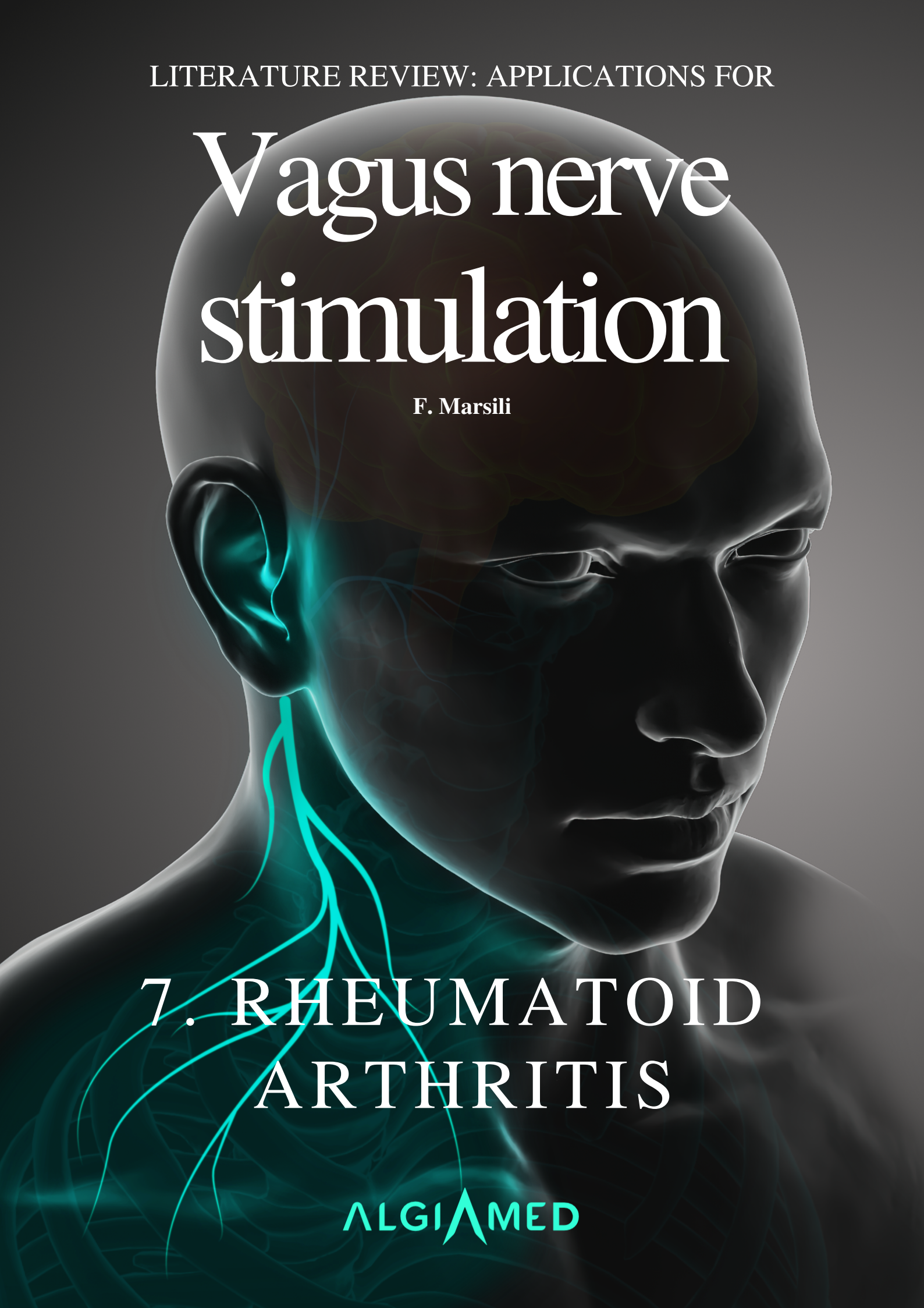
LITERATURE REVIEW: APPLICATIONS FOR

Vagus nerve stimulation

F. Marsili



7. RHEUMATOID ARTHRITIS

ALGIAMED

Author's choice

The papers in this collection focus on the application of Vagus Nerve Stimulation (VNS) as established therapeutic solution for difficult-to-treat conditions.

The vagus nerve is the longest cranial nerve and is widely distributed throughout the body, traversing the neck, thorax and abdomen. It is composed by motor fibres and sensory fibres from sympathetic and parasympathetic branches. [1], [2]. Afferent branches of the vagus nerve innervate brain behavioural areas involved in depressive states, and it desynchronises cortical activity with anti epileptic effects [3], [4]. Efferent branches of the vagus nerve regulate gastrointestinal secretory and motor function [5]. Recent advances in the field, have unraveled an anti-inflammatory role of the efferent vagus nerve via the Cholinergic Anti-inflammatory Pathway (CAP), a known mechanism for neural inhibition of inflammation linked to the activation of the autonomic nervous system (ANS) [6], [7].

Electrical stimulation of the VN modulates the nervous system at central, peripheral, and autonomic levels without the need for pharmacological interventions. For decades, invasive techniques of VNS have demonstrated their clinical efficacy in VN-related diseases and, to these days, efforts have been made to create a more safe, effective, and non-invasive solution to VNS.

The auricular branch is the only peripheral branch of the VN on the human body, it is part of the afferent portion of the VN that directly connects to the brainstem. Thus, auricular VN has become the most favourable access point for non-invasive VNS. Neuroimaging studies on animal models and humans have confirmed the modulatory efficacy of auricular VNS (aVNS). For examples, fMRI studies show identical activation patterns in the brain between invasive and aVNS, with significant inhibitory and anti-inflammatory effects. Due to the existence of different control systems, the anti-inflammatory effects of aVNS (i.e., release of norepinephrine and noradrenaline, and neurotrophic factors) seem to occur immediately after intervention, while neuroplastic changes only occur as a consequence of sustained regenerative efforts [7].

Colleciton 1 and collection 2 are the most extensive selections, since VNS has been standard-of-care for epilepsy and depression for decades. Collection 3 explores the possibility of using VNS for the treatment of posttraumatic stress disorders. Collection 4 focuses on fibromyalgia and collection 5 on multiple sclerosis. Collection 6 and 7 corroborates the hypothesis that VNS can be used to activate the cholinergic anti-inflammatory pathway to treat inflammatory diseases, such as inflammatory bowel disease or rheumatoid arthritis. Collection 8 and 9 focus on the use of VNS for ameliorating pain sensitivity in chronic pain conditions and for rehabilitating upper limb motor fibres after ischemic strokes, respectively. In conclusion, collection 10 opens up other possibilities for clinical applications of VNS, ranging from cardiovascular diseases, through ADHD disorders, to tinnitus.

To summarise, VNS is a novel technology and its non-invasive configuration is still under investigation. Further clinical examinations are mandatory in order to understand the underlying mechanism of VNS and to open the door to new possible therapeutic applications. However, being a non-invasive, safe, and efficient therapeutic solution, VNS is an attractive tool for further implementation and new creative clinical applications.

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7. VNS and rheumatoid arthritis

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Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis

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Rheumatoid arthritis (RA) is a heterogeneous, prevalent, chronic autoimmune disease characterized by painful swollen joints and significant disabilities. Symptomatic relief can be achieved in up to 50% of patients using biological agents that inhibit tumor necrosis factor (TNF) or other mechanisms of action, but there are no universally effective therapies. Recent advances in basic and preclinical science reveal that reflex neural circuits inhibit the production of cytokines and inflammation in animal models. One well-characterized cytokine-inhibiting mechanism, termed the "inflammatory reflex," is dependent upon vagus nerve signals that inhibit cytokine production and attenuate experimental arthritis severity in mice and rats. It previously was unknown whether directly stimulating the inflammatory reflex in humans inhibits TNF production. Here we show that an implantable vagus nerve-stimulating device in epilepsy patients inhibits peripheral blood production of TNF, IL-1β, and IL-6. Vagus nerve stimulation (up to four times daily) in RA patients significantly inhibited TNF production for up to 84 d. Moreover, RA disease severity, as measured by standardized clinical composite scores, improved significantly. Together, these results establish that vagus nerve stimulation targeting the inflammatory reflex modulates TNF production and reduces inflammation in humans. These findings suggest that it is possible to use mechanism-based neuromodulating devices in the experimental therapy of RA and possibly other autoimmune and autoinflammatory diseases.

vagus nerve | rheumatoid arthritis | inflammatory reflex | tumor necrosis factor | cytokines

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovial inflammation in the musculoskeletal joints resulting in cartilage degradation and bone destruction with consequent disability (1). The prevalence exceeds 1.3 million adult cases in the United States, with attributable medical costs estimated between $19–39 billion (2, 3). Standard therapies include glucocorticoids, methotrexate, monoclonal antibodies, and other pharmacological agents targeting inflammatory mechanisms (4). Despite these treatment options, many RA patients fail to respond, instead persisting with poor health, shortened life span, and significant impairments in quality of life affecting work, leisure, and social functions (5, 6). Thus, there remains a significant need for alternative therapeutic approaches.

Recent advances at the intersection of immunology and neuroscience reveal reflex neural circuit mechanisms regulating innate and adaptive immunity (7, 8). One well-characterized reflex circuit, termed the "inflammatory reflex," is defined by signals that travel in the vagus nerve to inhibit monocyte and macrophage production of tumor necrosis factor (TNF) and other cytokines (7). Electrical stimulation of the vagus nerve in animals (e.g., mouse, rat, and dog) stimulates choline acetyltransferase-positive T cells to secrete acetylcholine in spleen and other tissues (9). Acetylcholine is the cognate ligand for α-7 nicotinic acetylcholine receptors (α7nAChR) expressed on cytokine-producing monocytes, macrophages, and stromal cells (7, 10, 11). Ligand binding inhibits the nuclear translocation of NF-κB and inhibits inflammasome activation in macrophages activated by exposure to lipopolysaccharide (LPS), other Toll-like receptor (TLR) ligands, and other proinflammatory stimulating factors (12, 13).

Inflammatory reflex signaling, which is enhanced by electrically stimulating the vagus nerve, significantly reduces cytokine production and attenuates disease severity in experimental models of endotoxemia, sepsis, colitis, and other preclinical animal models of inflammatory syndromes (7, 8, 14–16). In experimental collagen-induced arthritis, vagotomy or selective disruption of α7nAChR worsened disease severity, and administration of nicotine or other selective α7nAChR agonists, ameliorated disease severity (17, 18). Vagus nerve stimulation delivered once daily for 60 s with an

Significance

Rheumatoid arthritis (RA) is a chronic, prevalent, and disabling autoimmune disease that occurs when inflammation damages joints. Recent advances in neuroscience and immunology have mapped neural circuits that regulate the onset and resolution of inflammation. In one circuit, termed "the inflammatory reflex," action potentials transmitted in the vagus nerve inhibit the production of tumor necrosis factor (TNF), an inflammatory molecule that is a major therapeutic target in RA. Although studied in animal models of arthritis and other inflammatory diseases, whether electrical stimulation of the vagus nerve can inhibit TNF production in humans has remained unknown. The positive mechanistic results reported here extend the preclinical data to the clinic and reveal that vagus nerve stimulation inhibits TNF and attenuates disease severity in RA patients.

