

Control of inflammation using non-invasive neuromodulation: past, present and promise

Aisling Tynan¹, Michael Brines¹ and Sangeeta S. Chavan^{1,2,3}

¹Laboratory of Biomedical Science, Institute of Bioelectronic Medicine, Feinstein Institutes for Medical Research, Northwell Health, 350 Community Drive, Manhasset, NY 11030, USA

²Elmezzi Graduate School of Molecular Medicine, 350 Community Drive, Manhasset, NY 11030, USA

³Donald and Barbara Zucker School of Medicine at Hofstra University, Hempstead, NY 11549, USA

Correspondence to: S. S. Chavan; E-mail: schavan@northwell.edu

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Abstract

The nervous system has been increasingly recognized as a novel and accessible target in the regulation of inflammation. The use of implantable and invasive devices targeting neural circuits has yielded successful results in clinical settings but does have some risk or adverse effects. Recent advances in technology and understanding of mechanistic pathways have opened new avenues of non-invasive neuromodulation. Through this review we discuss the novel research and outcomes of major modalities of non-invasive neuromodulation in the context of inflammation including transcutaneous electrical, magnetic and ultrasound neuromodulation. In addition to highlighting the scientific observations and breakthroughs, we discuss the underlying mechanisms and pathways for neural regulation of inflammation.

Keywords: auricular, inflammatory reflex, ultrasound, vagus

Introduction

Reducing inflammation and pain with non-invasive electrical neuromodulation can be traced back for about 5000 years, as Egyptian tomb reliefs (ca B.C.E. 2500) prominently feature the Nile electric catfish (*Malopturus*) and hint at its use for painful conditions (1) (Fig. 1). The effects of 'natural electricity' from contact with the electric ray (Torpedo) were noted by Hippocrates and Aristotle to cause a numbing effect (2, 3). The first person known to have been cured of an inflammatory condition by electricity was Anteros, a court official of Emperor Tiberius (42 B. C. E. to 37 C.E.). While walking on a seashore, he accidentally stepped on a Torpedo, and received a strong electric shock (Torpedo voltages range up to 225 volts). After the numbing effect of the shock subsided, Anteros realized that he has been freed from his gout, a type of arthritis (4).

Referring to this incident, Scribonius Largus (1–50 C.E.), the court physician to Roman emperor Claudius, suggested the use of electric shocks from the Torpedo as a therapy for arthritis (4), making the electric fish the first non-invasive neuromodulation device employed by humans to treat inflammatory diseases. Although Torpedo were in use for a long time for the regulation of inflammatory conditions, recent studies at the intersection of immunology, neuroscience and bioelectronic devices have provided important insights into

the molecular mechanisms of neuro-immune communication that underlie its efficacy (5, 6).

Inflammation is a dynamic protective immune response mechanism against endogenous cell damage, toxic/metabolic insults and pathogens. While essential for eliminating the inciting stimulus, inflammation promotes tissue healing and, in the case of infection, establishes immunological memory. However, unresolved or excessive inflammation can be deleterious, resulting in autoimmune or autoinflammatory disorders (7). Immune dysregulation and aberrant inflammation also play a major role in obesity, type 2 diabetes, metabolic syndrome and cancer pathogenesis affecting millions of people worldwide (8–10).

Identifying the molecular mechanisms of inflammatory responses has resulted in the development of an array of pharmaceutical and biological agents to treat inflammation, accounting for a multi-billion dollar drug industry (11, 12). Standard therapies include glucocorticoids, methotrexate, monoclonal antibodies and other pharmacological agents targeting inflammatory pathways (12). In spite of the availability of these therapies, a significant number of patients are either unresponsive or become resistant (13). Even more concerning are the adverse side-effects of treatment, including increased susceptibility to infections, elevated risk of

