CLINICAL SCIENCE

Transcutaneous auricular vagus nerve stimulation reduces pain and fatigue in patients with systemic lupus erythematosus: a randomised, double-blind, sham-controlled pilot trial

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ABSTRACT

Objectives Musculoskeletal pain and fatigue are common features in systemic lupus erythematosus (SLE). The cholinergic anti-inflammatory pathway is a physiological mechanism diminishing inflammation, engaged by stimulating the vagus nerve. We evaluated the effects of non-invasive vagus nerve stimulation in patients with SLE and with musculoskeletal pain.

Methods 18 patients with SLE and with musculoskeletal pain ≥4 on a 10 cm Visual Analogue Scale were randomised (2:1) in this double-blind study to receive transcutaneous auricular vagus nerve stimulation (taVNS) or sham stimulation (SS) for 4 consecutive days. Evaluations at baseline, day 5 and day 12 included patient assessments of pain, disease activity (PtGA) and fatigue. Tender and swollen joint counts and the Physician Global Assessment (PGA) were completed by a physician blinded to the patient's therapy. Potential biomarkers were evaluated.

Results taVNS and SS were well tolerated. Subjects receiving taVNS had a significant decrease in pain and fatigue compared with SS and were more likely (OR=25, p=0.02) to experience a clinically significant reduction in pain. PtGA, joint counts and PGA also improved. Pain reduction and improvement of fatigue correlated with the cumulative current received. In general, responses were maintained through day 12. Plasma levels of substance P were significantly reduced at day 5 compared with baseline following taVNS but other neuropeptides, serum and whole blood-stimulated inflammatory mediators, and kynurenine metabolites showed no significant change at days 5 or 12 compared with baseline.

Conclusion taVNS resulted in significantly reduced pain, fatigue and joint scores in SLE. Additional studies evaluating this intervention and its mechanisms are warranted.

Key messages

What is already known about this subject?

- Pain and fatigue are common symptoms voiced by patients with systemiclupus erythematosus (SLE).
- The inflammatory reflex is a physiological mechanism diminishing inflammation. The inflammatory reflex may be engaged by stimulation of the vagus nerve.
- Vagus nerve stimulation with a surgically implanted device has shown clinical benefit in uncontrolled studies in rheumatoid arthritis and inflammatory bowel disease.

What does this study add?

- Non-invasive stimulation of the vagus nerve in patients with SLE in a double-blind sham-controlled study resulted in a significant reduction of both pain and fatigue.

How might this impact on clinical practice or future developments?

- Transcutaneous auricular vagus nerve stimulation, a non-pharmacological, non-invasive, safe approach to alleviate pain and fatigue in SLE would fulfil an unmet clinical need.

INTRODUCTION

Musculoskeletal pain and fatigue are common symptoms in systemic lupus erythematosus (SLE), affecting up to 95% of patients and contributing to a reduced quality of life. Safe and efficacious treatment remains an unmet need. The inflammatory reflex is a physiological mechanism that attenuates the innate inflammatory response. Stimulation of the vagus nerve results in the reduction of inflammatory mediators and beneficial effects have been demonstrated in multiple animal models of disease.[1–13] Vagus nerve stimulation (VNS) administered by a surgically implanted stimulator has been shown to be efficacious in uncontrolled studies and safe in human inflammatory diseases such as rheumatoid arthritis (RA) and inflammatory bowel disease.[14 15] As the auricular branch of the vagus nerve innervates the cymba concha in the outer ear, the inflammatory reflex can be engaged non-invasively by stimulating this structure. Our objective was to obtain preliminary data evaluating the efficacy and safety of transcutaneous auricular vagus nerve stimulation (taVNS) in SLE and to explore the biological effects of this intervention.

PATIENTS AND METHODS

This pilot study was a randomised, double-blind, sham-controlled trial (ClinicalTrials.gov Identifier NCT02822989) of taVNS in subjects with SLE. Adult SLE subjects meeting 1997 Revised American College of Rheumatology (ACR) or Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE with self-reported pain of at least 4 on a 10 cm anchored Visual Analogue Scale (VAS) (corresponding to a high level of pain previously described in patients with SLE[16]) and inflammatory musculoskeletal symptoms (British Isles Lupus Assessment Group (BILAG) C or greater on the BILAG-2004 musculoskeletal domain) were recruited. Stable doses of Disease modifying Anti-Rheumatic Drugs (DMARDs), biological therapy and/or prednisone ≤10 mg/day were permitted, defined as no change of dose within 28 days prior to baseline. Pertinent exclusion criteria included a diagnosis of fibromyalgia, tobacco use and use of anticholinergic medication.