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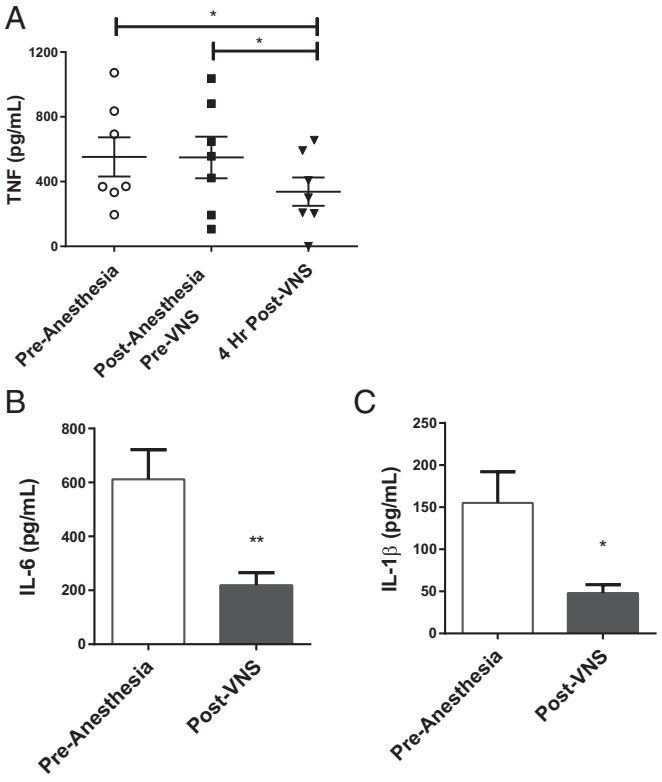

Fig. 1. Inflammatory reflex activation reduces whole-blood LPS-induced TNF production in epilepsy patients. Electrical stimulation of the vagus nerve in humans inhibits whole-blood LPS-induced TNF release. Blood was obtained from epilepsy patients ($n = 7$) undergoing implantation of a vagus nerve-stimulation device at different time points: before anesthesia induction and before vagus nerve stimulation; after anesthesia induction and before vagus nerve stimulation (pre-VNS); and 4 h after vagus nerve stimulation (post-VNS). Whole blood was incubated with LPS and TNF (*A*), IL-6 (*B*), and IL-1β (*C*) levels in plasma were determined after 4 h in culture. The significance of the differences between mean values at each time point was tested by unpaired ANOVA (*$P < 0.05$, **$P < 0.01$). Data are shown as mean ± SEM.

implanted device attenuated joint swelling, inhibited cytokine production, and conferred significant protection against synovitis and periarticular bone erosions (19, 20). Accordingly, we reasoned that it might be possible to modulate cytokine levels and inflammation using an active implantable medical device in humans (20).

Vagus nerve-stimulating devices have been used for decades in patients with refractory epilepsy and have been used more recently in patients with depression. These devices have been implanted in more than 100,000 patients, are relatively well tolerated, and have not been associated with immunosuppression or long-term complications (21, 22). We implanted a cohort of epilepsy patients with a vagus nerve-stimulating device and observed that transient delivery of electrical current during general anesthesia significantly inhibited TNF production in peripheral blood monocytes. A subsequent study of 17 RA patients in an 84-d open-label trial also revealed significantly decreased TNF production and significantly improved clinical signs and symptoms of disease.

Results

To determine whether vagus nerve stimulation inhibits TNF production in humans, we studied seven epilepsy patients [five male, two female; mean age 35 y (range 25–43 y)] who were implanted with a vagus nerve-stimulating device using a coiled cuff electrode (Cyberonics) on the left cervical vagus nerve. These patients had no history of inflammatory or autoimmune disorders. Peripheral blood was collected before, during, and after vagus nerve stimulator implantation surgery. Endotoxin was added to the whole blood to stimulate the production of TNF by monocytes for 4 h (13, 23). The application of current-controlled electrical pulses (single 30-s stimulation at 1.0-mA output current, 20-Hz pulse frequency, 500-μs pulse duration) significantly inhibited whole-blood TNF production compared with baseline levels before electrical stimulation (Fig. 1*A*). The inhibition of TNF release following vagus nerve stimulation during general anesthesia cannot be attributed to a placebo effect, because the subjects were unconscious and were not aware of the nerve stimulation. Whole-blood production of interleukin (IL)-6 and IL-1β was also inhibited significantly by vagus nerve stimulation (Fig. 1 *B* and *C*). To our knowledge, this is the first report that the delivery of electric current applied directly on the cervical vagus nerve to stimulate the inflammatory reflex inhibits the endotoxin-induced release of TNF, IL-1β, and IL-6 in humans.

We next studied the effects of vagus nerve stimulation in patients with RA. At enrollment the 18 study patients had active disease, with at least four tender and four swollen joints (of a 28-joint count), despite methotrexate therapy for at least 3 mo on a stable dose. One patient from cohort I, who fulfilled the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification for RA, was later diagnosed with Whipple disease and was excluded from the efficacy analysis. This patient is included in the baseline patient characteristics (Table 1) and adverse-event data (Table S1). The RA patients with active disease were studied in two cohorts. Cohort I ($n = 7$) included patients with active disease despite therapy with methotrexate. They had never received a biological TNF antagonist or had previously failed treatment with TNF antagonists because of drug toxicity. Cohort II ($n = 10$) included patients who had failed conventional therapy with methotrexate and also had failed treatment with at least two biological agents differing in mechanisms of action (e.g., anti-TNF, anti–IL-6 receptor, anti-CD20 antibodies, and/or T-cell costimulation inhibitor). There were no deaths, serious adverse events, withdrawals from the study because of adverse events, or infections in either cohort. In agreement with known risks of the procedure, nine patients experienced mild or moderate adverse events associated with implanting the vagus nerve stimulator on the left cervical vagus nerve (Table S1).

The study design schematic is shown in Fig. 2*A*. The vagus nerve was stimulated during surgery (day −14) to measure electrode impedance and to verify device function. During the 14-d postoperative recovery period (day −14 to day 0), the device was turned off, and no current was delivered to the vagus nerve. On the first treatment day (study day 0), patients received a single 60-s stimulation with electric current pulses of 250-μs duration at 10 Hz and an output current between 0.25–2.0 mA, as tolerated. No further stimulation was delivered for 7 d. On study day 7, the output current was adjusted to the highest amperage tolerated, up to 2.0 mA; this level of current was subsequently delivered once daily for 60 s in 250-μs pulse widths at 10 Hz. Current escalation up to the highest tolerated amperage (up to 2.0 mA) was repeated weekly until day 28. At that visit the frequency of daily stimulation events was increased to four times daily in patients who had not achieved a moderate or good clinical response according EULAR criteria (24). On day 28, the output current delivered was comparable in both cohorts: cohort I output current was 1.29 ± 0.37 mA (mean ± SD); cohort II output current was 1.60 ± 0.36 mA. In cohort I, two of seven patients received electric current pulses four times daily. In cohort II, 6/10 patients received electric current pulses four times daily.

We observed that TNF production in cultured peripheral blood obtained from the combined RA study cohort on day 42 was significantly reduced from baseline day −21 (TNF = 2,900 ± 566 pg/mL on day −21 vs. 1,776 ± 342 pg/mL on day 42, $P < 0.05$) (Fig. 2*B*). On day 42 the vagus nerve stimulator was turned off. After a 14-d hiatus, it was restarted on day 56, and patients were followed through day 84. After the vagus nerve stimulator was turned off, TNF production

Table 1. RA patient baseline demographics, medication history, and disease severity

Demographics	Cohort I	Cohort II	Combined
Total, *n*	8	10	18
Enrollment by country			
Bosnia	3	0	3
Croatia	2	0	2
The Netherlands	3	10	13
Mean age in years (range)	55 (36–69)	48 (36–56)	51 (36–69)
Sex, % female	50	100	78
Ethnicity, % Caucasian	88	100	94
Mean no. of years since RA diagnosis (SD)	9.9 (5.7)	11.8 (6.3)	11.0 (5.9)
No. rheumatoid factor-positive patients (%)	7 (88)	5 (50)	12 (67)
No. anti-citrullinated peptide Ab^+ patients (%)	6 (75)	6 (60)	12 (67)
No. patients receiving prior nonbiologic disease-modifying antirheumatic drugs (%)			
0 drugs	1 (13)	1 (10)	2 (11)
1 drug	2 (25)	3 (30)	5 (28)
2 drugs	2 (25)	2 (20)	4 (22)
3 or more drugs	3 (37)	4 (40)	7 (39)
No. patients receiving prior biologic disease-modifying antirheumatic drugs (%)			
0 drugs	3 (38)	0	3 (17)
1 drug	4 (50)	0	4 (22)
2 drugs	1 (12)	0	1 (6)
3 drugs	0	3 (30)	3 (17)
4 drugs	0	4 (40)	4 (22)
5 drugs	0	2 (20)	2 (11)
6 drugs	0	1 (10)	1 (6)
DAS28-CRP (SD)	6.05 (0.87)	5.94 (0.72)	5.99 (0.77)
High-sensitivity CRP, mg/L (SD)	17.5 (10.0)	17.5 (18.5)	17.5 (14.9)

increased significantly by day 56; when the stimulator was turned on again, TNF production again decreased significantly by day 84 (1,776 ± 342 pg/mL on day 42 vs. 2,617 ± 342 pg/mL on day 56 and 1,975 ± 407 pg/mL on day 84, $P < 0.01$ for both). This finding indicates that active electrical stimulation of the vagus nerve inhibits TNF production in patients with RA.

RA signs and symptoms are measured using a standard disease activity composite score [the 28-joint C-reactive protein (CRP)-based disease activity score, DAS28-CRP] derived from counting swollen joints and tender joints, a patient-defined visual analog score of disease activity, and serum CRP levels (25). We observed that DAS28-CRP values at day 42 were significantly improved (i.e., lower) from baseline day −21 in the combined cohorts (DAS28-CRP = 6.05 ± 0.18 on day −21 vs. 4.16 ± 0.39 on day 42, $P < 0.001$), when the device was delivering current (Fig. 2*C*). Within days after receiving electrical stimulation of the vagus nerve, the DAS28-CRP improved significantly in some patients (Fig. S1). When the device was turned off (at day 42), the DAS28-CRP increased significantly within 14 d (4.16 ± 0.39 on day 42 vs. 4.96 ± 0.31 on day 56, $P = 0.001$). Restarting the device (day 56) significantly reduced the DAS28-CRP (Fig. 2*C*). Linear regression analysis comparing the mean change in the DAS28-CRP and the percentage change in TNF release from baseline day −21 to day 42 revealed a highly significant correlation ($r = 0.384$, $P < 0.0001$) (Fig. 2*D*). The temporal pattern of TNF production in the combined cohort correlated with the DAS28-CRP (Fig. 2*E*).