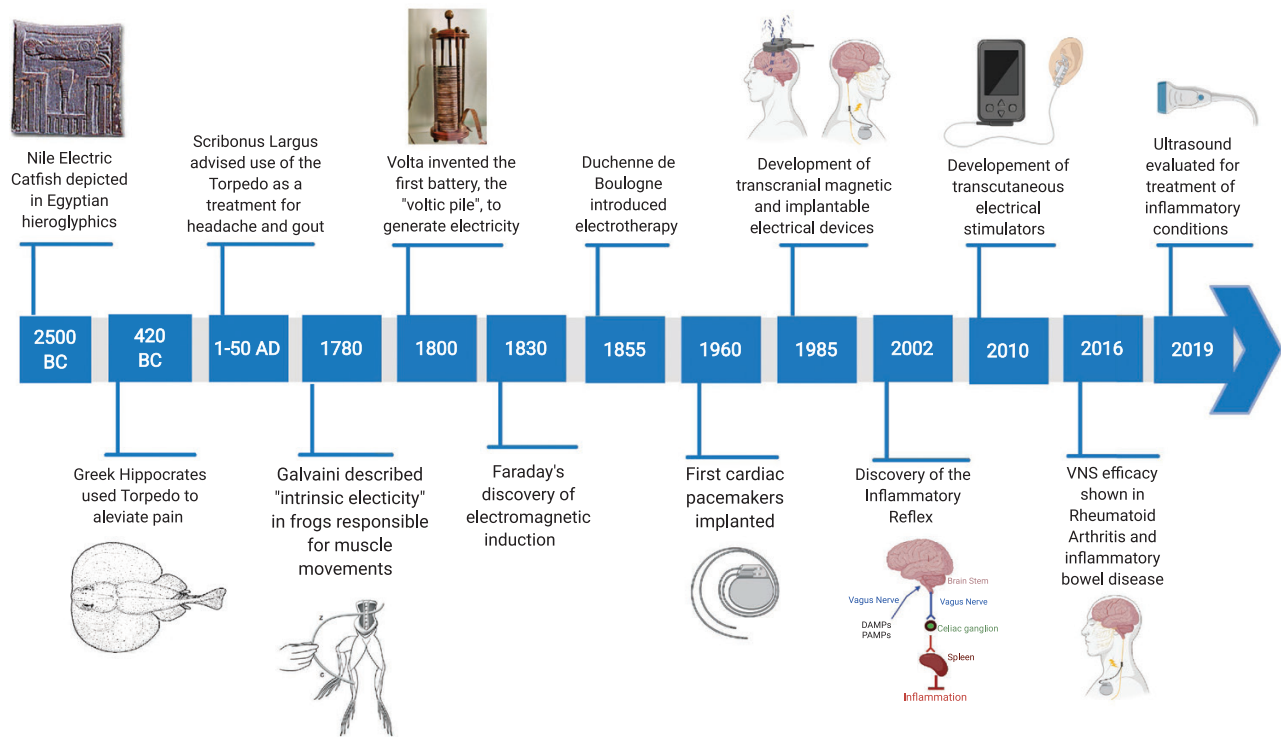


Fig. 1. Timeline indicating the important developments in neuromodulation therapy (Created with BioRender.com).

malignancy and off-target inflammatory responses (14–16). Accordingly, there is an unmet clinical need for alternative therapies that can directly treat inflammatory conditions.

Neuromodulation as therapy

Bioelectronic devices that harness electrical neuromodulation have been used to treat diverse diseases for decades. Based on the sequential discoveries of bioelectricity by Galvani (1780), the battery by Volta (1799) and electromagnetic induction by Faraday (1831), a variety of invasive and non-invasive electrical stimulators have been developed for the treatment of specific diseases (Fig. 1). For example, deep-brain stimulation improves quality of life for patients with depression or Parkinson's disease (17, 18); sacral-nerve stimulation helps people with bowel and bladder problems (19); pacemakers and defibrillators have revolutionized the treatment of patients with cardiac arrhythmias (20); and vagus-nerve stimulation (VNS) has been used to treat pharmaco-resistant seizures for more than 30 years (21).

Recently, VNS has shown efficacy in modulating inflammatory diseases such as rheumatoid arthritis (RA) and Crohn's disease (22, 23), following the discovery of a physiological mechanism accounting for its efficacy: the inflammatory reflex (Fig. 1) (24–26). The inflammatory reflex is a vagus nerve-based neural circuit in which afferent vagus nerve signaling activated by inflammatory mediators, such as cytokines or pathogen-derived molecular signals, functionally culminates into efferent vagus nerve activation that dampens pro-inflammatory cytokine production. To accomplish this, the motor (efferent) signals in the vagus nerve activate the splenic nerve (27), which culminates in the release of

acetylcholine (ACh) by a subset of T lymphocytes expressing choline acetyltransferase (ChAT) (28). ACh interacts with the $\alpha 7$ nicotinic ACh receptor ($\alpha 7$ nAChR) expressed by macrophages to inhibit cytokine production (29).

