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

Kusunose et al: Vagal Nerve Stimulation and LA Mechanics

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Abstract

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 220bpm for the first 4 weeks to develop HF and another 4 weeks at 180bpm 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 two groups. At the end of the terminal study, the left atrial 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 LA revealed that percent fibrosis was significantly lower in the VNS vs. the control group (8±1% vs. 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.

Key Words: strain echocardiography, left atrial function, 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) with TIC, and this benefit was associated with anti-inflammatory effects, even though 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 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 aim of this study was to 1) define changes in LA mechanics and volumes with the development of TIC and 2) compare LA mechanics and LA histology between control and VNS-treated animals.

**Methods**

**Study Population**

In brief, experiments were performed on 15 adult mongrel dogs (both sexes, body weight 22 to 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 VNS group dogs. 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

The details of the study protocol as well the impact of VNS on left ventricular size function in this group of animals have already been published. In brief, all dogs were anesthetized with thiopental (20 mg/kg IV), intubated and ventilated, with anesthesia maintained by 1% to 2% isoflurane. A bipolar screw-in endocardial pacing lead (model Tendril 1688TC/58 cm, St. Jude Medical, Inc, Sylmar, Calif.) 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, Tex.) 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 were 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 bpm to develop HF; the ventricular pacing rate was then reduced to 180 bpm for an additional 4 weeks. In the VNS group dogs, VNS (frequency 20 Hz, pulse width 0.5 ms, duty
cycle 14 s on to 12 s off) was delivered continuously concomitant with ventricular pacing for the entire 8 weeks. The VNS intensity (0.75 to 2.5 mA, average 1.5 ± 0.6 mA) was individually titrated to reduce the spontaneous sinus rate by 20 bpm. 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), to permit spontaneous sinus rhythm to return. After a stabilization period of ≥15 minutes, the 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, USA). Dogs were trained to lie down calmly on their side and were imaged in left decubitus while awake. The minimal frame rates acquired during standard two-dimensional echocardiography were 50 frames s⁻¹. Data were digitized and stored in a proprietary format for further analysis. Data were analyzed using EchoPAC PC (GE Medical Systems, Milwaukee, WI). LA volumes (maximum, minimum and pre-contraction volumes) were measured from the 4-chamber and 2-chamber apical views by the Simpson equation. Pulsed wave Doppler of the mitral inflow was used to assess early (E) and late, or atrial (A) wave velocities of the LV filling. We additionally assessed mitral regurgitation (MR) as its presence may modulate LV filling and atrial function, and it is known that TIC may induce functional mitral regurgitation. To do this we used a semiquantitative 5-point scale (with grades of none, trivial, mild, moderate, moderately severe, and severe) to stratify mitral regurgitation assessed by color 2-dimensional Doppler echocardiography clips obtained in apical 2- and 4-chamber views.
To obtain left atrial (LA) and LV strains we used a speckle-tracking algorithm incorporated into EchoPAC PC. Again, 4-chamber and 2-chamber apical views were used. The region of interest was overlaid on a cross section of the atrial silhouette at the image corresponding to the minimal endocardial area. The automated software then generated traces depicting regional longitudinal strain for each segment, and strain values were measured from these traces. The software calculated average strain values of six LA segments obtained from each apical view, and the global longitudinal LA strain values were then obtained as averages of the values of two apical views. The use of the P-wave as the reference point enabled the recognition of peak positive strain, which corresponded to LA conduit function, the peak negative strain, which corresponded to LA contractile function, and the sum of these values (LA strain total), which corresponded to LA reservoir function.13 LV segmental longitudinal strain curves were constructed in 4-chamber, 2-chamber and long-axis apical views. These three curves were averaged to obtain global LV strain curve with end-systolic value taken to represent global LV strain.14 For the analysis of all data, at least three heart beats were measured. The mean value was used for statistical analysis.