We assessed the fraction of patients who improved from baseline to achieve ACR 20%, 50%, and 70% clinical responses and also the number of patients who improved from baseline sufficiently to meet the definition for EULAR response and remission. The ACR response is defined as the percentage improvement in disease activity between two time points (ACR20 is ≥20%, ACR 50 is ≥50%, and ACR70 is ≥70% improvement). The EULAR response depends on the change in the DAS28-CRP and the absolute level achieved after treatment (24). As shown in Fig. S2, the ACR and EULAR response criteria were fulfilled in a large subset of patients in both cohorts. At the primary endpoint (day 42) the percentages of patients fulfilling the ACR response criteria for 20%, 50%, and 70% improvement were 71.4%, 57.1%, and 28.6%, respectively, for cohort I and were 70.0%, 30.0%, and 0.0%, respectively, for cohort II. The percentages of patients achieving DAS28 remission (DAS28-CRP <2.6) on day 42 in cohorts I and II were 28.6% and 0.0%, respectively. Improvement was observed in all constituent components of the composite end points (tender joint count, swollen joint count, patient's assessment of pain, patient's global assessment, physician's global assessment, and CRP) (Table S2). Together, these data indicate that vagus nerve stimulation inhibits TNF and significantly attenuates RA disease severity.

We measured a panel of serum cytokines to assess further the mechanisms of this experimental therapeutic intervention. Most, including serum TNF, IL-10, IL-12p70, IL-13, IL-1α, IL-1β, IL-2, IL-4, IL-5, and TNF-β, were below 1 pg/mL (unreliable limits of detection). Serum IL-6 levels in subjects who improved by EULAR criteria were significantly decreased compared with subjects who failed to improve: IL-6 levels were 15.4 ± 2.4 pg/mL in nonresponders ($n = 5$) vs. 5.0 ± 1.4 pg/mL in responders ($n = 12$) ($P = 0.001$) (Fig. 3*A*). Decreased IL-6 levels in the patients who responded to therapy correlated with improvement in disease severity between day −21 and day 42 ($r = 0.707$, $P = 0.002$) (Fig. 3*B*). The IL-6 responses are specific, because IL-8 and IL-17 levels did not change significantly [IL-8: 25.6 ± 9.1 pg/mL in nonresponders ($n = 5$) vs. 13.7 ± 1.7 pg/mL in responders ($n = 12$), $P = 0.29$ (Fig. 3*C*); IL-17: 2.8 ± 1.1 pg/mL in nonresponders ($n = 5$) vs. 1.8 ± 0.2 pg/mL in responders ($n = 12$), $P = 0.18$ (Fig. 3*E*)] and did not correlate to clinical response (Fig. 3 *D* and *F*).

Discussion

To our knowledge, this study is the first to assess whether stimulating the inflammatory reflex by directly implanting an electronic device modulates TNF and other cytokines in humans. Historically the development of electrically active implantable medical devices has been primarily empiric, based upon observing effects of devices that deliver

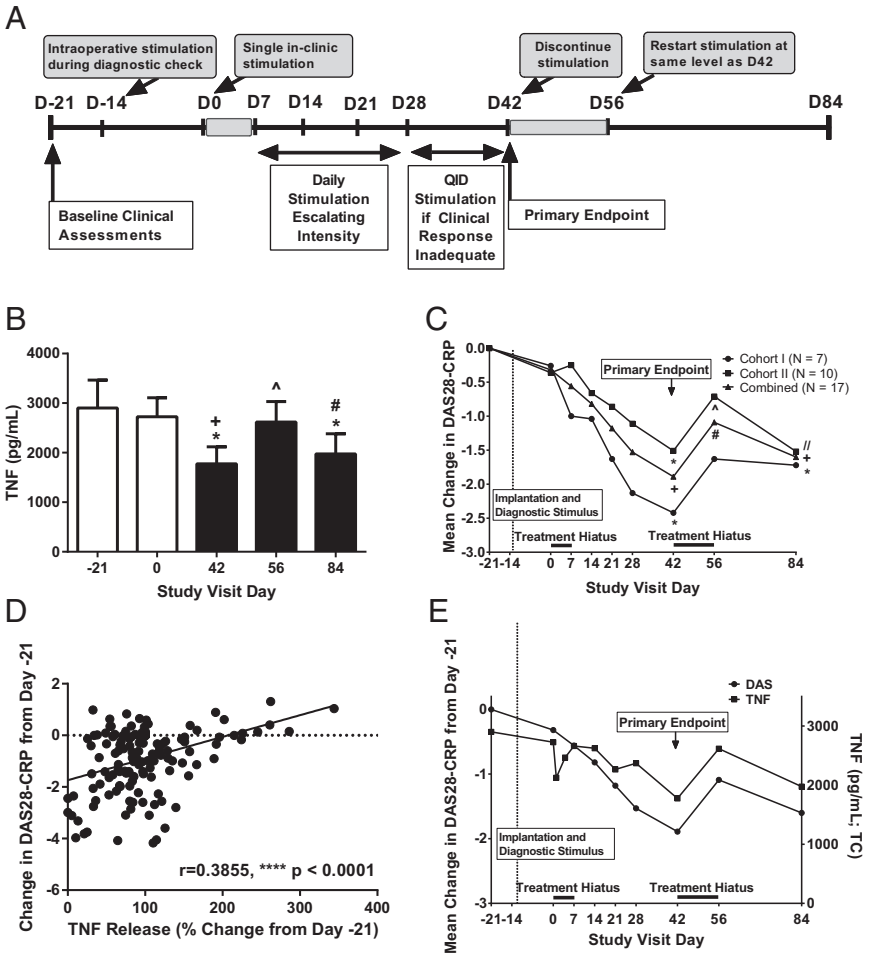

Fig. 2. The effects of inflammatory reflex activation on whole-blood LPS-induced TNF production and disease activity in RA patients. (*A*) Schematic of the RA study design. D −21 to D84 indicate study visit days. The stimulation schedule and timing of assessments are shown. (*B*) Mean LPS-induced TNF production in the combined RA cohort ($n = 17$) at study days −21, 0, 42, 56, and 84; visit means are designated by bars, and error bars indicate SEM. Differences in means were tested for significance by paired t test: $^{*}P < 0.05$ vs. d −21; $^{+}P < 0.01$ vs. d 0; $^{\wedge}P < 0.01$ vs. d 42; $^{\#}P < 0.01$ vs. d 56. (*C*) The mean change in DAS2 8-CRP from baseline by study visit day for cohort I (patients failing methotrexate treatment), cohort II (patients failing treatment by multiple biologic agents), and combined cohorts. The significance of the mean change by paired t test between visits is shown: $^{*}P < 0.05$ vs. d −21; $^{//}P < 0.01$ vs. d −21; $^{+}P < 0.001$ vs. d −21; $^{\#}P < 0.001$ vs. d 42; $^{\wedge}P < 0.05$ vs. d 42). (*D*) Linear regression analysis comparing the changes in the DAS28-CRP and the percent change in TNF release from study day −21 measured at each individual visit for each patient in the combined cohort. Changes in the DAS28-CRP and TNF release are significantly correlated by Pearson's test ($r = 0.384$, $P < 0.0001$). (*E*) Mean change in the DAS28-CRP and mean LPS-induced TNF release over time by study visit day. Changes in the DAS28-CRP and TNF release follow a similar temporal pattern in response to initial simulation, stimulation withdrawal, and stimulation reinitiation.

electrical current to depolarize neuronal or cardiac tissue. Absent appropriate biomarkers or mechanistic understanding, it has been difficult or impossible to develop or optimize the device parameters for current delivery, physiological effect in the targeted organ system, and clinical efficacy. Direct and accessible surrogate molecular markers of disease mechanism targeted by active implantable medical devices are uncommon. The discovery of the inflammatory reflex affords a unique opportunity for developing a neuromodulating device to regulate immune cell function by targeting a neural pathway that regulates cytokine production, a surrogate marker of molecular mechanism (26).

RA patients in cohort I are in early stages of disease not responding to therapy with methotrexate. These patients are frequently candidates for subsequent therapy with a biological agent that inhibits TNF. Cohort II patients are in later stages of disease, having failed multiple biological disease-modifying antirheumatic drugs. After electrical stimulation of the vagus nerve the DAS28-CRP improved significantly in both cohorts, and withdrawal of treatment significantly worsened the severity of disease. Reactivating the device on day 56 restored significant clinical improvement. The clinical responses were accompanied by significant reductions in TNF release during periods of disease remission and significant increases in TNF release during disease exacerbation. A large body of preclinical evidence has delineated the molecular and physiological mechanisms of the inflammatory reflex modulating TNF, IL-6, HMGB1, and other cytokines (7–9, 11–20). The molecular mechanisms of cytokine inhibition implicate acetylcholine derived from T_{ChAt} cells, the subset of choline acetyltransferase-positive T cells that we identified in the inflammatory reflex (9). In future clinical trials it should be interesting to study whether T_{ChAt} cells participate in mediating anti-inflammatory reflex mechanisms.

Vagus nerve stimulation has been used to treat medically refractory epilepsy in more than 100,000 patients, and it is generally well tolerated (21, 22). The adverse events reported here were mild to moderate in severity and were comparable in type and frequency to those seen in prior studies of vagus nerve stimulation therapy in epilepsy patients. These adverse events included transient hoarseness, postoperative hoarseness from neuropraxis, and transient intraoperative bradycardia during surgery. None of the patients developed infection. Larger clinical trials can be designed to

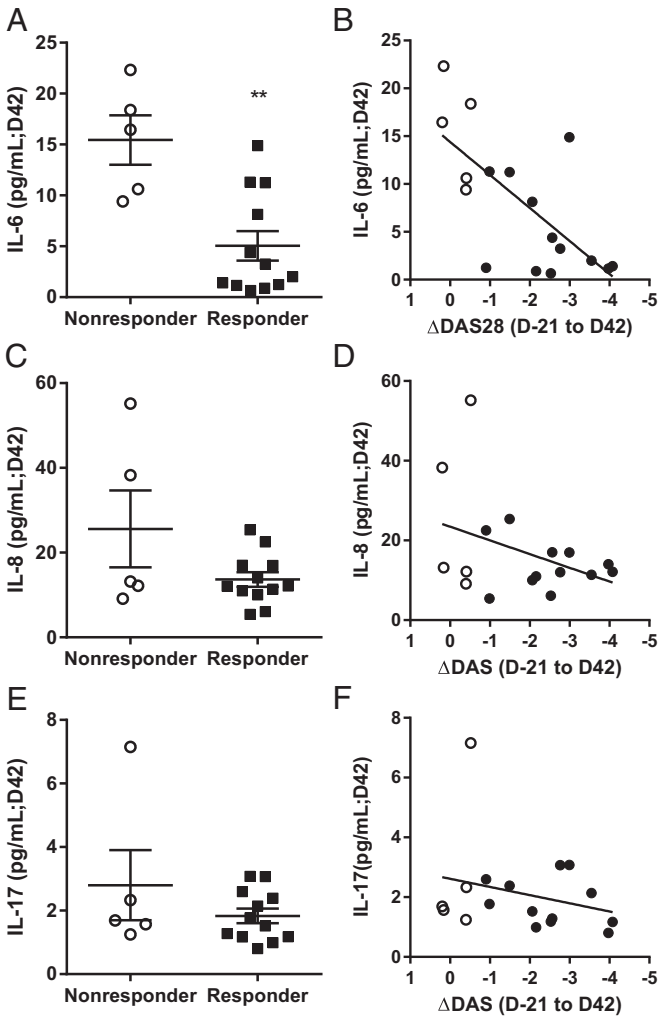

Fig. 3. Modulation of serum cytokines. Serum from each patient in the combined cohort was analyzed for multiple analytes at day 42. (*A*, *C*, and *E*) Individual patient values for EULAR nonresponders and responders are shown for IL-6 (*A*), IL-8 (*C*), and IL-17 (*E*) levels. The significance of differences between mean values at each time point was tested by unpaired *t* test (**$P < 0.01$). Horizontal bars indicate mean ± SEM. (*B*, *D*, and *F*) Linear regression analysis comparing analyte level at day 42 to the change in the DAS28-CRP from study day −21 to day 42. The change in the DAS28-CRP is significantly correlated to IL-6 release ($r = 0.707$, $P = 0.002$) (*B*) but not to IL-8 release ($r = 0.261$, $P = 0.31$) (*D*) or IL-17 release ($r = 0.384$, $P = 0.07$) (*F*).

determine the risk/benefit ratio for implantable electronic devices compared with the toxicity and side effects of pharmacological and targeted therapies for RA.