In addition to control of inflammation in peripheral tissues and organs, the inflammatory reflex may have therapeutic potential for central nervous system (CNS) inflammatory diseases, e.g. multiple sclerosis. Notably, 'gateway reflexes' have been discovered in which a variety of stimuli (gravity, pain, electricity, stress, light) activate neural circuits that result in focal inflammation of blood vessels of the blood–brain barrier (30). This inflammatory reaction allows for reactive lymphocytes and other immunocompetent cells to pass through the blood vessels and into the CNS, activating inflammatory disease. Modulation of this neuronal circuit reduces localized chemokine expression and suppresses the entry of pathogenic cells. Although not yet specifically explored, activation of the inflammatory reflex may be useful for therapeutic attenuation of gateway reflex pathways.

The discovery of the inflammatory reflex has prompted a substantial interest in developing strategies to target the nervous system as a key regulator of inflammatory responses and has been translated into therapeutic devices which have significantly improved disease-related end-points in clinical trials of patients with chronic inflammatory conditions (22, 23). In addition to these innovative programs, it is interesting to note that the traditional therapeutic technique of acupuncture is a form of neuromodulation and, as such, has been demonstrated to activate anti-inflammatory activity (31). For example, needle stimulation of nerve endings in the traditional ST36 Zusanli acupoint (i.e. in the anterior tibial muscle 2 mm lateral to the anterior tubercle of the tibia and 4 mm

distal to the knee joint lower point, adjacent to the common peroneal and tibial branches of the sciatic nerve) has been shown to potently reduce inflammation and increase survival following endotoxin administration (32, 33); and using electroacupuncture reduces polymicrobial peritonitis (32) in the mouse. These effects depend upon an intact inflammatory reflex, as transection of the vagus nerve abolishes the anti-inflammatory effects of acupuncture.

These pre-clinical and clinical studies focusing on the anti-inflammatory effects of the vagus nerve resulted in the current development of bioelectronic medicine. This growing field combines new insights into neurally mediated regulation of the immune system and advances in bioelectronic technology to offer novel approaches to both diagnosis and treatment of diseases (5). Studies are identifying novel targets for neuro-immune regulation, including the sciatic/vagus pathway that regulates catecholamine production in the adrenal gland (32), central neural circuits maintaining glucose and energy homeostasis (34) and intestinal nerve pathways modulating gut immune responses to bacterial infection (35). Moreover, the scope of the neuromodulation as a therapeutic approach has been extended into a number of chronic inflammatory conditions from asthma (36), Alzheimer's disease (37), diabetes (38) and digestive disorders (39) to cardiovascular diseases (40).

Although initial studies targeting neuromodulation have focused on using implantable bioelectronic devices, the development of non-invasive technologies could ultimately lead to conceptually personalized treatment for disease management. In this review, we briefly summarize key aspects of some of the non-invasive neuromodulation platforms, leading to current clinical translation for the treatment of inflammatory conditions.

Non-invasive VNS

VNS at the cervical region has been established as a non-pharmacologic therapeutic approach for control of inflammation in a number of pre-clinical disease models (5, 10, 41–43). Earlier clinical studies of VNS using implantable bioelectronic devices have shown the efficacy of VNS for the treatment of RA (22), Crohn's disease (23) and fibromyalgia (44), validating the translational applicability of pre-clinical findings. The current bioelectronic device requires surgical implantation of a fine-wire electrode wrapped around the left cervical vagus nerve in the neck, which is associated with technical and surgical challenges including electrode fracture, dislocation, generator malfunction, wound infection, recurrent laryngeal nerve palsy and cardiac arrhythmias under test stimulation (45). It is also associated with potential adverse side-effects including syncope, asystole, bradycardia, late-onset bradyarrhythmia, paresthesia, pain, sleep apnea, cough, hoarseness, dysphagia, dyspnea and thermal injury to the vagus nerve and adjacent area because of the radiofrequency exposure (46–48).