Eighteen subjects were randomised (2:1) using the Biostatistics Randomization Management System, a web-based HIPAA compliant software package to receive 5 min of taVNS or sham stimulation (SS) for 4 consecutive days at the Feinstein Institutes for Medical Research. For taVNS, a spring-loaded clip consisting of opposing conductive silicone electrodes was placed around the left ear with one electrode on the concha and the other behind the ear. Stimulation pulses (30 Hz frequency, 300 μs pulse width) were generated by a commercial transcutaneous electrical nerve stimulation (TENS) unit (Roscoe TENS 7000), and the amplitude was increased to the maximum amount tolerated by the subject without pain. All subjects were told that they may or may not feel any sensation from the stimulation. For SS, the battery was removed from the TENS unit, the electrode clip placed on the ear lobe (a location without vagus nerve innervation) and the dial on the TENS unit advanced. After each advance of the dial, the subject was asked if they felt anything. After three advances, subjects receiving SS were informed that the 'target stimulation had been reached'. SS was then delivered for 5 min. To evaluate the effect(s) and durability of taVNS, subjects received comprehensive assessments at baseline, day 5 and day 12 by a physician blinded to the subject's treatment; all patient assessments were performed by an investigator who was not present during the stimulation. To provide additional assurance that the assessing physician would not inadvertently uncover a subject's treatment allocation, all participants were reminded not to mention any aspects of the stimulation procedures to the evaluating physician and Case Report Forms (CRFs) containing data relevant to the stimulation were maintained in a separate location.

The primary objective was the effect of taVNS on musculoskeletal pain. Safety and tolerability were also assessed throughout the study. Secondary objectives included determination of effects of taVNS on fatigue, tender and swollen joint counts and patient and physician assessments (PtGA and PGA) of disease activity at days 5 and 12. Patients were additionally asked if they felt better, worse or the same. Mechanistic objectives aimed to explore potential mechanisms known to be involved in pain and inflammation that might be affected by VNS.

In this pilot study, per protocol, subjects not receiving four consecutive stimulations were replaced. This study was approved by the Northwell Health Institutional Review Board (HS16-0171) and informed consent was obtained from all study participants prior to the initiation of any study procedures. Patients were not directly involved in the design, recruitment or conduct of the study.

Table 1 Baseline characteristics of 18 subjects

	taVNS (n=12)	SS (n=6)
Female, n (%)	12 (100)	6 (100)
Age (years), mean (SD)	45.7 (11.7)	54.2 (15.3)
Race, n (%)		
Black/African American	4 (33)	3 (50)
White	7 (58)	3 (50)
Other	1 (9)	–
Baseline pain on 10 cm VAS, mean (SD)	6.7 (1.0)	5.6 (1.5)
Baseline fatigue on FACIT-F,* mean (SD)	23.0 (9.1)	15.8 (5.4)
Tender joints, mean (SD)	7 (8.7)	13.3 (8.9)
Swollen joints, mean (SD)	2 (2.2)	4.8 (4.1)
Baseline musculoskeletal BILAG C	3 (25%)	1 (16.7 %)
Baseline musculoskeletal BILAG B	9 (75%)	5 (83.3%)
Baseline SLEDAI-2K, mean (SD)	4.8 (2.1)	5.8 (2.2)

*Higher FACIT-F scores correspond to lower fatigue.
BILAG, British Isles Lupus Assessment Group; FACIT F, Functional Assessment of Chronic Illness Therapy Fatigue Subscale; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index-2K; SS, sham stimulation; taVNS, transcutaneous auricular vagus nerve stimulation; VAS, Visual Analogue Scale.