**Histology Analysis**

At the end of the terminal study, under anesthesia (Isoflurane Inhalation 1.5-2%), ventricular fibrillation was induced using 9 volt battery to euthanize the animals and the heart was removed. The left atrial appendage was obtained in 10 dogs (5 from control and 5 from VNS), immersion-fixed with 4% formalin, and then processed by paraffin embedding. The 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’s 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 the 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, Wash), per the manufacturer’s directions. Plasma NE concentrations were determined by a validated radioimmunoassay method supplied by American Laboratory Products Company (17-NORHUR50, ALPCO, NH), and plasma was processed for radioimmunoassay method according to the manufacture’s published procedure. Plasma Ang-II contents were determined using a validated radioimmunoassay method supplied by American Laboratory Products (01-RK-A22, ALPCO).

**Statistical Analysis**

Continuous data are presented as mean ± 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 two-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 significance of parameter change over time in individual groups. To eliminate the impact of non-normal distribution and the presence of outliers, we also correlated pericardial thickness and strain using Spearman Rho ($\rho$) coefficient. To assess the intra- and inter-observer variability of the three components of LA strain, six 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 inter-observer standard error of measurement (SEM) using the method of Eliasziw et al.\textsuperscript{15} 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 as the echo images were inadequate. Figure 1 shows examples of characteristic 2-dimensional echocardiographic image, individual LA strain profiles, and LV filling profiles obtained in two representative dogs belonging to the control and VNS groups, respectively.

As shown in Figure 2A to D, LA volumes and LA EF were comparable in the control and VNS treated dogs at baseline (control vs. VNS, LA minimum volume; 7±2ml vs. 6±3ml, $p=0.46$, LA pre-contraction volume; 11±3ml vs. 10±4ml, $p=0.74$, LA maximum volume; 18±5ml vs. 15±4ml, $p=0.20$, LA ejection fraction; 62±8% vs. 62±10%, $p=0.97$). In both groups, LA volumes showed an overall increase; while 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 by time interaction for all comparisons). Further, 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 on Figure 3A to C. Baseline LA strains were
comparable in the control and VNS treated dogs (LA positive peak strain; 20±3% vs. 19±6%, p
=0.58, LA negative peak strain; -10±2% vs. -9±3%, p =0.65, LA total strain; 30±4% vs. 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 by 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 VNS group (p <0.001 for group by 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 by time
interaction), and when only the VNS group was assessed in isolation, changes in total LA strain
were borderline (p =0.1).

Left atrial systolic function follows the Frank Starling relationship; thus LA systolic
output increases with the increase of LA preload.16 Figure 3D illustrates the Frank Starling
relationship by plotting negative LA strain against LA pre-contraction volume, a measure of LA
preload. In the control group, 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 of 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 by time interaction).

The early (E) component of LV filling is determined by the opposing effects of increased LV filling pressure and worsened LV relaxation, while the late atrial (A) component of LV filling is strongly affected by LA systolic function, and these two components showed different patterns of change in our study. Again, baseline transmitral flows were comparable in the control and VNS group (E wave; 70±10 cm/sec vs. 87±11 cm/sec, p =0.21, A wave; 47±16 cm/sec vs. 49±19 cm/sec, p =0.81) (Figure 5). At both 4 and 8 weeks of ventricular pacing, E wave velocity decreased in the control group but was unchanged in the VNS group (p = 0.02 for group by time interaction). As a result E/A ratio increased in the control group, while 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, while MR degrees in the control group were trivial in 2, mild in 3 and moderate in 2 animals, with no significant difference between 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 LA in 10 dogs (5 control and 5 VNS) revealed the interstitial fibrosis was significantly suppressed in the VNS group. Significant
fibrosis was notably more common among dogs in the control group (13±1% vs. 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 blood sample, and the correlation between inflammatory markers and left atrial function (LA strain) is stronger than the correlation between markers and left ventricular function (LV strain) (p <0.05). This result may support the hypothesis that the VNS effects are based on the down-regulation of the anti-inflammatory pathway and renin-angiotensin system in left atrial fibrosis.