The electrical stimulation parameters used in this study were previously established to stimulate the inflammatory reflex in preclinical studies and differ significantly from the stimulation protocols used in epilepsy (19, 27). Here, electrical current (up to 2.0 mA) was delivered to the cervical vagus nerve for 60 s one to four times daily; the maximum time of electrical current flow for any patient in this study was 4 min daily. This stimulation protocol differs significantly from the protocols for treating epilepsy, in which current (up to 2.25 mA) is delivered at 60-s intervals, followed by an OFF interval of 5–180 min, repeated continuously. Thus, epilepsy patients may receive electrical current delivery for up to 240 min daily. Preclinical studies have established that stimulation of the inflammatory reflex for as little as 60 s confers significant inhibition of cytokine production for up to 24 h. The present study was not designed or powered to evaluate the relationship between specific electrical current dose–response and clinical outcomes or the longer-term durability of therapeutic benefit, and the effects of under- or overstimulation of the inflammatory reflex are also an important area for future study.

The primary objective of this study was to determine whether activating the inflammatory reflex with an implanted electronic device inhibits cytokine production in humans. It is reasonable to consider whether placebo mechanisms contribute to these findings, because some patients are aware when the device is delivering current. There are several arguments against a placebo effect explaining the observed inhibition of TNF and IL-6 and the significant clinical improvements. First, we observed that intraoperative vagus nerve stimulation significantly inhibited TNF release in epilepsy patients who were unconscious during the implantation. These patients could not be aware of the stimulation, indicating that the suppression of cytokine release immediately following vagus nerve stimulation cannot be attributed to a placebo effect. Second, we also observed that the suppression of TNF release during vagus nerve stimulation in RA patients occurred only when the device was functioning. It has been established previously that biomarkers are not modifiable by placebo effects in RA studies of this duration (28, 29). Third, we observed reduced TNF and IL-6 production and positive clinical responses in the subset of therapy-resistant patients who had failed both methotrexate therapy and treatment with multiple biologic agents with differing mechanisms of action. It has been established in prior studies that placebo response rates in drug-resistant cohorts are extremely low (ACR20 responses 5–11%). The findings here of significantly higher ACR20 responses (between 70% and 71.4%) argue strongly against a placebo effect being the mechanism. Fourth, a recent study reported clinical improvement using vagus nerve-stimulation therapy to treat another disease mediated by TNF, Crohn's disease (30). Although the investigators in that study did not measure the activity of the inflammatory reflex or cytokine production, they did examine endoscopic biopsies and observed that vagus nerve stimulation significantly inhibited inflammation in the colonic tissues, an objective histological tissue response that cannot be attributed to placebo effects. Finally, our recent prospective observational studies indicate that impaired constitutive vagus nerve activity precedes the development of clinically manifest RA (31). Therefore, when considered together with extensive preclinical data that identify molecular and neurophysiological mechanisms, the inhibition of TNF during electrical stimulation and the significant clinical responses shown give evidence that the clinical mechanism is mediated by the inflammatory reflex.

This first-in-class study supports a conceptual framework for further studies of electronic medical devices in diseases currently treated with drugs, an approach termed "bioelectronic medicine" (32). Larger clinical trials in RA can be designed and powered to assess clinical efficacy, but our findings encourage pursuing this strategy in RA and in other cytokine-mediated autoimmune and auto-inflammatory disorders.

Materials and Methods

Study of Vagus Nerve Stimulation in Epilepsy Patients. The study of vagus nerve stimulation in epilepsy patients was performed at the Hofstra Northwell School of Medicine and was approved by the Clinical Research Center (CRC) and the Institutional Review Board. All patients provided informed consent before participation. The study population consisted of seven epilepsy patients being implanted with a Cyberonics Vagus Nerve Stimulation System (Cyberonics) according to the manufacturer's instructions as part of their standard care for the treatment of refractory epilepsy (Fig. S3). During the intraoperative diagnostic procedure, the pulse generator produces a 30-s stimulation at a 1.0-mA output current with pulse frequency of 20 Hz and pulse width of 500 μs. Blood samples were taken before anesthesia induction, after anesthesia induction but before intraoperative vagus nerve stimulation, and 4 h after intraoperative vagus nerve stimulation.

The LPS-induced cytokine release assay was performed as previously described (33). Cytokine levels were analyzed using the MSD multiplex cytokine assay (Meso Scale Discovery) per the manufacturer's instructions. TNF, IL-6, and IL-1β

release across time points was analyzed using the Prism analytical software package (GraphPad).

Study of Vagus Nerve Stimulation in RA Patients. The study of vagus nerve stimulation in RA patients was performed at one center in The Netherlands (the Academic Medical Center of the University of Amsterdam), at two centers in Bosnia and Herzegovina (the University Clinical Hospital in Mostar and Sarajevo University Clinical Center in Sarajevo), and at in one center in Croatia (Clinical Hospital Center Sestre Milosrdnice, Zagreb) and was approved by the respective national and institutional Ethics Committees. All patients provided informed consent before participation. The investigational study device was a Cyberonics Vagus Nerve Stimulation System, implanted as described above. The systems were treated as investigational study devices because of their off-label use in patients with RA. The study recruited two separate patient cohorts. Cohort I consisted of RA patients who had failed to respond to methotrexate and who were either TNF-antagonist naive or had previously failed treatment with a TNF antagonist because of safety reasons rather than lack of efficacy. Cohort II included patients who had not responded adequately to at least two biologic agents with at least two different mechanisms of action. Major inclusion and exclusion criteria are given in *SI Materials and Methods*. The use of prednisone at a stable daily dose of less than 10 mg and other nonbiological disease-modifying antirheumatic drugs at stable doses was allowed.

The design schematic of this single-arm study is shown in Fig. 2*A*. At the conclusion of the study, patients were offered the options of having the device surgically removed or left in place and inactivated or continuing treatment in a long-term extension study. All recruited subjects opted to continue in the extension study, which will be reported separately.

The primary study end point was mean change in the DAS28-CRP between visits on baseline day −21 and day 42 (25). Mean changes in the DAS28-CRP between day −21 and day 42 or day 84, and between day 42 and day 56 also were assessed for significance at $P < 0.05$ using a Student's paired t test in the SAS 9.2 statistical analysis package (SAS). Because this was an exploratory study, no formal statistical power calculations were performed, and no adjustments for multiple comparisons were made. Adverse events were collected from the day of implantation through the day 84 visit, coded using the Medical Dictionary for Regulatory Activities (MedDRA), and presented by MedDRA term as subject incidence rates.

Whole-Blood Cytokine Release Assay in the RA Study. The TruCulture system (Myriad RBM), an assay system suitable for use at clinical sites and scalable to larger studies, was used. Venous blood was drawn into tubes containing endotoxin at 100 ng/mL and was incubated at 37 °C for 24 h. Supernatant TNF was measured by ELISA (R&D Systems). Comparisons of changes in TNF release between baseline and subsequent visits [with three statistical outlier exclusions; robust regression and outlier removal (ROUT) method with the maximum false discovery rate at 1%] by paired t test and linear regression analysis of relationships between changes in the DAS28-CRP and TNF release were performed using the Prism analytical software package.

Serum Cytokines in the RA Study. Serum cytokine levels from day 42 were analyzed using the MSD multiplex cytokine assay as above. Analysis of IL-6, IL-8, and IL-17 release on day 42 and linear regression analysis of relationships (Pearson's test) between the change in the DAS28-CRP and serum cytokine release at day 42 were performed using the Prism analytical software package.

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Bioelectronic Medicine

RESEARCH ARTICLE Open Access

Investigational treatment of rheumatoid arthritis with a vibrotactile device applied to the external ear

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic and debilitating inflammatory disease characterized by extensive joint tissue inflammation. Implantable bioelectronic devices targeting the inflammatory reflex reduce TNF production and inflammation in preclinical models of inflammatory disease, and in patients with RA and Crohn's disease. Here, we assessed the effect of applying a vibrotactile device to the cymba concha of the external ear on inflammatory responses in healthy subjects, as well as its effect on disease activity in RA patients.

Methods: Six healthy subjects received vibrotactile treatment at the cymba concha, and TNF production was analyzed at different time points post-stimulation. In a separate study, nineteen healthy subjects were enrolled in a randomized cross-over study, and effects of vibrotactile treatment at either the cymba concha or gastrocnemius on cytokine levels were assessed. In addition, the clinical efficacy of vibrotactile treatment on disease activity in RA was assessed in nine patients with RA in a prospective interventional study.

Results: Vibrotactile treatment at the cymba concha reduced TNF levels, and the suppressive effect persisted up to 24 h. In the cross-over study with 19 healthy subjects, vibrotactile treatment at the cymba concha but not at the gastrocnemius significantly reduced TNF, IL-1β, and IL-6 levels compared to pre-treatment baseline (TNF $p < 0.05$, IL-6 $p < 0.01$, IL-1β $p < 0.001$). In healthy subjects, vibrotactile treatment at the cymba concha inhibited TNF by 80%, IL-6 by 73%, and IL-1β by 50% as compared to pre-treatment baseline levels. In RA patients, a significant decrease in DAS28-CRP scores was observed two days post-vibrotactile stimulation at the cymba concha (DAS28-CRP score pre-treatment $= 4.19 \pm 0.33$ vs post-treatment $= 3.12 \pm 0.25$, $p < 0.05$). Disease activity remained significantly reduced 7 days following vibrotactile treatment (DAS28-CRP score 7 days post-treatment $= 2.79 \pm 0.21$, $p < 0.01$). In addition, a persistent improvement in visual analogue scale scores, a patient derived measure of global health assessment, was observed in RA patients following vibrotactile treatment.