Laboratory assessments

Laboratory assessments were performed on specimens collected at baseline before taVNS/SS, day 5 and day 12. Serum and plasma were batched and stored at −80°C until analysis. Commercial laboratory assessments including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), C3, C4 and anti-dsDNA were conducted at the Northwell Core Laboratories, Manhasset, New York, USA. HMGB1 ELISA (IBL International GmbH (Hamburg Germany), substance P ELISA (Cayman Chemical, catalogue 583751), neuropeptide Y ELISA (Millipore Sigma, catalogue EZHNPY-25K), calcitonin gene-related peptide (CGRP) EIA kit (Cayman Chemical, catalogue 589101), IL1RA, interleukin (IL)-18 multiarray assay (Meso Scale Discovery, catalogue K15067M-1) were performed according to the manufacturers' instructions. Serum levels of interferon (IFN)α, IL-1, IL-8, IL-10 and tumour necrosis factor (TNF) were determined at the Myriad RBM Central Laboratory using standardised Luminex multianalyte profiling. Assessments of components of the kynurenine pathway of tryptophan degradation (tryptophan, kynurenine and quinolinic acid) were performed by Charles River Laboratories (San Francisco, California, USA) using high-performance liquid chromatography with tandem mass spectrometry.

Cytokine release by unstimulated whole blood or whole blood stimulated by TLR 4, 7 and 9 agonists was determined using the TruCulture Myriad self-contained system. Whole blood was collected into null TruCulture tubes or TruCulture tubes containing 0.1 µg/mL LPS, 1 µg/mL gardiquimod or 30 µg/mL CpG/ODN2216. After incubation for 24 hours at 37°C, the supernatant was removed and stored at −80°C. Two panels of stimulated inflammatory mediators, HumanCytokine MAP A and MAP B, were measured in the supernatant by Myriad RBM Laboratories using a bead-based multiplex immunoassay.

Statistical analyses

The sample size of 18 subjects was based primarily on feasibility as there was no previous experience of taVNS in SLE.

The Wilcoxon Rank Sum test was used to compare the change in endpoints from baseline to day 5 and from baseline to day 12 in subjects receiving taVNS or SS and the Spearman Rank Order correlation was used to assess the strength of potential

Table 2 Change from baseline of trial endpoints

	Day 5–Day 1			Day 12–Day 1		
T	taVNS median (IQR)	SS median (IQR)	P value	taVNS median (IQR)	SS median (IQR)	P value
Δ VAS pain (0–10 mm)	−5.00 (−5.80 to −3.10)	0.10 (−10.0 to 1.0)	0.049	−5.35 (−5.80 to −1.45)	0.15 (−0.60 to 0.70)	0.079
Δ FACIT-F†	11.00 (4.50 to 16.00)	0.00 (−2.00 to 1.00)	0.003	12.00 (5.50 to 18.25)	−2.00 (−3.00 to 1.00)	0.003
% Tender joint reduction	100.0 (100.0 to 100.0)	5.27 (-11.1,80.0)	0.005	98.49 (50.0 to 100.0)	10.00 (0.00 to 34.61)	0.050
% Swollen joint reduction‡	100.0 (100.0 to 100.0)	9.09 (−8.33 to 57.15)	0.019	100.0 (80.0 to 100.0)	14.29 (−100.0 to 59.09)	0.056
Δ PtGA (0–100 mm)	−22.50 (−46.50 to −2.50)	4.00 (−2.00 to 9.00)	0.125	−18.50 (−64.00 to −2.00)	−0.52 (−16.00 to 9.00)	0.301
Δ PGA (0–3)	−0.51 (−0.99 to −0.30)	0.04 (−0.06 to 0.12)	0.053	−0.50 (−0.84 to −0.08)	0.03 (0.03 to 0.12)	0.107
Δ CRP (mg/dL)	0.00 (−0.23 to 0.00)	0.05 (-0.10 to 0.15)	0.165	0.00 (−0.20 to 0.00)	-0.05 (−0.23 to 0.08)	1.000
Δ Serum cytokine						
IFNα	0.00 (0.00 to 0.00)	0.12 (−0.12 to 1.10)	0.871	0.00 (−0.01 to 0.00)	0.25 (0.00 to 1.00)	0.080
IL-1β	−0.01 (−0.40 to 0.20)	−0.01 (−0.02 to 0.02)	0.820	−0.01 (−0.04 to 0.00)	−0.04 (−0.05 to 0.04)	0.874
IL-8	−0.21 (−2.25 to −0.01)	0.77 (−1.10 to 1.80)	0.144	−0. 17 (−2.54 to 0.45)	−0. 20 (−2.80 to 0.10)	0.963
IL-10	0.05 (−0.10 to 1.10)	−0.08 (−0.16 to 0.30)	0.600	0.12 (−0.17 to 0.23)	0.04 (−0.10 to 0.40)	0.569
TNF	−0.05 (−0.25 to 0.12)	0.00 (−0.80 to 0.20)	0.943	−0.05 (−0.25,0.12)	−0.05 (−0.20 to 0.20)	0.848
Il-6	−0.39 (−2.89 to −0.03)	0.17 (−3.35 to 2.6)	0.112	−0.17 (−3.13 to 0.67)	−0.20 (−3.35 to 0.25)	0.960
IL1-RA	−0.06 (−1.73 to 0.66)	2.92 (−2.63 to 10.30)	0.603	0.41 (−10.68 to 1.73)	−0.39 (−2.73 to 2.32)	0.741
Il-18	0.71 (−0.23 to 8.20)	−0.21 (−2.47 to 0.92)	0.208	−0.55 (−2.98 to 0.48)	0.10 (−9.37 to 1.21)	0.603
Δ Plasma neuropeptide						
Substance P	−2.76 (−4.79 to 0.94)	0.09 (−5.57 to 4.48)	0.008	4.90 (−2.41 to 8.23)	5.08 (0.24 to 9.98)	0.335
Neuropeptide Y	−0.80 (−7.43 to 2.28)	−2.25 (−4.38 to 5.48)	0.509	0.30 (−6.05 to 5.20)	2.0 (−1.38 to 14.98)	0.242
CGRP	0.0 (−2.0 to 4.7)	0.0 (−1.98 to 2.40)	0.960	−0.7 (−17.15 to 0.83)	0.0 (−1.68 to 0.99)	0.484
Δ Kynurenine pathway						
Kynurenine	−0.02 (−0.35 to 0.18)	0.155 (−0.06 to 0.51)	0.121	−0.17 (−0.56 to 0.18)	−0.27 (−0.01 to 0.65)	0.055
Quinolinic acid	−51.0 (−99.5 to −14.75)	−35 (−173.5 to 28.75)	0.674	−100.0 (−138 to −41.75)	−12.5 (−74.75 to 62.0)	0.121
Kynurenine/tryptophan	0.001 (−0.004 to 0.005)	−0.002 (−0.011 to 0.010)	0.603	−0.003 (−0.007 to 0.008)	0.001 (−0.004 to 0.008)	0.603