Given the number of conditions that VNS has the potential to benefit, two types of transcutaneous VNS have been developed: transcutaneous cervical VNS (TC-VNS) and transcutaneous auricular VNS (TA-VNS). Both treatments do not require surgical implantation of the device; therefore,

exhibiting a much broader therapeutic potential. Functional magnetic resonance imaging demonstrated that both mechanisms of non-invasive VNS activate known brain projections of the vagus nerve, including the nucleus tractus solitarius (NTS), parabrachial area, hypothalamus, amygdala, nucleus accumbens, anterior insula and locus coeruleus (49–53).

Transcutaneous cervical VNS

TC-VNS can be accomplished by delivering a low-voltage electrical signal to the cervical vagus nerve with electrodes placed over the sternocleidomastoid muscle (54). Although implanted electrodes for VNS are positioned at a similar location, the position of the vagus nerve beneath the skin within the carotid sheath, superficial fascia and sternocleidomastoid muscle makes the transcutaneous electrical stimulation of the vagus fibers difficult; with current bioelectronic devices most likely stimulating both afferent and efferent fibers in the vagus nerve bundle (55).

GammaCore® transcutaneous device has been approved by the Food and Drugs Administration (FDA) for acute treatment of migraine and acute or prophylactic treatment of cluster headaches (56). It uses high- and low-frequency stimulation to penetrate deep into the neck as a clinical application for cluster headaches or migraines. The anti-inflammatory effects of TC-VNS have been explored using this device. TC-VNS (up to three times in a day) in healthy subjects resulted in a significant decrease in whole-blood cytokine and chemokine levels (57), and improved fatigue and immune responses in patients with primary Sjögren's syndrome (58). Preliminary clinical data also demonstrated beneficial effects in hemicrania continua (59), asthma (60), asthma patients with bronchoconstriction (60) and respiratory distress associated with chronic obstructive pulmonary disease (COPD) (ClinicalTrials.gov Identifier: NCT01679314). These findings paved the way for the gammaCore® device to receive emergency use authorization from the FDA as an additional therapy for coronavirus disease 2019 (COVID-19) patients experiencing asthma-related breathing difficulties and reduced airflow (61). Computational modeling analysis indicated the utility of TC-VNS in models of spinal-cord stimulation, deep-brain stimulation and stimulation of other peripheral nerves; however, more work is needed to elucidate the clinical benefit in these disease conditions (62).

A number of clinical studies of TC-VNS are in progress including pancreatitis (ClinicalTrials.gov Identifier: NCT03357029), pain perception (ClinicalTrials.gov Identifier: NCT01174498), dyspepsia and irritable bowel syndrome (ClinicalTrials.gov Identifier: NCT02388269), depression (ClinicalTrials.gov Identifier: NCT04037111) and Raynaud's phenomena (ClinicalTrials.gov Identifier: NCT03869008).

RA is a debilitating chronic autoimmune and inflammatory disease that affects more than 1.3 million people in the USA alone and is expensive to treat. Current therapy (e.g., anti-tumor necrosis factor (TNF), anti-interleukin (IL)-6 receptor, and anti-CD20 biologics, T-cell co-stimulation inhibitors, or methotrexate) is associated with significant toxicity and is not effective in all patients. Previous studies using implantable bioelectronic vagus-nerve stimulators demonstrated significant improvement in disease activity in RA patients for up to

84 days (22). A recent study targeted the cervical vagus nerve transcutaneously in RA patients using a portable electrical stimulator with two steel contact electrodes (63). TC-VNS performed three times per day for 4 days significantly decreased the disease activity score 28-C-reactive protein (DAS28-CRP) values in RA patients, with a concurrent decrease in circulating CRP levels and swollen-joints scores (63).

Pre-clinical studies have also shown the efficacy of TC-VNS in alleviating CNS inflammation and brain derangements by attenuating cerebral ischemic injury and altering microglial activation toward a neuroprotective phenotype (37, 64, 65). Zhao *et al.* stimulated the right cervical vagus nerve in a mouse model of focal cerebral ischemia (64) and identified microglial M2 polarization as an important mechanism underlying the TC-VNS-mediated neuroprotection against cerebral ischemia–reperfusion injury. This effect of TC-VNS was dependent on the inhibition of IL-17A production (64). Similarly, a single TC-VNS treatment in a mouse model of Alzheimer’s disease resulted in significantly altered morphological changes in microglia from a neuroinflammatory to a neuroprotective phenotype (37). Interestingly, only the older animals in this study showed the morphological changes following TC-VNS, indicating that primed or activated microglia, and not healthy microglia, are more susceptible to morphological changes as a result of TC-VNS. These studies suggest a stabilizing effect of TC-VNS on microglia morphology to a neuroprotective phenotype.

