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RESEARCH PROTOCOL

Title: Noninvasive Neuromodulation to Reverse Diastolic Dysfunction

Principal Investigator: Stavros Stavrakis, MD, PhD; Assistant Professor of Medicine; Department of Medicine/ Cardiology

Abstract:

Heart failure with preserved ejection fraction (HFpEF) has become a major public health concern. Epidemiological studies have shown that the prevalence and hospitalizations related to HFpEF are increasing, and the growing elderly population is expected to further worsen these trends. Outcomes of patients with HFpEF are poor, and so far, no treatment has been shown to decrease morbidity or mortality. HFpEF is associated with various cardiovascular risk factors, including hypertension, atrial fibrillation and aging. The net result is impaired diastolic relaxation and filling of the left ventricle (LV), increased myocardial stiffness, impaired vascular compliance, and increased diastolic pressure. Recent animal and human studies support the notion that proinflammatory stimuli play a central role in the development of HFpEF. Specifically, comorbid conditions such as diabetes, obesity, hypertension and aging induce a systemic proinflammatory state, which in turn leads to decreased nitric oxide (NO) bioavailability, left ventricular fibrosis, increased myocardial stiffness, diastolic dysfunction and heart failure. Therefore, attenuating the proinflammatory state is an attractive therapeutic target for HFpEF.

The anti-inflammatory properties of the vagus nerve have been well established. In a recent proof-of-concept study in humans, we showed that in patients with drug-refractory atrial fibrillation, low-level transcutaneous vagus nerve stimulation (LLTS), delivered at the tragus of the ear for just one hour, significantly suppressed atrial fibrillation and decreased inflammatory cytokines. These results support the use of LLTS as a novel non-pharmacological treatment modality for atrial fibrillation and possibly other conditions, where inflammation plays a key role, including HFpEF. In this proposal, we hypothesize that short-term LLTS may reverse diastolic dysfunction in a 2x2 cross over study in patients with diastolic dysfunction. These proof-of-concept investigations will establish the first evidence of the effects of LLTS on LV diastolic dysfunction in humans and may provide the basis for the design of further human studies using this modality to target selected populations with HFpEF. In light of the increasing number of patients with HFpEF and the poor success of the currently available treatment options, an alternative approach such as the one tested in this grant proposal may benefit a large number of patients in Oklahoma.

A. Specific aims:

Specific Aim 1:

To examine the effect of short-term LLTS on echocardiographic markers of diastolic dysfunction in patients with diastolic dysfunction.

Specific Aim 2:

To examine the effect of short-term LLTS on heart rate variability in patients with diastolic dysfunction.

B. Background and significance

Heart failure with preserved ejection fraction (HFpEF) has become a major public health concern. Epidemiological studies have shown that the prevalence and hospitalizations related to HFpEF are increasing[1,2], and the growing elderly population is expected to further worsen these trends. Despite normal or near-normal LV ejection fraction, the rates of morbidity and mortality among these patients are high and similar to those of patients with reduced LV ejection fraction [3-5]. Unfortunately, no pharmacologic therapy has been shown to improve outcomes in patients with HFpEF [6]. HFpEF is associated with various cardiovascular risk factors, including hypertension, atrial fibrillation (AF), diabetes and aging. The net result is impaired diastolic relaxation and filling of the LV, increased myocardial stiffness, impaired vascular compliance, and increased diastolic pressure [7,8]. Recent animal and human studies support the notion that systemic inflammation plays a central role in the development of HFpEF [9-15]. As illustrated in Figure 1 [13], comorbidities, such as obesity, hypertension and diabetes induce a systemic proinflammatory state, which leads to recruitment of monocytes into the heart. These inflammatory cells contribute to LV fibrosis by promoting the differentiation of fibroblasts into myofibroblasts through the release of transforming growth factor (TGF)-β. The resulting increase in LV collagen content is a major contributor to the increase in passive myocardial fiber stiffness, which eventually leads to diastolic dysfunction and HFpEF. In addition, lower NO bioavailability leads to decreased protein kinase G activity, which increases myocardial tension and induces hypertrophy, leading to diastolic dysfunction and HFpEF. Therefore, attenuating the proinflammatory state is an attractive therapeutic target for HFpEF.