*Negative scores correspond to a reduction in the measured endpoint from baseline.
†Higher FACIT-F scores correspond to lower fatigue.
‡Data shown for seven taVNS and five SS subjects with swollen joints at baseline.
CGRP, calcitonin gene-related peptide ; CRP, C-reactive protein; IFNα, interferon alpha; IL, interleukin; IL1-RA, interleukin-1 receptor antagonist; PGA, Physician Global Assessment ; PtGA, Patient Global Assessment of disease activity ; SS, sham stimulation ; taVNS, transcutaneous auricular vagus nerve stimulation; TNF, tumour necrosis factor; VAS, Visual Analogue Scale.

associations between endpoints. A response was defined as a reduction in pain from baseline to day 5 of at least 1.58, as a 1.58 decrease on a 10 cm VAS is considered clinically meaningful.[17] An odd's ratio (OR) was determined to compare the odds of achieving a response between the two treatment groups. The odds of achieving a meaningful change in fatigue measured by the FACIT-F (a 4-point change)[18] was similarly determined. The Spearman rank-order correlation coefficient was used to examine the relationships between pain and fatigue and between cumulative current and changes in pain and fatigue.

RESULTS

Baseline characteristics of the 18 subjects completing the four daily stimulations (taVNS or SS) are shown in table 1, with no significant differences in any parameter between the two arms. All subjects noted musculoskeletal pain with tender and/or swollen joints and fatigue at baseline. One subject was replaced following two stimulations after developing an upper respiratory infection during the influenza season.

After four consecutive stimulations, subjects receiving taVNS achieved a significantly greater reduction in their pain compared with SS, (−5.00 vs 0.10, p=0.049) (table 2 and figure 1). As a clinical response was noted in 10 of 12 (83.3%) taVNS subjects, and 1 of 6 (16.7%) SS subjects, the odds of achieving a meaningful reduction in pain was 25 times greater in subjects receiving taVNS compared with subjects receiving SS (p=0.02). Subjects receiving taVNS also experienced a significant improvement of fatigue compared with subjects receiving to SS (table 2 and figure 2) and the odds of achieving a meaningful reduction in fatigue was 54.6 times greater in subjects receiving taVNS compared with those receiving SS (p=0.014), 10 of 12 taVNS subjects and 0 of 6 SS subjects achieved a meaningful reduction in fatigue. The change of reported pain at day 5 from baseline correlated significantly with the change in fatigue (r=0.69, p=0.013). Moreover, subjects receiving taVNS were more likely to report an overall improvement in their condition on day 5 compared with baseline on a Likert scale. Additionally, a greater numerical decrease from baseline to day 5 of both PtGA and