Conclusion: Application of a vibrotactile device to the cymba concha inhibits peripheral blood production of TNF, IL-1β, and IL-6 in healthy subjects, and attenuates systemic inflammatory responses in RA patients.

Trial registrations: ClinicalTrials.gov Identifier: NCT01569789 and NCT00859859. The AMC trial conducted in The Netherlands does not have a ClinicalTrials.gov Identifier.

Keywords: Auricular vagus nerve, taVNS, rheumatoid arthritis, TNF

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Introduction

Rheumatoid arthritis (RA) is a progressive and debilitating disease characterized by an inflammatory pannus whose growth is stimulated by TNF and IL-6 (Smolen et al. 2016). Early work identified anti-TNF monoclonal antibodies as protective against lethality in animal models of septic shock (Tracey et al. 1987), and set the stage for the clinical translation of anti-TNF agents to a variety of inflammatory diseases. Anti-TNF biologics are widely-prescribed and efficacious in rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, and psoriasis. Despite their clinical efficacy, these agents are costly (>$25,000 per patient annually), and render patients susceptible to serious infections as a direct result of TNF blockade (Monaco et al. 2015). To circumvent these limitations of conventional anti-TNF agents, bioelectronic medicine has emerged as a promising alternative approach to target systemic inflammation (Olofsson and Tracey 2017). Bioelectronic devices targeting the inflammatory reflex reduce TNF and inflammation in preclinical models of inflammatory disease, and in patients with rheumatoid arthritis and Crohn's disease (Chavan et al. 2017; Andersson and Tracey 2012a; Levine et al. 2014; Koopman et al. 2016; Bonaz et al. 2016).

Neural reflexes control the cardiovascular, pulmonary, gastrointestinal, renal, hepatic, and endocrine systems. Recent studies revealed that innate and adaptive immunity are also controlled by neural reflex mechanisms (Chavan et al. 2017; Chavan and Tracey 2017; Pavlov et al. 2018). The vagus nerve-based inflammatory reflex is a physiological mechanism though which the vagus nerve signals regulate immune function (Borovikova et al. 2000; Andersson and Tracey 2012b; Tracey 2002). Molecular mediators of innate immunity activate the afferent signals in the vagus nerve which are transmitted to the brainstem that controls outgoing efferent signals in the vagus nerve. Efferent signals arising in the vagus nerve are then transmitted to the splenic nerve and synapse on splenic lymphocytes causing them to release acetylcholine, which binds the α7 nicotinic acetylcholine receptor (α7nAChR) expressed on macrophages and monocytes (Chavan et al. 2017; Wang et al. 2003; Olofsson et al. 2012). Signal transduction induced by acetylcholine binding increases intracellular calcium, decreases nuclear translocation of NFκB, stabilizes mitochondrial membranes, and inhibits inflammasome activity. These events result in the reduction of the pro-inflammatory cytokines TNF, IL-1β, and IL-6 produced by the spleen (Olofsson et al. 2012). α7nAChR-mediated inhibition of TNF is dependent on CREB and c-FOS (Tarnawski et al. 2018).

Positive pilot clinical trial results have been reported in patients with RA and Crohn's disease treated with implanted stimulators that deliver electrical impulses to the cervical vagus nerve (Koopman et al. 2016; Bonaz et al. 2016). The vagus nerve is a bilateral cranial nerve which arises from brainstem nuclei and innervates the viscera. The auricular branch of the vagus nerve arises from the vagus and supplies cutaneous regions of the concha and posterior aspect of the lower ear with afferent innervation (Henry 2002; Peuker and Filler 2002; Trevizol et al. 2016; Kong et al. 2018). Transcutaneous auricular vagus nerve stimulation (taVNS) is an investigational therapy in which electrical signals are applied to the cutaneous territory supplied by the auricular branch of the vagus nerve. There are numerous studies of taVNS currently planned or recruiting in the United States and abroad for a range of diseases, including depression, stroke, atrial fibrillation, and heart failure. Functional MRI studies have demonstrated that taVNS activates known brain projections of the vagus, including the nucleus tractus solitarius, dorsal raphe, locus coeruleus, parabrachial area, hypothalamus, amygdala, anterior cingulate cortex, anterior insula, and nucleus accumbens (Yakunina et al. 2017; Kraus et al. 2007; Dietrich et al. 2008; Kraus et al. 2013; Krause et al. 2013; Frangos et al. 2015; Fang et al. 2017). Previous clinical studies have evaluated the effect of taVNS on a variety of conditions and physiological states, including pharmacoresistant epilepsy, depression, pre-diabetes, tinnitus, memory, stroke, and oromotor dysfunction (Kong et al. 2018; Rong et al. 2014; Rong et al. 2016; Stefan et al. 2012; Huang et al. 2014; Shim et al. 2015; Jacobs et al. 2015; Badran et al. 2018; Redgrave et al. 2018). These studies have made use of a range of electrical stimulation settings, as well as several stimulation sites on the auricle or mastoid process, however, it is unknown whether applying a stimulus to the cutaneous region innervated by the auricular branch of the vagus nerve could attenuate systemic inflammatory responses in human subjects. Moreover, it remains unknown whether a mechanical stimulus applied to the cutaneous region innervated by the auricular branch of the vagus nerve, i.e., the cymba concha can attenuate systemic inflammatory responses.

We have reported that transcutaneous mechanical activation of the cervical vagus nerve is therapeutically efficacious in murine models of sepsis (Huston et al. 2007). Pressure applied directly to the skin overlying the cervical vagus nerve dose-dependently attenuated serum TNF levels during lethal endotoxemia. These studies demonstrated that transcutaneous mechanical stimulation of the vagus nerve attenuates serum inflammatory cytokine levels, and enhances survival in model of cytokine-mediated disease. Here we show that application of a vibrotactile device to the cymba concha

of the external ear inhibits peripheral blood production of TNF, IL-1β, and IL-6 in healthy subjects, and attenuates disease activity in RA patients.

Methods

Vibrotactile device

An oscillatory device (X/Y Axial Stimulator, Mass Bay Engineering, Norwell, MA) was designed and fabricated for the clinical studies. The device consists of a hand-held probe containing a motor which spins an eccentric counterweight, producing radial displacement in a circular pattern at the probe tip. Application of the probe tip to the skin of a human subject is sensed as vibration. The device was powered by a 4602PS DC power supply (MPJA, West Palm Beach, FL). For treatment of study subjects, the device was manually applied by a device operator. It was positioned perpendicular to and with its tip in contact with the cymba concha or gastrocnemius, and applied with a force of 0.15 kg. The specifications of the device were measured using an experimental set-up representative of the device treatment administered to study subjects. All tests were performed at 4VDC; current fluctuated from 0.25A to 0.28A influenced by loading. For testing, the handle of the device was cushioned by a ¼″ thick sleeve of closed cell vinyl foam closely resembling the grip and flexibility of human fingers. Device frequency with zero load was measured to be 6600 RPM on the vertical axis. The frequency and amplitude of the device were recorded by applying the device to tissue-matched durometers (Sorbothane Inc., Kent, OH) overlying a #DS2–110 digital force gauge (Imada Instruments, Northbrook, IL). Durometers were matched to the cymba concha and gastrocnemius by tactile sensation; for the cymba concha, a 30 "A" urethane durometer was used, and for the gastrocnemius, a 30 "00" Sorbothane durometer was used. A microscope with reticle was used to assess amplitude. The frequency of device oscillation was measured using a Strobotac 1531-AB strobe light (General Radio Company, Cambridge, MA). When applied to the 30 "A" urethane cymba concha-matched durometer, device frequency was 10,100 RPM, horizontal amplitude was 0.008″, and vertical amplitude was 0.005″. When applied to the 30 "00" Sorbothane durometer, device frequency was 9330 RPM, horizontal amplitude was 0.015″, and vertical amplitude was 0.005″.

Study design

Human subjects participated at two institutional sites, the Academic Medical Center (AMC), University of Amsterdam, Amsterdam, The Netherlands, and the Feinstein Institute for Medical Research (FIMR), Manhasset, New York, USA. A third cohort of subjects with rheumatoid arthritis were treated at the Feinstein Institute for Medical Research. The study at the AMC was approved by the Institutional Review Board (IRB) of the University of Amsterdam (IRB# MEC 07/095), and the study protocols for healthy subjects (IRB# 11-122B) and RA patients (IRB# 06.02.027) at the FIMR were approved by the Clinical Research Center (CRC) and the Institutional Review Board of Northwell Health, and performed at the CRC of Northwell Health. Healthy subjects at the AMC received vibrotactile stimulation at the cymba concha of the ear (Fig. 1). Blood was collected pre-stimulation and at 30 min, 2 h, 4 h and 24 h post-stimulation from these subjects, and subjected to the whole blood assay (Fig. 2). A randomized cross-over study design was used for the healthy subjects at the FIMR site. Healthy subjects participated in two visits, separated by at least one week. At each visit, the subjects received vibrotactile stimulation either at the cymba concha or at the gastrocnemius muscle (control) using a cross-over design. The subjects were

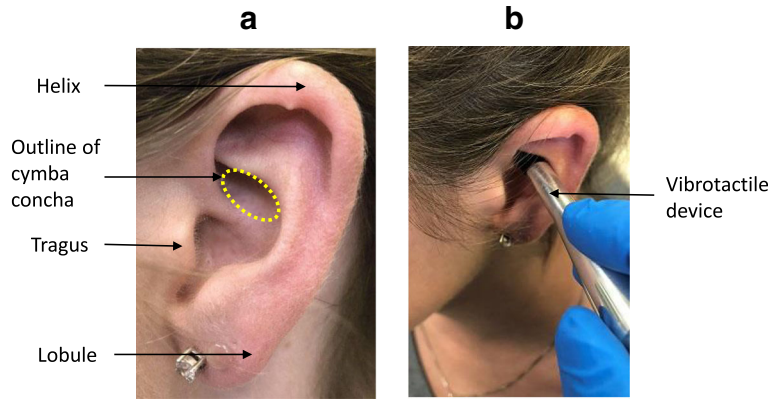

Fig. 1 Site of application of vibrotactile device to the cymba concha of the ear. (**a**) Major anatomical landmarks of the external ear (pinna) with approximate outline of the cymba concha. The cymba concha is a highly-conserved anatomical feature of the external ear that was identified by the device operator. (**b**) Representative device placement of the vibrotactile device in contact with the cymba concha