The anti-inflammatory properties of the vagus nerve have been well established. Current evidence suggests that the vagus nerve provides the efferent and possibly the afferent limb of the cholinergic anti-inflammatory pathway, by which the brain modulates inflammation [16,17]. The cholinergic anti-inflammatory pathway can be activated experimentally by electrical vagus nerve stimulation to inhibit inflammatory cytokine production (including TNF-α), prevent tissue injury and improve survival in multiple experimental models of systemic inflammation and sepsis [16-21]. In addition, vagus nerve stimulation through an implantable device has been used successfully in a preliminary clinical trial in patients with rheumatoid arthritis [17] and is currently being tested in patients with inflammatory bowel disease (NCT02311660 and NCT01569503). Preliminary studies in animal models of systolic heart failure indicated that the attenuation of heart failure development was associated with pronounced anti-inflammatory effects [22,23]. We [24-27] and others [28] have shown that low level vagus nerve stimulation (LLVNS), at voltages substantially below the threshold for slowing the sinus rate, suppressed AF inducibility and decreased the duration of AF episodes. In those experiments, LLVNS applied to both vagal trunks dissected in the neck [24,26,27] or to the right vagus nerve alone [25], exerted equally strong anti-arrhythmic effects. Based

on the observation that, transcutaneous electrical stimulation of the tragus, the anterior protuberance of the outer ear, where the auricular branch of the vagus nerve is located, elicits evoked potentials in the brainstem in humans [29], we examined the effects of low-level tragus stimulation (LLTS) for inhibiting AF in a canine model of rapid atrial pacing [30]. Notably, the anti-arrhythmic effects of LLTS were comparable to those of LLVNS delivered to the cervical vagal trunk [30]. In contrast, LLTS after transection of both vagus nerves failed to show any antiarrhythmic effect, indicating that the efferent vagus nerves are essential for its antiarrhythmic effects [30]. Consistent with the anti-inflammatory effects of vagus nerve stimulation, we have recently shown in a first-in-man clinical trial, that in patients with drug-refractory AF, LLTS for just one hour, significantly suppressed AF and decreased systemic (but not cardiac) inflammatory cytokines, including tumor necrosis factor (TNF)-α [31]. These results support the use of LLTS as a novel non-pharmacological treatment modality for AF and possibly other conditions, where inflammation plays a major role, including HFpEF. Currently, there are no animal models that sufficiently recapitulate all the features of the HFpEF syndrome, to allow drug and device testing in animals before application to human studies [6]. It has been argued, however, that the lack of animal models should not prevent conducting human studies to test promising therapies [6]. In this proposal, we hypothesize that short-term LLTS may reverse diastolic dysfunction in a 2x2 cross over study in patients with diastolic dysfunction. These proof-of-concept investigations will establish the first evidence of the effects of LLTS on LV diastolic dysfunction in humans and may provide the basis for the design of human studies using this modality to target selected populations with HFpEF.

C. Preliminary studies

Neuromodulation suppresses AF and inflammation in humans:

We have recently shown that transcutaneous electrical stimulation of the auricular branch of the vagus nerve at the tragus (low-level tragus stimulation; LLTS) in humans has antiarrhythmic and anti-inflammatory effects [31]. Forty patients with paroxysmal AF who presented in sinus rhythm for AF ablation, were randomized to either 1 hour of LLTS (n=20) or control (n=20). LLTS in the right ear, 50% lower than the voltage that slowed the sinus rate, was accomplished by attaching a flat metal clip onto the tragus. Under general anesthesia, AF was induced at baseline and after 1 hour of LLTS or sham stimulation and were analyzed for inflammatory cytokines, including TNFα and CRP, using a multiplex immunoassay. Pacing-induced AF duration decreased significantly by 6.3±1.9 min compared to baseline in the LLTS group, but not in the control ($p=0.002$ for comparison between groups; Figure 2A). AF cycle length increased significantly from baseline by 28.8±6.5ms in the LLTS group, but not in the control ($p=0.0002$ for comparison between groups). Systemic (femoral vein) but not coronary sinus TNFα and CRP levels decreased significantly only in the LLTS group (Figure 2B). Importantly, the magnitude of decrease in TNF-α levels by LLTS was comparable with the difference between patients with active vs. inactive inflammatory diseases[32]. We concluded that LLTS acutely suppresses AF and decreases inflammatory cytokines in patients with paroxysmal AF, supporting the emerging paradigm of neuromodulation to treat AF [31].