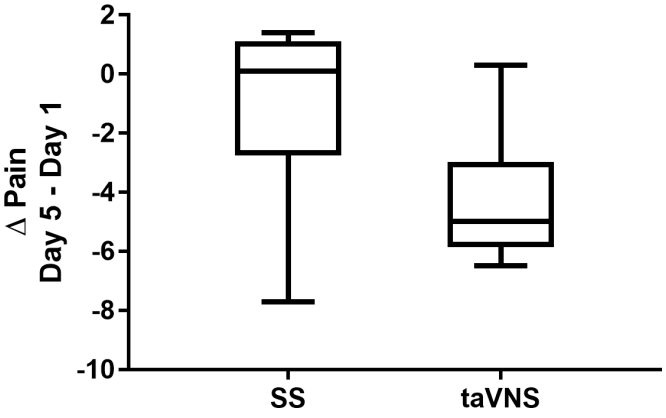

Figure 1 Change in patient-reported pain determined by a 10 cm VAS from baseline to day 5 (day 5–day 1) in subjects receiving SS or taVNS (p<0.05). SS, sham stimulation; taVNS, transcutaneous auricular vagus nerve stimulation; VAS, Visual Analogue Scale.

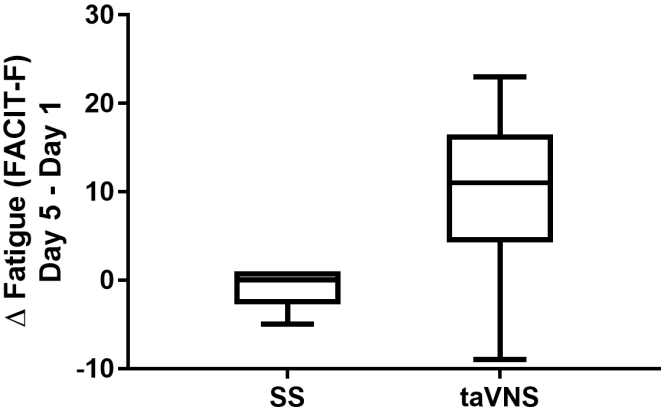

Figure 2 Change in patient-reported fatigue measure by the FACIT-F from baseline to day 5 (day 5–day 1) in subjects receiving SS or taVNS (p=0.003). An increase in the Functional Assessment of Chronic Illness Therapy Fatigue Subscale (FACIT-F) score correlates with less fatigue. SS, sham stimulation; taVNS, transcutaneous auricular vagus nerve stimulation; VAS, Visual Analogue Scale.

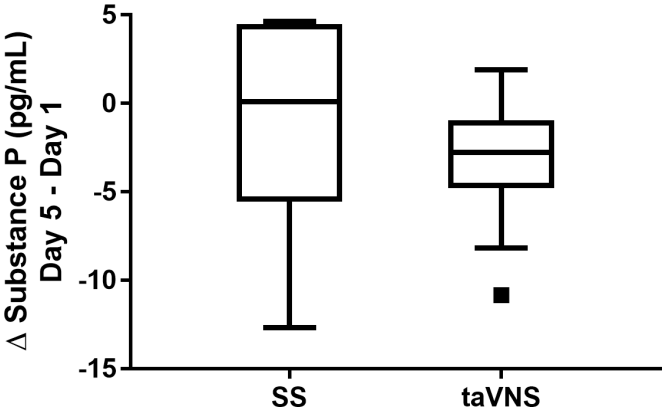

Figure 3 Change in plasma levels of substance P (pg/mL) from baseline to day 5 (day–5 day 1) in subjects receiving SS or taVNS (p=0.008). SS, sham stimulation; taVNS, transcutaneous auricular vagus nerve stimulation.

PGA was observed in subjects receiving taVNS compared with subjects receiving SS (table 2), however these differences were not statistically significant (p=0.125, p=0.053, PtGA, PGA respectively). Both the reduction in pain and the improvement of fatigue significantly correlated with the cumulative current received over 4 days of VNS (r=0.49, p=0.04, pain, r=0.83, p=0.003, fatigue). In general, these improvements continued through day 12.