Transcutaneous auricular VNS

TA-VNS is an emerging therapeutic approach in the field of bioelectronic medicine. The external ear is the only location on the body where afferent fibers of the vagus nerve innervate the skin, specifically the antihelix, tragus, cymba concha and concha (66, 67). The proportion of vagus fibers varies in different areas of the ear and the cymba concha is the only distinct region with 100% innervation by the vagus nerve (67, 68). In brief, the auricular branch of the vagus nerve allows for an accessible external site for electrical stimulation, which then connects and transmits signals to the brainstem. In particular, the auricular branch of the vagus nerve within the cymba concha sends signals to nearby brainstem nuclei, including the NTS, the locus coeruleus and the dorsal raphe nucleus (66), and regulates the heart rate and sympathetic nerve activity (69).

A number of pre-clinical studies have highlighted the efficacy of TA-VNS in reducing inflammation. Zhao *et al.* originally demonstrated the protective effects of TA-VNS against endotoxemia in rats by reducing TNF levels after administration of lipopolysaccharide (LPS; also known as endotoxin) (70). These data were recently recapitulated in a mouse model of endotoxemia, with TA-VNS reducing a number of pro-inflammatory cytokines, including TNF, IL-6 and IL-1 β (71). In a pre-clinical model of postoperative ileus, TA-VNS significantly attenuated intestinal cytokine expression, improved gastrointestinal transit and lowered leukocyte recruitment to the area of manipulation (71). TA-VNS has also been used to elicit neuroprotective effects in cerebral ischemia–reperfusion injury, significantly attenuating the volume of the infarct and inducing angiogenesis in rats (72).

Recently, two pilot open-label studies evaluated the effects of TA-VNS in normal subjects and RA patients. First, we demonstrated the efficacy of TA-VNS using a vibrotactile device at the cymba concha in attenuating endotoxin-induced inflammatory responses in healthy subjects (73). The therapeutic efficacy of this neuromodulation was also demonstrated in RA patients. Stimulation of the auricular branch of the vagus nerve at the cymba concha twice per day for 2 days significantly improved DAS28-CRP disease activity scores in patients with RA (73). In addition, a persistent improvement in visual analogue scale scores—a patient-derived measure of global health assessment—was observed following TA-VNS treatment (73). Initial results of a 12-week proof-of-concept pilot study of TA-VNS using a wearable device showed that the device is well-tolerated in RA patients, with significant reductions in the disease severity. Out of 30 RA patients receiving TA-VNS, 11 attained low disease activity and 7 achieved remission. The TA-VNS treatment was well-tolerated over the study period with American College of Rheumatology 20 (ACR20), ACR50 and ACR70 response rates of 53%, 33% and 17%, respectively, at 3 months (74). Follow-up studies demonstrated sustained, long-term benefits of TA-VNS in RA patients for up to 1 year (75). Fifteen out of 30 patients continued the study for another 9 months and used the wearable device for up to 30 min daily as in the first 12 weeks of the study. A significant reduction in the disease activity (DAS28-CRP) was observed, without significant adverse effects (75).

The use of TA-VNS as a treatment for postoperative ileus has also been suggested (71, 76). TA-VNS activated efferent vagus nerve signaling to the viscera and increased gastrin levels (a surrogate marker for vagus nerve activation) in 14 patients requiring open laparotomy. TA-VNS led to suppression of the action potential frequency and an increase in action potential amplitude, as analyzed by a free-running electromyography in the stomach of these patients (76). The protective effects of TA-VNS have been successfully explored in depression, epilepsy and cardiovascular diseases (77–80). Interestingly, these clinical studies have highlighted the anti-inflammatory effects of TA-VNS, including suppression of inflammatory cytokines, and attenuation of heart-rate irregularities in patients undergoing cardiac surgery or with paroxysmal atrial fibrillation (80, 81).