Observer variability

The intra- and inter-observer 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 paper, 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 for the assessment of LA function. Our findings show that, while LA contractility deteriorates during the development of TIC,17 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

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 semi-automatic, with feasibility studies showing acceptable measurement error,\textsuperscript{13, 18} which we reaffirmed in this study. Measurement of LA strain has been clinically validated. For example, LA strain is sensitive to known age-induced changes of LA function.\textsuperscript{11} More importantly, LA strain correlates with LA wall fibrosis in various disease states.\textsuperscript{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 detection of LA remodeling. In addition, HF may affect the left atrium both immediately and long-term. 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 development of TIC, VNS-treated dogs had a preserved left atrial function with less atrial dilatation and fibrosis. While previous studies have shown that VNS decreases ventricular fibrosis in dogs with infarct-induced HF,\textsuperscript{20} this is the first study of the same phenomenon also occurring in atrial myocardium. Although worsened left atrial function and increased left atrial size can be expected due to worsening of LV function,\textsuperscript{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 left atrial function rather than acting solely through improved LV function.\textsuperscript{17}

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

\textit{Limitations}

Our main limitation was that we were unable to have no histological inflammation analysis in left atrium to enable the mechanistic insight into VNS effects. We initiated VNS simultaneously 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, previous study showed a similar magnitude of VNS effect on LV function in dogs with ischemic HF.\textsuperscript{20} We did not assess LV histology, but Hanna et al.\textsuperscript{26} have shown that TIC results in increased left atrial fibrosis from less than 1\% to close to 10\%, while LV fibrosis is minimal (<1\%). There remains a possibility that at least part of the changes seen with VNS is mediated by, or occurs in parallel to, changes in LV structure. We cannot exclude that the effects of VNS on LA in TIC are solely...
mediated by improved LV function. However, experimental uncoupling of left atrial 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 vs. 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.

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**Disclosures**

None.

**References**


Table. The relationship between biochemical data, left atrial strain, left atrial fibrosis, and left ventricular strain

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**Figure Legends**

**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 (Panel A) and in the vagal nerve stimulation (VNS)-treated group (Panel B).

**Figure 2.** Left atrial (LA) volumes (Panel A: the minimum volume; Panel B: the pre-contraction volume; and the Panel C: maximum volume) and LA ejection fraction (EF) (Panel C) 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.

**Figure 3.** Left atrial (LA) strains (Panel A: LA positive peak strain; Panel B: LA negative peak strain; and Panel C: LA total strain) at baseline, at 4- and 8-week ventricular pacing in control (n=7) and vagal nerve stimulation (VNS)-treated group (n=7). Panel D shows a plot of the LA Frank Starling relationship at baseline, at 4- and 8-week ventricular pacing in the control (n=7) and VNS-treated groups (n=7). LA negative strain is shown on the y axis while LA pre-contraction volume (i.e. LA preload) is shown on the x axis. Circles represent VNS, while triangles represent 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.
**Figure 4.** Left ventricular (LV) global longitudinal strain 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.

**Figure 5.** Mitral inflow parameters 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.

**Figure 6.** Impact of heart failure (HF) with and without vagal nerve stimulation (VNS) on left atrial fibrosis. Panels on the left show Masson-trichrome staining of left atrial appendage tissue from three HF dogs (upper panel) and three HF dogs treated by VNS (lower panel). The areas of fibrosis, stained blue, are much more evident in the HF dog. Scale bar = 100 µm. Panel on the right shows mean percent interstitial fibrosis in both groups of dogs. Error bars represent standard deviation.
Control group

Figure 1A
VNS group

Figure 1B

Baseline → 8 week
Figure 2

A. Minimum LA volume
B. Pre-contraction LA volume
C. Maximum LA volume
D. LA EF

- VNS
- Control

Baseline 4 week 8 week
Baseline 4 week 8 week
Baseline 4 week 8 week
Baseline 4 week 8 week

p < 0.001 for time x group interaction
p < 0.001 for time x group interaction
p = 0.001 for time x group interaction
p = 0.006 for time x group interaction
Figure 3

A) LA positive strain

B) LA negative strain

C) LA total strain

D) Pre-contraction LA volume

- VNS
- Control

p = 0.04 for time x group interaction

p < 0.001 for time x group interaction

Baseline 4 week 8 week

p = 0.001 for time x group interaction

Baseline 4 week 8 week

Pre-contraction LA volume
Global LV strain (%)

Baseline  4 week  8 week

VNS  Normal

p = 0.05 for time x group interaction

Figure 4
Figure 5
Figure 6

Interstitial fibrosis tissue (%)

HF dogs
Number = 5

HF + VNS dogs
Number = 5

p<0.001
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