Tender and swollen joints were present at baseline with no significant differences between the two groups. The median reduction of both tender and swollen joints for subjects receiving taVNS was 100%, compared with a median reduction of 5.3% tender and 9.1% reduction of swollen joints in subjects receiving SS (p=0.005, p=0.019, tender and swollen, respectively) (table 2).

Safety

taVNS was well tolerated with no adverse events attributed to the stimulation. There were no reports of headache, lightheadedness, tinnitus, ear irritation or changes to the external skin of the outer ear.

Mechanistic analyses

Baseline ESR and serum levels of High Mobility Group Box 1 (HGMB1) and CRP were low in this population with no significant changes from baseline to day 5 in either arm. Similarly, there were no significant changes in serum levels of IFNα, IL-1β, IL-6, IL-8, IL-10, IL1RA, IL-18 or TNF (table 2), and no significant changes in levels of C3, C4 or anti-DNA antibody titers. Levels of proinflammatory cytokines after stimulation with TLR 4, 7 or 9 agonists for 24 hours were variable with no differences observed from baseline to day 5 between subjects receiving taVNS or SS (data not shown). However, plasma levels of the neuropeptide substance P, were significantly lower in subjects receiving taVNS compared with those receiving SS at day 5 than at baseline p=0.008 (table 2, figure 3). Given the significant change observed between groups in levels of substance P, we evaluated two additional neuropeptides, neuropeptide Y and CGRP, but detected no significant difference between groups in plasma levels of either of these neuropeptides from baseline to day 5 (table 2). Lastly, the examination of changes from baseline of kynurenine and quinolinic acid levels and the kynurenine/tryptophan ratio, which have previously been shown to associate with severe fatigue in SLE,[19] did not correlate with a reduction in fatigue and showed no significant change at day 5 or 12 from baseline between subjects receiving taVNS compared with SS (table 2).

DISCUSSION

VNS is an approved treatment for refractory epilepsy, depression and migraine headaches. As stimulation of the vagus nerve engages the cholinergic anti-inflammatory pathway, this modality offers a promising, non-toxic intervention for the treatment of inflammatory disease. Clinical efficacy of VNS has been suggested in uncontrolled studies in other inflammatory diseases. In one small open-label pilot study, five of seven biologically naïve patients with active Crohn's disease received daily VNS administered by a surgically implanted device. Significant improvement was demonstrated on the Crohn's Disease Activity Index (CDAI). Moreover, CDAI remission was achieved in four of five patients at 6 months with only one patient requiring ongoing immunosuppressive medication. Inflammatory markers, that is, serum CRP and faecal calprotectin, were also significantly diminished.[15] A second pilot study included eight subjects with active Crohn's disease who were refractive to biological treatment and given 16 weeks of daily VNS delivered by a surgically implanted device.[20] At week 16, CDAI scores were significantly reduced meeting a predefined target reduction of 70 in six of eight patients; three patients achieved CDAI remission. Inflammatory markers (CRP and faecal calprotectin) were reduced in patients who exhibited clinical response.

An open-label study of VNS was completed in 18 patients with RA.[14] Subjects (eight non-responsive to methotrexate and 10 non-responsive to biologics) received daily stimulation delivered by an implanted device. At day 42, the significant improvement of Disease Activity Score (DAS) disease activity was observed in both cohorts and a EULAR response was achieved in 7 of 8 and 6 of 10 patients in each group. TNF secretion by ex vivo LPS-stimulated whole blood was attenuated by daily VNS and circulating levels of IL-6 were significantly reduced in those patients with a EULAR response. The treatment was well tolerated and the observed adverse events were those known to associate with an implanted device (transient hoarseness and events related to the actual surgery). Importantly, no infections were observed during this study. A study of VNS in treatment-resistant RA is ongoing in US centres (ClinicalTrials.gov Identifier: NCT03437473).

More recently, the effect of VNS on fatigue in patients with Sjogren's disease was evaluated using the gammaCore device.[21] This device stimulates the vagus nerve transcutaneously at the neck. In this uncontrolled 26-day open-label study, 15 patients received stimulation two times per day. Patients reported a significant reduction of fatigue. Moreover, LPS-stimulated production of IL-6, IL-1β, IP-10, MIP1α, IL-1β, TNF-α, IL-6 and IP-10, was also significantly reduced.