A recent randomized, double-blind, placebo-controlled pilot study demonstrated TA-VNS reduces pain and fatigue in patients with systemic lupus erythematosus (82). The same device was recently used in a clinical trial for stroke patients to enhance muscle recovery. Although results have yet to be published, it is reported that patients who received TA-VNS exhibited enhanced muscle recovery after 3 weeks of stimulation (ClinicalTrials.gov Identifier: NCT03592745). In light of the most recent COVID-19 pandemic, studies have highlighted the possible effects of TA-VNS in an acute respiratory distress syndrome with compelling evidence for this hypothesis yet to be tested (83).

The Cerbomed device NEMOS (Erlangen, Germany) utilizes a special earphone-like electrode for TA-VNS to ensure correct placement of electrodes for at-home use (84), and has received European clearance for the treatment of epilepsy, depression and pain relief. Its use in drug-resistant epilepsy patients has been associated with significant reductions

in seizure frequency and severity, with corresponding improvements in quality of life (85). TA-VNS using NEMOS in recovering stroke patients demonstrated increased motor recovery, but no mechanistic insight has been evaluated (86).

Other clinical trials using the NEMOS device are investigating the effects of TA-VNS in juvenile inflammatory arthritis (ClinicalTrials.gov Identifier: NCT01924780) and on peripheral glucose metabolism (ClinicalTrials.gov Identifier: NCT03615209). An individualized approach is utilized for NEMOS, with the stimulation intensity chosen by the individuals on the basis of the intensity needed to elicit a non-painful stinging sensation, with a recommended stimulation duration up to 4 h per day. This non-standard individualized approach, however, impedes the establishment of a standardized protocol. Other TA-VNS devices, NET-1000 and NET-2000, developed by Auri-Stim, have been approved by the FDA for the treatment of depression, anxiety and insomnia (87), but the effects on inflammation have yet to be established.

Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) transcutaneously delivers a rapidly pulsed, high-intensity magnetic field to cause an electric current at a specific area of the brain via electromagnetic induction (88). With a variable magnetic field, a voltage difference between two points is induced resulting in current flow which subsequently stimulates the neural circuits. As cell bodies have higher stimulation thresholds, TMS preferentially stimulates axons (89, 90).

In clinical practice, the non-invasive nature of TMS has several advantages: the magnetic field can pass through any medium without attenuation, and the field decreases inversely proportionally to the distance from the generator coil. TMS has an excellent safety profile, with patients rarely reporting pain due to stimulation; no charged particles are injected into the skin; and magnetic stimulation has only a weak recruitment ability for cutaneous sensory afferent fibers. TMS is delivered using stimulation coils, with the focal ability and depth of stimulation established by changing the type of coil attached to a high-current pulse generator. Several stimulation parameters have been proposed with TMS, with variations in duty cycle, frequency and intensity being actively explored.

TMS has seen ample clinical applications including depression, pain management, neural reinforcement after trauma, spasticity reduction, increased muscle strength after surgery and reduction of dysphagia. Repetitive TMS has been shown to reduce apoptotic cell death and neuroinflammation after hemispherectomy-induced focal brain injury in rats (91). Activation of the inflammatory reflex via TMS has yet to be explored; however, targeting brain areas associated with the modulation of immune function, including the dorsal motor nucleus (the efferent outflow of the vagus nerve), NTS (the afferent inflow of the vagus nerve), insular cortex and the hypothalamic–pituitary–adrenal axis, is achievable. Also of interest is the use of peripheral magnetic stimulation on sites other than the brain. Early studies have shown success but are mainly limited to pain-management models (92–95).

Ultrasound technology

Since the first report of ultrasound as a therapeutic tool in the 1920s, it has been widely used in clinical practice and clinical/translational research for the treatment of various human malignancies (96–98) and pathologies including Parkinson's disease (99), stroke (100), prostatic hyperplasia (101), renal masses (102), treatment of abdominal subcutaneous adipose tissue (103), bone repair (104), osteoarthritis (105) and carpal tunnel syndrome (106). Ultrasound waves are sound waves generated by cyclic mechanical vibrations with frequencies higher than the upper audible range for the human (>20 kHz). Whereas diagnostic ultrasound uses frequencies in the MHz range, therapeutic ultrasound uses frequencies in the kHz range, leading to focused beams of ultrasound energy with higher levels of precision that target deeper tissues compared with existing non-invasive neuromodulatory approaches.