D. Research Design and Methods

Specific Aim 1:

To examine the effect of short-term LLTS on echocardiographic markers of diastolic dysfunction in patients with diastolic dysfunction. This is a prospective, randomized, 2x2 cross over pilot study. Patients who have been diagnosed with diastolic dysfunction by echocardiogram within 36 months of study enrollment will be eligible for enrollment in the study. Patients will be excluded if they have any of the following: left ventricular dysfunction (left ventricular ejection fraction <40%), significant valvular disorder (i.e., prosthetic valve or hemodynamically significant valvular diseases), recent (<6 months) stroke or myocardial infarction, severe heart failure (class III or IV), recurrent vasovagal syncope, unilateral or bilateral vagotomy, pregnancy or nursing and sick sinus syndrome (without a pacemaker), 2nd or 3rd degree AV block, bifascicular block or prolonged (PR>300ms) 1st degree AV block**.** Patients will be recruited from the outpatient clinics of OUHSC. All patients will receive 2 separate, 1-hour sessions, at least 1 day apart, of active and sham LLTS, with the sequence of the sessions being randomized. Patients will be randomly assigned (1:1) to active/sham or sham/active LLTS. LLTS will be performed using a transcutaneous electrical nerve stimulation (TENS) device with electrodes attached to the tragus of the ear, which is innervated by auricular branch of the VN [33]. The SaluSTIM Digital TENS Unit distributed within the US by US Medical Inc will be used for LLTS or sham stimulation. The device will be connected to a clip electrode that will be attached to the external ear. In the active group, the ear clip electrode will be attached to tragus in the active stimulation group (Figure 3). This device was deemed as non-significant risk by the FDA (see attached letter). The same TENS protocol will be followed in sham LLTS arm, but the electrode will be placed on the ear lobe, which is devoid of vagus innervation [33]. The TENS unit will be set at a pulse width of 100 μs and a pulse frequency of 20 Hz. Amplitude will be titrated to the level of sensory threshold, typically in the range of 10–50 mA. In patients with pacemakers, the pacemaker will be tested with the TENS unit to assess for interaction. In case of interaction (the stimulation artifact of the TENS unit picked up by the pacemaker), the patients will be excluded from the study. Echocardiography will be performed after 30 minutes of LLTS or sham stimulation to assess diastolic function (Acuson SC2000, Siemens). Two-dimensional long axis and short axis LV images will be obtained and Pulse-wave Doppler spectra of mitral inflow (E and A waves), as well as mitral annulus tissue Doppler spectra will be recorded. The early diastolic mitral annulus velocity (e') will be used to assess diastolic function as previously described [34,35]. The ratio of early to late mitral inflow Doppler velocity (E/A ratio) is a marker of LV diastolic relaxation and stiffness, and the E/e' ratio correlates well with LV filling pressures [34,35]. In addition, LV diastolic strain, a sensitive marker of diastolic LV function, which independently predicts outcomes in patients with HFpEF [35,36], will be obtained off-line using a speckle-tracking algorithm (Acuson SC2000 eSie VVI ™).

Specific Aim 2:

To examine the effect of short-term LLTS on heart rate variability in patients with diastolic dysfunction. For this Aim, the same group assignment as in Aim 1 will be used. HRV is a marker

of vagus nerve activity and can be easily measured by software calculating the distance between consecutive R waves on the ECG [37]. Importantly, measures of HRV have been inversely correlated with inflammatory cytokines (C-reactive protein, IL-6) in the general population [38-40]. At each visit, serial 5-minute ECGs will be obtained for HRV analysis every 15 minutes of stimulation (total of 4 recordings). ECG will be obtained in the supine position after resting for 15 minutes. This test will done using a PC-Based ECG device. Analysis and interpretation of the HRV data will be performed in a blinded fashion.

Duration of participation:

Enrolled patients will participate for two 1-hour sessions, at least 1 day apart. No follow up is required for this study

Patient enrollment:

A maximum of 26 patients meeting the inclusion criteria and not meeting the exclusion criteria will be enrolled in the study. The enrollment period is expected to last for at least 12 months.

