1161/CIRCHEARTFAILURE.113.000937
Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/7/2/320

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Heart Failure can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Heart Failure is online at:
http://circheartfailure.ahajournals.org//subscriptions/