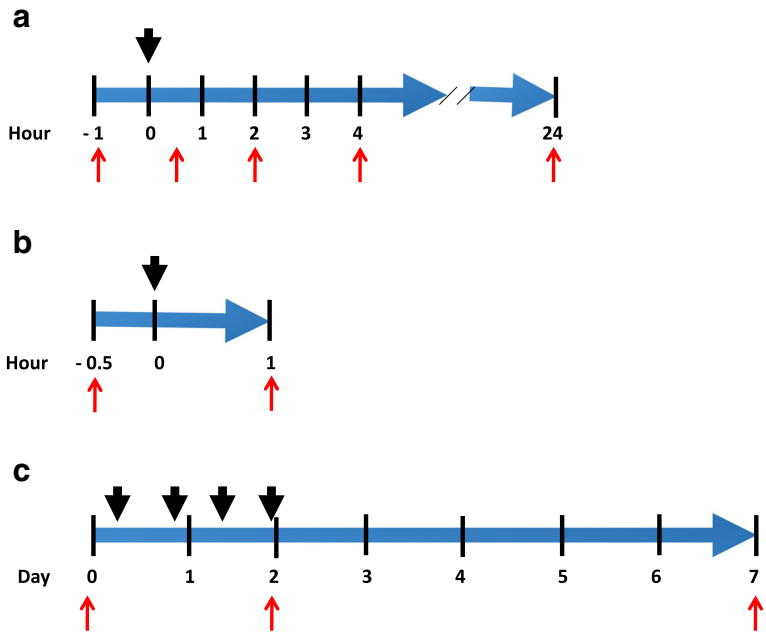

Fig. 2 Timeline of investigational studies. (**a**) Study design for healthy subjects at AMC; (**b**) Study design for healthy subjects at FIMR; (**c**) Study design for RA patients at FIMR. Black arrows indicate application of vibrotactile treatment. Red arrows indicate time of blood draws for healthy subjects (**a**, **b**) and clinical assessments for RA patients (**c**)

instructed that both the types of treatment were comparable types of nerve stimulations, and not informed about the order of the respective treatment. All treatments with the vibrotactile device on the cymba concha or gastrocnemius were performed between 9:30 a.m. and 11:30 a.m. A washout period of at least one week, but no more than two weeks, was observed between the two stimulations. Blood was collected pre-stimulation and at 1 h post-stimulation during both visits, and subjected to the whole blood assay. RA patients were admitted to the CRC inpatient unit at the Northwell Health for a 48-h period, and received vibrotactile treatment at the cymba concha twice daily (8:00 a.m. and 8:00 p.m.) for two days. Clinical assessments were performed by the same examiner at the time of admission, 48 h and 7 days after vibrotactile treatment.

Subjects

The study population of healthy subjects at the Academic Medical Center, University of Amsterdam, The Netherlands consisted of 6 participants. The study population at the Feinstein Institute for Medical Research consisted of two groups: healthy subjects ($n = 19$) and RA patients ($n = 9$). Healthy subjects were enrolled to determine whether vibrotactile stimulation inhibits TNF, IL-1β, and IL-6 production in whole blood assay. Informed consent was obtained from all the study participants. Individuals of both genders between the ages of 18 and 60 years were screened. Exclusion criteria were a history of smoking, ear infection (otitis media or otitis externa), arrhythmia, coronary artery disease, chronic inflammatory disease, anemia, malignancy, depression, connective tissue disease (osteoarthritis, vasculitis), neurologic disease, diabetes mellitus, renal disease, malignancy, dementia, psychiatric illness including active psychosis, or any other chronic medical condition. In addition, subjects using cholinergic, anti-cholinergic, or beta-blocking medications were excluded, as were pregnant patients. Study subject characteristics are summarized in Table 1. Nine rheumatoid arthritis patients were recruited from the North American Rheumatoid Arthritis Consortium and by affiliated rheumatology clinic referrals. Exclusion criteria were a history of smoking, immunosuppressive condition (including malignancy and chronic alcoholism), severe dementia, psychiatric illness with active psychosis, current intravenous or other serious illicit drug use, ischemic cardiovascular disease (including myocardial infarction, unstable angina, and bradytachyarrythmias), moderate or severe anemia, pregnancy, and use of anti-cholinergic or beta-blocking medications. Three RA patients had hypertension controlled with medication. No other cardiovascular risk factors were identified in the RA subjects. Patients had an established diagnosis of RA according to American College of Rheumatology criteria. Two patients were excluded from data analysis as they had DAS28 scores at baseline indicating low disease activity.

Table 1 Study participant characteristics

	Healthy Subjects AMC cohort	Healthy Subjects FIMR cohort	Rheumatoid Arthritis Patients
Number of subjects (male/female)	6 (6/0)	19 (9/10)	9 (2/7)
Age at stimulation (range)	25-49	22-59	28-70
C-reactive protein (mg/L)	NA	NA	6.63 ± 7.04
Disease activity score-28 (mean ± SD)	0	0	3.7 ± 1.4
co-Morbidities:	Number of subjects	Number of subjects	Number of patients
Hypertension	0	0	3
Hypothyroidism	0	0	1
SLE	0	0	0
Medications:	Number of subjects	Number of subjects	Number of patients
Corticosteroids	0	0	2
NSAIDS	0	0	3
β-Blockers	0	0	0
DMARDs	0	0	5
TNF Inhibitors	0	0	4

AMC Amsterdam Medical Center, *FIMR* Feinstein Instituted for Medical Research CP and Disease activity score-28 are expressed as mean± SD, *NA* Not Available
Subjects participated in three studies at two institutional sites. Six healthy subjects were enrolled at the Academic Medical Center in Amsterdam for a pilot study. Nineteen healthy subjects were enrolled in a controlled cross-over study at the Feinstein Institute for Medical Research. Nine patients with RA were enrolled at the Feinstein Institute for Medical Research

Vibrotactile device treatment

For the stimulation at the external ear in the healthy subjects and RA patients, the stimulation with the vibrotactile device was delivered at the right cymba concha. For the study of healthy subjects at the AMC, subjects underwent a one-time stimulation of two minutes duration at the right cymba concha. For the study of healthy subjects at FIMR, a cross-over design was utilized in which each subject received a one-time stimulation of two minutes duration at the right cymba concha, and a one-time stimulation of two minutes duration at the right gastrocnemius. RA patients received a total of four stimulations of five minutes duration each using a vibrotactile device (Brookstone). Two stimulations were delivered on the first day, and two stimulations were delivered on the second day. After cleansing the skin with a disposable alcohol prep pad, the device was placed in direct contact with the skin either at the right cymba concha or at the posterior aspect of the leg at the right gastrocnemius muscle (three inches inferiorly from the head of the fibula, and two inches posteriorly), and the treatment was delivered as indicated.

Disease activity in RA patients

Disease activity in RA patients was measured using DAS28-CRP with four variables, a validated combined index that includes enumeration of tender and swollen joints, visual analog score (VAS), and measurement of high-sensitivity CRP (Fransen et al. 2003). Joint tenderness and swelling were assessed by a single physician to limit variability. The score was calculated as follows: DAS28-CRP = 0.56*sqrt(TJC28) + 0.28*sqrt(SJC28) + 0.36*ln(CRP + 1) + 0.014*GH + 0.96 (https://www.das28.nl/das28/en/). A DAS score of less than 2.6 indicates remission; a score between 2.6 and 3.2 indicates low/minimal disease activity; a score between 3.2 and 5.1 indicates moderate activity; a score of more than 5.1 is considered high/severe disease activity (Fransen and van Riel 2009; Anderson et al. 2012).

Whole blood assay

Venous blood was drawn into either sodium heparin tubes (AMC) or EDTA tubes (FIMR) (Becton Dickinson, NJ, USA). Blood was aliquoted (0.5 ml) in the assay tubes and stimulated with endotoxin. Endotoxin [lipopolysaccharide (LPS) at FIMR: *Escherichia coli* 0111:B4, Sigma cat. no. L4130 or at AMC: ultrapure, Invivogen, cat. no. tlrl-pelps) was re-suspended to 5 mg/ml, sonicated for 30 min, vortexed, and diluted with RPMI (at AMC) or PBS (at FIMR) to generate a working 1 mg/ml stock. This stock was serially diluted with 1x PBS to final concentrations of 1 ng/ml, in 500 μL

blood aliquots. Microfuge tubes aliquoted with blood and endotoxin were incubated on a rocking platform at 37 °C with 5% CO_2. After a 3-4 h incubation, plasma was collected by centrifugation [5 min, 2000 g (5000 rpm in Microfuge 5415C; Brinkmann, Westbury, NY)] and frozen at − 20 °C for future analysis. All assays were performed in duplicate.

Cytokine analysis

Interleukin (IL)-6, IL-1β, and TNF levels were determined by using the Cytometric Bead Array (from BD Biosciences) or the V-PLEX proinflammatory panel 1 human kit (Meso Scale Discovery, Gaithersburg, MD, USA), according to manufacturer's instructions. TNF levels in plasma samples were analyzed by commercially available ELISA kits (R&D Systems, Minneapolis, MN) according to manufacturer's instructions. High sensitivity CRP serum analysis was performed using a Hitachi 917 automated analyzer (Roche Diagnostics, Indianapolis, IN) at the core laboratory at Northwell Health. The reference value for hsCRP is 0.0–3.0 mg/L.

Statistical analysis

All statistical analyses were carried out using GraphPad Prism 6 software (GraphPad Software, La Jolla, CA). Data are presented as mean ± SEM where applicable. Repeated measures analysis of variance (RMANOVA) with the Friedman test was used to determine if healthy subjects or RA patients behaved differently over time (pre- vs. post-taVNS). For all analyses, the standard assumptions of Gaussian distribution were tested. The mean changes in TNF release between pre- and post-taVNS or pre- and post-gastrocnemius stimulation in healthy subjects were assessed for significance using a paired Student's t test. All *p*-values and n values are indicated in figure legends. *P*-values < 0.05 were considered significant.

Results

Application of a vibrotactile device at the cymba concha decreases TNF in a pilot study of healthy subjects

To assess the effect of vibrotactile treatment on modulating inflammatory cytokines in healthy subjects, we conducted a pilot study at the Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands. Vibrotactile treatment was applied to the cymba concha in 6 healthy subjects (6 males, age range 25–49 years, Table 1). No adverse effects after vibrotactile stimulation was reported. TNF production in response to endotoxin challenge was analyzed in the whole blood (Rosas-Ballina et al. 2009) pre- and post- vibrotactile stimulation. We observed that vibrotactile stimulation at the cymba concha in healthy subjects significantly attenuated endotoxin induced TNF by 56, 55, 69% ($p < 0.01$, RMANOVA- Friedman test), and 42% at 30 min, 2 h, 4 h and 24 h post-stimulation respectively (Fig. 3). Interestingly, the suppressive effect persisted up to 24 h post-stimulation (pre-treatment = 1633 ± 417, $n = 6$ vs. 24 h post-treatment = 940 ± 177, $n = 6$).