Data collection and analysis:

The final data will be pooled and analyzed by the investigators.

E. Statistical methods

Continuous variables will be compared between the groups using repeated measures analysis of variance (ANOVA). Significant interactions will be followed by time trend analyses stratified by intervention group. For all pair-wise testing, we will adjust for multiple comparisons using Tukey's method. Statistical significance will be declared at $p<0.05$.

Sample size and power calculations:

For purposes of sample size calculations we have simplified the analysis to focus on the difference in the E/e' ratio between the 2 intervention groups. Assuming a mean ± standard deviation E/e' ratio 15.2±6.4 [41] and 40% reduction in the LLTS group [31], 26 patients would provide greater than 80% power to detect the specified effect sizes at a two-sided α level of 0.05.

F. Gender/Minority/Pediatric Inclusion for Research

Participants will be age 18 or older. Race, minority status and gender will not affect enrollment.

G. Human participants

Twenty six participants of any gender/ethnic group, age 18 to 100 will be included.

Inclusion Criteria:

Male and female patients older than 18 year old

1. Patients who have been diagnosed with diastolic dysfunction by echocardiogram within 36 months of study enrollment, will be eligible for enrollment in the study.

Exclusion Criteria:

1. Left ventricular dysfunction (Left ventricular ejection fraction <40%)
2. Significant valvular disorder (i.e., prosthetic valve or hemodynamically relevant valvular diseases)
3. Recent (<6 months) stroke or myocardial infarction
4. Severe heart failure (NYHA class III or IV)
5. Recurrent vaso-vagal syncopal episodes
6. Unilateral or bilateral vagotomy
7. Pregnancy or breast feeding
8. Sick sinus syndrome (without a pacemaker), 2^{nd} or 3^{rd} degree AV block, bifascicular block or prolonged (PR>300ms) 1^{st} degree AV block
9. Hypotension due to autonomic dysfunction

2. ***Sources of research material:***

The research information will consist of echocardiographic parameters and HRV parameters.

3. ***Plans for recruitment and consent procedures to be followed:***

a. ***Location where consent is most likely to take place:***

Patients will be recruited from the outpatient clinics of the University of Oklahoma Health Sciences Center. The consent process will likely take place at the OUHSC Heart Rhythm Institute, 6^{th} Floor Children's Hospital. All candidate subjects will have the purpose of the study explained to them, including benefits,risks and options. Subjects, will be asked to read the consent form. After all questions have been answered, they will be asked to participate. Finally, they will be asked to sign the consent form.

b. Only English-speaking persons will be recruited. Non-English-speaking adults constitute less than 1% of the patients referred for ablation.

c. ***Measures to decrease coercion of participants:***

Patients will have adequate time to review the consent. Recruiting of employees or staff by the principal investigator will be avoided.

2. **Risks and assess likelihood and seriousness:**

Transcutaneous VNS has generally been shown to be safe and well tolerated with only minor side effects, like tingling sensation, dysesthesia, skin redness and pressure marks at the site of stimulation, painful stimulation, dizziness, mild dyspnea and headaches [42,43] [44]. No significant effects on heart rate, blood pressure, or peripheral microcirculation could be detected during short term tVNS in a sample of 10 patients with tinnitus [45] and in 22 healthy volunteers [46]. In a pilot study of 24 patients with tinnitus treated with tVNS over 3-10 weeks, 2 adverse cardiac events (one classified as a severe adverse event) were registered but considered very unlikely to have been caused by tVNS since other explanations for the symptoms were evident [42]. One patient had experienced sinus arrhythmic episodes already in the past, and in the other patient comorbid hypertension had caused concentric cardiac hypertrophy which might have contributed to the described temporary left bundle branch block. Retrospective analyses of ECG parameters revealed a trend toward shortening of the QRS complex by tVNS. This was observed after the 2 patients with cardiac adverse events were excluded from the analysis, but not when the whole sample of patients was analyzed. There was definitely no prolongation of the QRS complex which is a known predictor of cardiac morbidity and mortality. In conclusion, in subjects with no known pre-existing cardiac pathology, there has been no indication of arrhythmiogenic effects of tVNS [45] [42] [44] [43]. This is in line with the low incidence of adverse cardiac reactions during the long-term experience in more than 50,000 patients with implanted left VNS for treatment of epilepsy and depression [47].