We now show that a short course of taVNS administered once daily for 4 consecutive days via non-invasive external electrodes to the auricular branch of the vagus nerve results in a significant reduction of pain and fatigue in patients with SLE. Our study population included individuals with significant pain and exemplifies the unmet need for adequate control of pain and fatigue in SLE. Importantly, this was a double-blind, sham-controlled study and neither the subject nor assessor was aware of a subject's intervention. Objective outcomes, that is, tender and swollen joint counts, were also significantly reduced in subjects receiving taVNS compared with those receiving SS. The stimulation was well tolerated with no adverse events attributed to the intervention, and, clinical benefits continued after taVNS was stopped.

Despite the impressive clinical benefits observed on pain and fatigue in our study in SLE after only 4 days of stimulation, we did not detect significant changes in circulating levels of most potential biomarkers. Reductions in serum proteins observed in studies in RA, inflammatory bowel disease or Sjogren's disease following VNS were reported following weeks or months of VNS[14 15 20] and four daily stimulations may not have been sufficient to effect changes in circulating levels of inflammatory markers, cytokines or components of the kynurenine pathway.

Previous studies investigating the effects of VNS have used ex vivo stimulation of whole blood with LPS before and after VNS to demonstrate the anti-inflammatory effects of engaging the inflammatory reflex and have shown that levels of LPS-stimulated proinflammatory cytokines including IL-1, IL-6 and TNF are reduced following VNS.[1 22–24] Decreased measurements of TNF, IL-1b, MCP-1 and IL-8 have also been observed in whole blood incubated, but not stimulated for 24 hours.[25] We, therefore, stimulated whole blood ex vivo, but did not observe reductions of inflammatory mediators or chemokines on day 5 or 12 in whole blood stimulated with TLR 4, 7 or 9 agonists, nor did we demonstrate a reduction of mediators in unstimulated whole blood after incubation for 24 hours. These assays were performed on day 5, 24 hours following the last stimulation and day 12. We do not know whether analysis of whole blood obtained shortly after stimulation of the vagus nerve would have resulted in different findings. Alternatively, the whole blood may have been overstimulated ex vivo with the stimulant concentrations used so that the anti-inflammatory biological effects of VNS could not be detected by these assays.

We did observe the change in plasma levels of substance P following 4 days of stimulation suggesting that the biological responsiveness of this neuropeptide to taVNS may be more rapid or sensitive than that of cytokines. Our finding of a reduction of plasma levels of substance P in subjects receiving taVNS but not in control subjects receiving SS is of interest, because substance P not only facilitates the transmission of nociceptive signals from the periphery to the brain but also has proinflammatory properties. In RA, a positive association between the levels of substance P and inflammation has been proposed.[26] The role of substance P in the inflammatory pain in SLE merits additional investigation.

The results of our short, sham-controlled pilot study engaging the cholinergic anti-inflammatory pathway by non-invasive stimulation of the vagus nerve for treatment of inflammatory musculoskeletal pain and fatigue in SLE are promising. Although we have not yet fully identified the molecular pathway(s) responsible for the observed clinical response, our findings suggest that SLE inflammatory symptoms are responsive to VNS and that substance P is affected by the cholinergic anti-inflammatory pathway. A non-toxic, non-pharmacological approach for control of these common SLE symptoms would be welcome. Additional studies of this intervention applied over a longer period of time are needed to assess the durability of the effects of VNS on pain, fatigue and other manifestations of SLE. A better understanding of the cellular and molecular pathways downstream of VNS are needed as well as biomarkers to identify those who will respond or that are early indicators of sustained response.

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Contributors CA, MM, CB, KJT and BD: study design. CA, YA-F, EA, SC, TPZ, TD-C: data acquisition. CA, YA-F, ML, MM, EA, SC, TDZ, TD-C, CB, KJT and BD: data analysis. CA, YA-F, ML, MM, EA, SC, TDZ, TD-C, CB, KJT and BD: interpretation of findings. CA, YA-F, ML, MM, EA, SC, TDZ, TD-C, CB, KJT and BD: preparation of manuscript. All authors approved the final manuscript.

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Competing interests KJT reports a financial relationship with Set Point Medical and My String; Prof. CB and Assistant Professors TPZ and Datta-Chaudhuri have a provisional patent application: "Auricular stimulation device, system and methods of use".

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. All data relevant to the study are included in the article or uploaded as supplementary information.

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