Original Article

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, transmitral 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.

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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...
Vagal Nerve Stimulation and LA Mechanics

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Tendril 1688TC/58 cm; St Jude Medical, Inc, Sylmar, CA) was implanted under sterile conditions into the right ventricular apex through the right jugular vein under fluoroscopic guidance. The lead was connected to a custom high-rate pacemaker (St Jude Medical), which can deliver high-rate ventricular pacing suitable for induction of HF. Dogs in the VNS group were also implanted with a right cervical vagus nerve stimulator. The right cervical vagus nerve was isolated and a cervical VNS electrode (Cyberonics Inc, Houston, TX) was placed around the nerve. The electrode was connected to a nerve stimulator (Cyberonics Inc). Both the right ventricular pacemaker and the nerve stimulator were buried in pockets at the neck area.

Study Protocol

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. All 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 echocardiography 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 2-dimensional echocardiography was 50 frames/second. Data were digitized and stored in a proprietary format for further analysis. Data were analyzed using EchoPAC PC (GE Medical). LA volumes (maximum, minimum, and precontraction volumes) were measured from 2-dimensional echocardiography at 50 frames/second. Data were used to assess the significance of parameter change over time in 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. 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, 6 randomly selected data sets were evaluated 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. Statistical significance was defined by P<0.05.

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 (0.6-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 time×group 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, 6 randomly selected data sets were evaluated 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. 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-
sons). 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

**Figure 1.** Examples of left atrial (LA) strain tracings obtained by 2-dimensional speckle tracking and corresponding mitral inflow obtained 
from a single dog in the control group (A) and in the vagal nerve stimulation (VNS)-treated group (B). A indicates atrial; and E, early.

**Figure 2.** Left atrial (LA) volumes (A, the minimum volume; B, the precontraction volume; and C, maximum volume) and LA ejection frac-
tion (D) at baseline, and at 4- and 8-week ventricular pacing in the control (n=7) and vagal nerve stimulation (VNS)-treated groups (n=8). 
Error bars represent standard error.
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 (\( P = 0.8 \)).

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, a measure of LA preload. In the control group, the absolute values of negative LA strain can be seen to decrease concomitantly with increasing LA preload, indicative of decreasing LA contractility. In contrast, the VNS group shows a small increase in both strain and preload, indicative of preserved LA contractility throughout the study period.

**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.

Figure 5. Mitral inflow peak early (A) and atrial (B) wave velocities and their ratio (C) at baseline, at 4- and 8-week ventricular pacing in the control (n=7) and vagal nerve stimulation (VNS)-treated groups (n=8). Error bars represent standard error. A indicates atrial; and E, early.

Figure 6. Impact of heart failure (HF) with and without vagal nerve stimulation (VNS) on left atrial (LA) fibrosis. Left: Masson trichrome staining of LA appendage tissue from 3 HF dogs (top) and 3 HF dogs treated by VNS (bottom). The areas of fibrosis, stained blue, are much more evident in the HF dog. Scale bar, 100 μm. Right: Mean percent interstitial fibrosis in both groups of dogs. Error bars represent standard deviation.
function,21 the impact of VNS was more pronounced on con-
tractile rather than diastolic LA function. Moreover, our find-
ings suggest that VNS affects negative LA strain (LA pump
function) more than it does LV strain (LV systolic function).

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 dilata-
tion and fibrosis. Although previous studies have shown that VNS
decreases ventricular fibrosis in dogs with infarct-induced HF,20 this is the first study of the same phenomenon also occur-
ing in atrial myocardium. Although worsened LA function and
increased LA size can be expected due to worsening of LV
function,21 the impact of VNS was more pronounced on contrac-
tile rather than diastolic LA function. Moreover, our find-
ings 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.17

Although we show that dogs subjected to rapid ventricu-
lar pacing have preserved atrial function and less fibrosis if
treated with VNS, the possible molecular pathways responsi-
ble 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.22 It attenuates the production
of tumor necrosis factor and interleukins in endotoxic shock.23

It decreases oxidative stress after experimental myocardial
infarction.24 Furthermore, in an ischemic HF model, the
VNS attenuates inducible nitric oxide synthase synthesis, an
enzyme associated with both fibrosis and inflammation.25

Limitations
Our main limitation was that we were unable to perform his-

tological inflammation analysis in the left atrium to enable the
mechanistic insight into VNS effects. We initiated VNS simul-
taneously with rapid ventricular pacing and, therefore, 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.20 We did not
assess LV histology, but Hanna et al26 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 protect-
ing LA myocardium. This insight may be useful in further
therapeutic studies of VNS in human HF.

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Research Alliance).

Disclosures
None.

References

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| Table. Relationship Between Biochemical Data, Left Atrial (LA) Strain, LA Fibrosis, and Left Ventricular (LV) Strain |
|-----------------|-----------------|-----------------|-----------------|
|                | Mean            | Spearman ρ      | P Value         | Spearman ρ      | P Value         | Spearman ρ      | P Value         |
| High-sensitivity C-reactive protein, mg/L | 7.9±15.7        | 0.461           | 0.044           | −0.679          | <0.001          | −0.427          | 0.005           |
| Angiotensin II, pg/mL | 52±15           | 0.62            | 0.004           | −0.612          | <0.001          | −0.335          | 0.04            |
| Norepinephrine, pg/mL | 109±135         | 0.624           | 0.006           | −0.545          | <0.001          | −0.342          | 0.03            |


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**CLINICAL PERSPECTIVE**

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. However, the magnitude of VNS effects and its exact anatomic targets are still not well understood. We investigated the impact of VNS on left atrial (LA) mechanics and volumes in a canine high-rate ventricular pacing model and compared LA mechanics and LA histology between control and VNS-treated animals. Our study shows that although LA contractility deteriorates during the development of tachycardia-induced cardiomyopathy, VNS treatment significantly blunted 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. In addition, there are significant correlations between inflammatory markers and LA function and LA fibrosis. 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. We think these insights will be useful in further therapeutic studies of VNS in human HF.
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