The risks of the study are considered minimal. The potential benefits in knowledge and determining whether there may be potential usefulness of this treatment therefore justify these risks. TENS is a noninvasive, well- tolerated modality that has been extensively used for treatment of pain in various settings. TENS has not been associated with side effects more that minimal discomfort at the area of application. Based on the FDA information sheet guidance for IRBs, clinical investigators, and sponsors, it is considered a non-significant risk device: http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM126418.pdf. Previous studies examining TENS for vagus nerve stimulation in healthy individuals [48] and in patients with tinnitus [45] and epilepsy [49] showed that this approach was safe and well tolerated. The ECG procedure for recording of HRV is similar to a routine ECG and is not associated with side effects or additional risks. The TENS unit used in this study has been deemed non-significant risk by the FDA.

5. ***Procedures for protecting against or minimizing potential risks:***

Risk should be no greater than minimal risk. The TENS unit used in this study has been deemed non-significant risk by the FDA.

a. ***Measures instituted to protect the privacy and/or confidentiality of participant PHI:***

The patient charts will be obtained after informed consent. A representative data sheet will be constructed and approved for confidentiality. Names will be replaced by numbers and the subject's code and name will be kept in separate repositories.

6. ***Potential benefits and importance to the participants and others:***

Heart failure with preserved ejection fraction (HFpEF) has become a major public health concern. Epidemiological studies have shown that the prevalence and hospitalizations related to HFpEF are increasing, and the growing elderly population is expected to further worsen these trends. Outcomes of patients with HFpEF are poor, and so far, no treatment has been shown to decrease morbidity or mortality. If this therapy proves to be effective in reversing diastolic dysfunction, a hallmark of HFpEF, both study participants and others may benefit from this non-invasive treatment option with less risk involved. The results of this study may provide the basis for the design of human studies using this modality to target selected populations with HFpEF.

7. ***Why risks are reasonable in relation to benefits:***

Heart failure with preserved ejection fraction (HFpEF) has become a major public health concern. Epidemiological studies have shown that the prevalence and hospitalizations related to HFpEF are increasing, and the growing elderly population is expected to further worsen these trends. Outcomes of patients with HFpEF are poor, and so far, no treatment has been shown to decrease morbidity or mortality. These proof-of-concept investigations will establish the first evidence of the effects of LLTS on LV diastolic dysfunction and may provide the basis for the design of further human studies using this modality to target selected populations with HFpEF. In light of the increasing number of patients with HFpEF and the poor success of the currently available treatment options, an alternative approach such as the one tested in this study, may benefit a large number of patients in Oklahoma.

H. Data and Safety Monitoring Plan

1. ***Data and Safety Monitoring Plan (DSMP):***

An independent safety officer (Dr. Sunny Po) will be appointed to monitor for any potential risks associated with this therapy.

a. ***Reporting mechanisms for adverse events to the IRB, FDA, and NIH:***

Clinical staff will track the following from patient report, physical assessments and documentation of clinical emergencies, adverse events according to federally published grading (0=no adverse event; 1=mild; 2=moderate; 3=severe and undesirable adverse events; 4=life threatening/disabling; or 5=death) and attribution (1=unrelated; 2=unlikely; 3=possible; 4=probable; 5=definite) scales. Clinical staff will report findings to the Investigator, who will then advise the IRB, NIH and FDA as indicated. Given the mild risk nature of the study, composite reports will be reviewed every ten patients to assure that untoward events do not occur systematically, and dropout cases will be reviewed singly to learn whether further inquiry or modifications should be made to improve study implementation.

b. Adverse event (AE) grading

0=no AE; 1=mild; 2=moderate; 3=severe and undesirable AE; 4=life threatening/disabling; or 5=death

c. Plan for unanticipated AE reporting:

Unanticipated AE will be reported to the IRB.

d. Plan for annual reporting of AEs:

Use of IRB forms for reporting. Our data sheet will have a column detailing any AEs that might occur.

e***. Interim efficacy analysis is not applicable to this study.***

2. ***Data and Safety Monitoring Board (DSMB) that will be responsible for monitoring the study:***

- Institutional studies provide:
  a. Chair, members

  Dr. Sunny Po

  b. Frequency of safety reviews:

  After each study

I. Literature sited

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