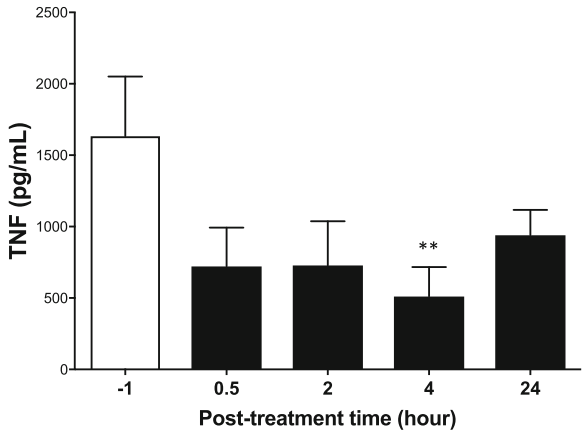

Fig. 3 Vibrotactile stimulation at the cymba concha reduces whole-blood LPS-induced TNF production. In a pilot study, six healthy subjects were enrolled at the Academic Medical Center, the Netherlands, and subjected to vibrotactile stimulation at the cymba concha. Blood was obtained before stimulation (− 1) and at different time points after stimulation (0.5, 2, 4 and 24 h). Whole blood was incubated with LPS, and TNF levels in plasma were determined after 4 h in culture. Data are shown as mean ± SEM, $^{**}p < 0.01$; RMANOVA with Friedman test

Application of a vibrotactile device at the cymba concha, and not the gastrocnemius, attenuates inflammatory responses in healthy subjects

Having established the time kinetics for the suppression of inflammatory cytokines in the pilot study of healthy subjects at the Amsterdam site, we next conducted a blinded cross-over study at Northwell Health in Manhasset, NY, USA. In this controlled study, we enrolled 19 healthy subjects (9 males, 10 females; age range 22–59 years of age, Table 1) to determine whether vibrotactile stimulation inhibits TNF, IL-1β, and IL-6 production in whole blood assay. All subjects were scheduled for two visits, separated by at least a week, and received stimulation at either the cymba concha or gastrocnemius. No adverse effects after vibrotactile stimulation at either anatomical location were reported, and the stimulation was well-tolerated. Peripheral blood was collected 30 min before and 1 h after the stimulation, and subjected to endotoxin stimulation in a whole blood assay. As shown in Fig. 4a, vibrotactile stimulation at the cymba concha significantly reduced TNF, IL-1β, and IL-6 levels compared to pre-stimulation baseline (TNF $p < 0.05$, IL-1β $p < 0.001$, IL-6 $p < 0.01$, Wilcoxon matched-pairs test), whereas stimulation at the

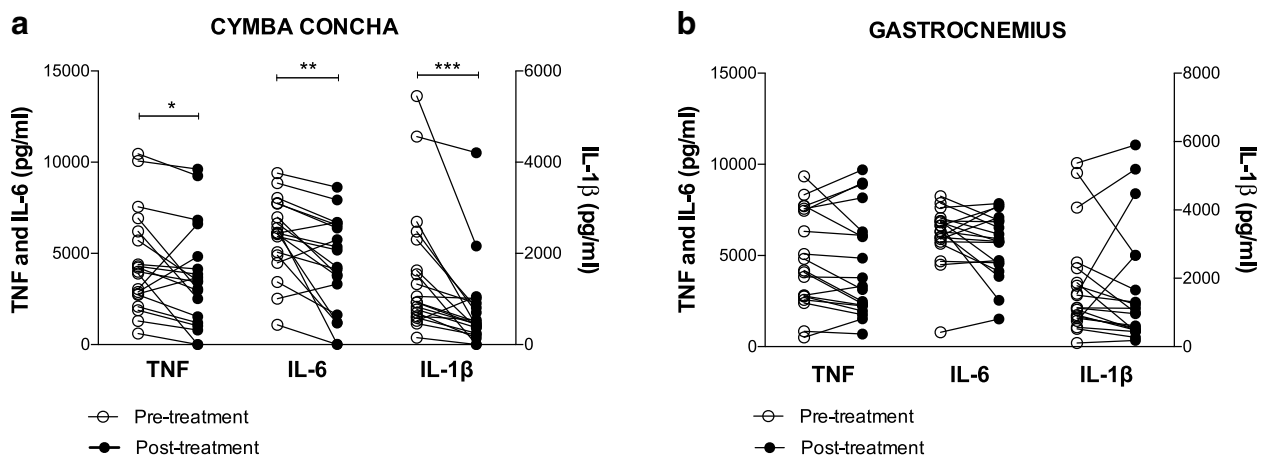

Fig. 4 Vibrotactile stimulation at the cymba concha but not at the gastrocnemius, attenuates inflammatory responses in healthy subjects ex vivo. Nineteen healthy subjects enrolled in a cross-over study at the Feinstein Institute for Medical Research received stimulation at either the cymba concha or gastrocnemius on two different days. Blood was collected 30 min prior and 1 h post stimulation. Whole blood was incubated with LPS, and cytokine levels in plasma were determined 4 h after. (**a**) Effect of vibrotactile stimulation at the cymba concha on cytokine responses in healthy subjects. (TNF *$p < 0.05$, IL-6 ***$p < 0.001$, IL-1β **$p < 0.01$, Wilcoxon matched-pairs test) (**b**) Effect of vibrotactile stimulation at the gastrocnemius on cytokine responses in healthy subjects. Open circles represent the pre-treatment and close circles represent post-treatment cytokine levels with lines connecting data points for each individual study participant

gastrocnemius did not attenuate inflammatory responses. Vibrotactile stimulation at the cymba concha inhibited TNF by 20% (pre-stimulation = 4541 ± 624 pg/ml vs. post-treatment = 3625 ± 645 pg/ml), IL-6 by 27% (pre-stimulation = 5979 ± 480 pg/ml vs. post-treatment = 4342 ± 597 pg/ml), and IL-1β by 50% (pre-treatment = 1527 ± 328 pg/ml vs. post-treatment = 765 ± 222 pg/ml) as compared to the baseline levels before treatment. The attenuation of cytokine levels following vibrotactile treatment of the cymba concha cannot be attributed to a placebo effect, as vibrotactile treatment at the gastrocnemius for one minute did not change endotoxin-induced TNF (pre-treatment = 4796 ± 607 pg/ml vs. post-treatment = 4477 ± 653 pg/ml), IL-6 (pre-treatment = 6137 ± 369 pg/ml vs. post-treatment = 5577 ± 401 pg/ml) and IL-1β (pre-treatment = 1787 ± 344 pg/ml vs. post-treatment = 1667 ± 398 pg/ml) levels in whole blood (Fig. 4b). To our knowledge, this is the first report that application of a vibrotactile device at the cymba concha inhibits endotoxin-induced whole-blood TNF, IL-1β, and IL-6 in humans.

Application of a vibrotactile device at the cymba concha decreases disease activity scores in patients with rheumatoid arthritis

Next we investigated whether vibrotactile treatment confers clinical benefit to rheumatoid arthritis patients. Nine RA patients with active disease (DAS-28 score 4.19 ± 0.33) were enrolled in the study and received vibrotactile treatment twice daily for two days. Disease activity was reassessed 2 days and 7 days post-vibrotactile treatment. As shown in Fig. 5a, vibrotactile treatment at the cymba concha significantly decreased DAS-28 scores in RA patients at two days post-treatment (DAS28 score pre-treatment = 4.19 ± 0.33 [3.16–5.96] vs. post-treatment = 3.12 ± 0.25 [2.43–4.37], $p < 0.05$, RMANOVA, Friedman test). Disease activity remained significantly reduced 7 days following vibrotactile treatment (DAS28 score 7 days post-stimulation = 2.79 ± 0.21 [2.14–3.96]; $p < 0.01$, RMANOVA, Friedman test).

At the time of the study, nine RA patients had active disease (moderate to severe, DAS28 score > =3.2) based on the criteria by American College of Rheumatology (Anderson et al. 2012); two additional patients had inactive disease (DAS28 score < 2.6) and underwent the study, but these data were not included as part of the analysis. Disease activity was significantly attenuated in RA patients with active disease (24–51% reduction in DAS28 scores over 7 days) after vibrotactile treatment at the cymba concha. 85% of the patients with moderate disease (5 out of 7 patients DAS28 scores 3.16–4.38) reached inactive disease stage by 7 days (DAS28 scores 2.14–2.41); whereas in two of the study subjects with severe disease (100%, 2 out of 2 subjects), vibrotactile treatment at the cymba concha reduced disease activity by 34–51% (DAS28 score 5.67 and 5.96 reduced to 2.78 and 3.96 respectively). Following taVNS, circulating CRP levels were significantly reduced after 2 days (pre-treatment = 6.66 ± 2.5 [0.2–21] vs. post-treatment = 4.71 ± 1.71 [0.3–14], $p < 0.05$, RMANOVA, Friedman test) but returned back to the baseline after 7 days (6.97 ± 2.56 [0.7–16.2]).

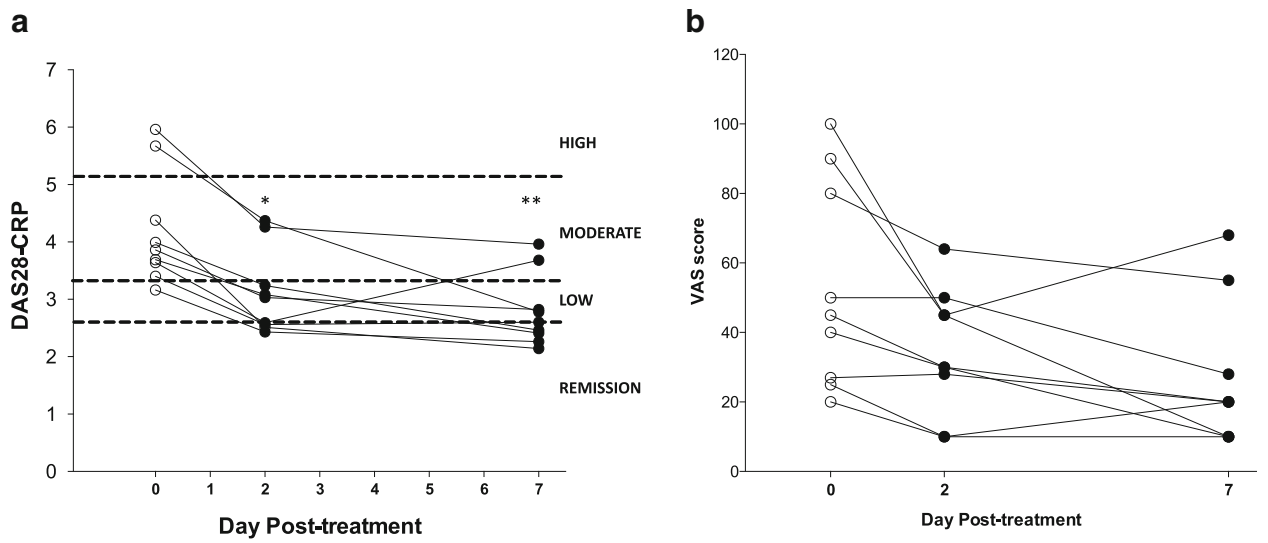

Fig. 5 The effect of vibrotactile treatment at the cymba concha in patients with rheumatoid arthritis. (**a**) The DAS28-CRP scores, (**b**) The visual analog scores in nine RA patients enrolled in the prospective interventional study. DAS28-CRP scores indicate a composite score of enumeration of swollen and tender joints, measurement of high-sensitivity CRP in serum, and scores on the visual analog scale, a validated patient-reported assessment of functional status and well-being. These data were obtained at day 0 (pre-treatment), day 2 and day 7 (post-treatment) following vibrotactile treatment at the cymba concha. Lines connect data points for the DAS28-CRP score of each individual study participant at these timepoints. Hashed lines indicate the cut-off points for categories of disease severity. A DAS score of less than 2.6 indicates remission; a score between 2.6 and 3.2 indicates low/minimal disease activity; a score between 3.2 and 5.1 indicates moderate activity; a score of more than 5.1 is considered high/severe disease activity (Fransen and van Riel 2009; Anderson et al. 2012). The significance of the change by RMANOVA using Friedman test between visits is shown: *$p < 0.05$ vs. day 0; **$p < 0.01$ vs. day 0)

Application of a vibrotactile device at the cymba concha decreases global health VAS in patients with rheumatoid arthritis

Self-reported well-being and pain are routinely used in the assessment of RA (Amaya-Amaya et al. 2012). Vibrotactile treatment of the cymba concha reduced visual analogue scale (VAS) scores, a patient derived measure of global health assessment when assessed 2-days post-treatment (VAS score pre-treatment = 53.0 ± 9.9 vs. post-treatment = 34.7 ± 6.0; $p < 0.05$, RMANOVA, Fig. 5b); this effect persisted throughout the 7 day study period (VAS score 26.8 ± 6.95; $p < 0.05$, RMANOVA, Fig. 5b). Together, these results indicate application of a vibrotactile device to the cymba concha of the external ear may confer therapeutic benefit to RA patients.

Discussion

Our results demonstrate that vibrotactile stimulation at the cymba concha attenuates inflammatory responses in the settings of both health and disease, and further suggest that the auricular branch of the vagus nerve is a functional component of the inflammatory reflex. In healthy subjects, vibrotactile stimulation at the cymba concha attenuated endotoxin induced TNF responses in whole blood assay for up to 24 h. Using a cross-over study, we found that vibrotactile stimulation at the cymba concha but not at the gastrocnemius reduces TNF, IL-6, and IL-1β responses ex vivo following endotoxin challenge in healthy patients, and attenuates disease severity in RA patients with moderate or severe disease. Reduction of systemic inflammation in patients may be achieved by delivering electrical impulses to the vagus nerve at the cervical level (Koopman et al. 2016; Bonaz et al. 2016). Moreover, in healthy subjects, transcutaneous cervical vagus nerve stimulation decreases cytokines and chemokines in whole blood cultures (Lerman et al. 2016). These signals travel distally to the spleen, where they terminate on acetylcholine-synthesizing T cells, which in turn inhibit the inflammatory responses of splenic macrophages (Rosas-Ballina et al. 2011). Previous work has anatomically demonstrated projections of the auricular branch of the vagus. Activation of the NTS has been demonstrated following vibrotactile treatment of the cavum concha in rats (Ay et al. 2015), and afferent fibers of the vagus are known to terminate primarily in the nucleus tractus solitarius (Goehler et al. 2000). The modulation of immunological endpoints both in endotoxin assays performed on blood from healthy subjects and attenuation of disease severity in RA patients strongly suggests that the vibrotactile stimulus at the cymba concha in this study induces neural signals which converge on the efferent neural signaling pathway of the inflammatory reflex. While the origin of descending motor fibers in the vagus that ultimately target

the spleen was beyond the scope of the present study, it is nonetheless a subject of interest for future study.

The stimulation modality, anatomical location, and treatment duration in the present study are different in important ways from these previous studies. The auricular branch of the vagus nerve supplies several regions of the auricle with sensory fibers. The cymba concha region has been shown by cadaveric studies to receive all of its sensory innervation from the auricular branch of the vagus nerve, with no additional innervation from other cutaneous nerves (Peuker and Filler 2002). Integration of anatomical studies, functional studies, and the modulation of clinical endpoints by different approaches to vibrotactile treatment will be important to define both the neuroanatomy and biology of the auricular branch of the vagus nerve as well as its role as a therapeutic target.

While this study was not designed to assess the dose-dependence of vibrotactile treatment, it is interesting to note that the disease attenuation observed in RA patients – as indicated by both DAS28 scores and VAS scores – persisted for up to 7 days in the majority of patients. The RA patients in this study received a total of 4 separate stimulations, each lasting 2 min, delivered on two sequential days, totaling 8 min of vibrotactile treatment in sum. That the reduction of TNF, IL-6 and IL-1 β observed in ex vivo assays following endotoxin challenge in healthy subjects were likewise achieved with minimal stimulation duration. These results demonstrate that in the absence of systemic inflammation, the motor arm of the inflammatory reflex may still be targeted in healthy subjects to reduce the levels of inflammatory cytokines below their physiological set points.

The primary objective of this study was to determine whether activating the auricular branch of the vagus nerve using vibrotactile device inhibits cytokines in humans and improves disease severity in RA. It is reasonable to consider whether placebo mechanisms contribute to these findings as study subjects are aware when the device is delivering the stimulation. It is plausible that placebo mechanisms may contribute to attenuating disease scores in RA. However, lack of any cytokine suppressing effects following vibrotactile stimulation at the gastrocnemius in healthy subjects argues against the placebo effect. A related and open question regarding the nature of vagus nerve activation is the identity of the fibers that carry the signals which culminate in anti-inflammatory effects. Trains of electrical taVNS have been shown to modulate heart rate in a parameter-dependent fashion (Badran et al. 2018). It follows therefore, that similar parametric determinants of vagus nerve signaling may differentially regulate inflammatory cytokines by engaging different types of fibers, a concept which we recently demonstrated in afferent cytokine-mediated signaling of the vagus nerve (Zanos et al. 2018). A limitation of our study is the inability to control for the sound of the vibrotactile device during simulation. These sound waves are produced in close proximity to the external auditory meatus, and could simultaneously engage other neural pathways. Whether sound waves themselves can activate neural signals to control immune responses is an additional interesting subject of study. Taken together, the data in this study support the mechanistic and therapeutic framework for the use of bioelectronic devices to target neural circuits previously mapped to control inflammatory responses. Vibrotactile taVNS warrants further study for the treatment of RA and other disorders of systemic inflammation, and may be of particular relevance in patients for whom implantable vagus nerve stimulators are not tolerated or are otherwise contraindicated or unavailable.

Conclusion

Together, these studies establish that vibrotactile stimulation at the cymba concha modulates TNF, IL-6 and IL-1β production and reduces inflammation in humans. These findings also demonstrate that transcutaneous vagus nerve stimulation reduces the disease severity in RA patients. This pilot study supports the future development of novel non-invasive bioelectronic treatment modalities for diseases currently treated with drugs. Clinical trials in RA are warranted to address the clinical efficacy, as our findings suggest that it is possible to use the vibrotactile taVNS in the experimental therapy of RA and possibly other cytokine-mediated auto-inflammatory disorders.

Abbreviations

AMC: Academic Medical Center; CRC: Clinical Research Center; CREB: cAMP response element-binding protein; DAS: Disease activity score; EDTA: Ethylenediaminetetraacetic acid; ELISA: Enzyme-linked immunosorbent assay; FIMR: Feinstein Institute for Medical Research; hsCRP: High-sensitivity c-reactive protein; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; LPS: Lipopolysaccharide; NFκB: Nuclear factor kappa-light-chain-enhancer of activated B cells; RA: Rheumatoid arthritis; RMANOVA: Repeated measures analysis of variance; taVNS: Transcutaneous auricular vagus nerve stimulation; TNF: Tumor necrosis factor; VAS: Visual analog scale; VDC: Volts direct current; α7nAChR: α7 nicotinic acetylcholine receptor

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Availability of data and materials

Please contact authors for data requests.

Authors' contributions

AFdV, TvdP, VAP, SSC and KJT designed research; MEA, AFdV, RSG and SSC performed research; MEA, GHI, AFdV, TvdP, RSG, SSC, and KJT analyzed and interpreted data; SF designed, and fabricated the vibrotactile device; GHI, SSC and KJT wrote the manuscript; VAP, HY and BD provided additional comments and contributed to finalizing the manuscript. All authors read and approved the final manuscript.

Authors' information
There is no additional author information to be included.

Ethics approval and consent to participate
Human subjects participated at two institutional sites. The study protocol for healthy subjects at the Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands was approved by the Institutional Review Board of the University of Amsterdam. The study protocols for healthy subjects and RA patients at the Feinstein Institute for Medical Research, Manhasset, New York, USA were approved by the Clinical Research Center (CRC) and the Institutional Review Board of Northwell Health, and performed at the CRC of Northwell Health. Informed consent was obtained from all the study participants.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Safety and efficacy of neurostimulation with miniaturised vagus nerve stmulation device in patients with multidrug-refracotry rheumatoid arthritis: a two-stage multicentre, randomised pilot study

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SUMMARY

Background
The inflammatory reflex plays a role in regulating innate and adaptive immunity by modulating cellular and molecular inflammatory pathways. The vagus nerve is a major constituent of the inflammatory reflex and studies have shown that the reflex can be activated by electrical stimulation of the vagus nerve. In this first in-human pilot study, we assessed the safety and efficacy of a novel miniaturised vagus nerve stimulation (VNS) device for the treatment of multidrug-refractory rheumatoid arthritis.

Methods
Participants with moderately to severely active rheumatoid arthritis and prior insufficient response to two or more biological disease-modifying anti-rheumatic drugs or Janus kinase inhibitors with at least two different modes of action were enrolled in a two-stage study done at five clinical research sites in the USA. Stage 1 was open label; participants were implanted with a miniaturised VNS device, which was activated for 1 min once a day. In stage 2, participants were randomly assigned (1:1:1) to receive active stimulation (1 min once a day or 1 min four times a day) or sham stimulation (device implanted but not activated), with the sites and participants masked to treatment assignment. The primary outcome was incidence of treatment-emergent adverse events. Clinical efficacy was assessed as a key secondary outcome. The study was registered with ClinicalTrials.gov, NCT03437473.

Findings
14 patients were enrolled between March 13 and Aug 8, 2018. Three patients received stimulation in stage 1 and, following safety review board approval, the remaining 11 patients were implanted during stage 2 and randomly assigned to receive 1 min of stimulation once daily (n=3), 1 min of stimulation four times daily (n=4), or no stimulation (n=4) for 12 weeks. There were no device-related or treatment-related serious adverse events. Surgery-related adverse events were Horner's syndrome and vocal cord paralysis (in one patient each), which resolved without clinically significant sequelae. No deaths were recorded.

Interpretation
VNS with a miniaturised neurostimulator was safe and well tolerated and reduced signs and symptoms of rheumatoid arthritis in patients with multidrug-refractory disease. These results support further evaluation in a larger randomised sham-controlled study.

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VNS for treatment of inflammatory joint diseases

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ABSTRACT

The cholinergic anti-inflammatory pathway regulates innate and adaptive immunity during normal physiological function, and activation of the pathway by electrical stimulation of the vagus nerve (VNS) can reduce pathological levels of inflammation in animal models of autoimmune disorders. A proof-of-concept human study of VNS in rheumatoid arthritis (RA) has shown that VNS can ameliorate inflammation in humans. Future clinical studies will employ a novel, application-specific investigational stimulation system. In concept, this system is capable of being evolved to function in a closed-loop manner, adjusting therapy delivery to the patient's level of disease activity.

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