Several modalities of action of focused ultrasound have been proposed; including mechanical force, local heating and bubble cavitation, described in detail elsewhere (107). High-intensity focused ultrasound is currently approved by the FDA for thermal ablation in many pathologies, including atrial fibrillation (108), uterine fibroids (109) and visceral tumors (110). Although in clinical interest for more than half a century (111), the interest for focused ultrasound as a non-invasive neuromodulation approach for regulating inflammatory responses has increased recently (112–114).

Ultrasound stimulation targeted to the spleen in mice reduced antibody responses to sheep erythrocytes in a manner dependent on the dose of ultrasound energy, whereas ultrasound delivered to an area devoid of major lymphoid tissue was not immunosuppressive (115). Additionally, exposure to ultrasound impaired the phagocytic and bactericidal activity of peritoneal macrophages (116). Recently, our knowledge about the immunomodulatory functions of ultrasound was considerably advanced when ultrasound energy was characterized as a major regulator of inflammation (112, 113). Delivery of pulsed ultrasound to the spleen using a non-invasive clinical ultrasound machine diminished inflammation and tissue damage during renal ischemic–reperfusion injury (112, 113). While attempting to image the kidney vasculature before reperfusion, Gigliotti *et al.* demonstrated that ultrasound conferred a significant protection from renal ischemia–reperfusion (112). The protective effect of a single ultrasound stimulation lasted for 2 days and waned in a time-dependent manner when ultrasound was applied up to 7 days before kidney injury (112). Moreover, ultrasound treatment was also protective in reducing acute kidney injury in the cecal ligation–puncture model of induced sepsis (113).

A growing body of experimental evidence in recent years indicates that targeting the spleen with focused ultrasound controls peripheral immune responses and inflammation (114, 117). When applied either prior to or at the time of endotoxin challenge, focused ultrasound treatment was found equally effective in TNF reduction as compared with traditional VNS using implanted electrodes (114). In this study, an ultrasound transducer was focused directly to the center of the spleen, using a second imaging transducer to align the ultrasound delivery, and pulsed ultrasound energy was delivered to

the spleen prior to and after endotoxin administration (114). A single session of ultrasound stimulation suppressed TNF in rodent models. In addition, ablating the ACh-producing T cells or blocking $\alpha 7nAChR$ suppressed the immunomodulatory effect of ultrasound stimulation (114), confirming the role of the inflammatory reflex. Although ultrasound stimulation at several distinct locations within the spleen provided similar modulation of the TNF response, stimulation at the off-target sites (i.e. liver) did not modulate the LPS-induced inflammatory response (114). Interestingly, splenic ultrasound stimulation showed no effect on the heart rate, a known side-effect of stimulation of the vagus nerve. This study also demonstrated the ability of site-specific effects of ultrasound stimulation that cannot be achieved with traditional cervical VNS. Cotero and colleagues demonstrated that targeting the ultrasound energy to the porta hepatis region of the liver, which contains glucose-sensitive neurons, but not at the liver lobes or the spleen, reduced LPS-induced hyperglycemia.

In line with the effects seen in clinical trials studying efficacy of VNS in RA (22), Zachs *et al.* demonstrated that focused splenic ultrasound significantly attenuates the disease severity in a model of inflammatory arthritis (117). Importantly, using single-cell RNA sequencing, their study showed ultrasound stimulation-induced changes in gene expression in splenic lymphocytes from arthritic but not from non-arthritic mice, suggesting a unique therapeutic effect in the setting of inflammation (117). A clinical study is in progress to study the effects of focused splenic ultrasound in RA (ClinicalTrials.gov Identifier: NCT03690466).

The mechanism of this splenic ultrasound-mediated immunomodulation is unknown, but several findings suggest the protective effect is mediated via activation of the inflammatory reflex circuit. First, the immunomodulatory effect of ultrasound is dependent on the spleen, as splenectomized animals fail to respond to ultrasound treatment (112). Second, targeting the spleen is crucial in achieving these protective effects, since ultrasound stimulation of other body locations is ineffective (114, 115). Third, catecholamine depletion by reserpine (114) or chemical sympathectomy by using splenic administration of 6-hydroxydopamine (a neurotoxin that destroys catecholaminergic neurons) (113) abolishes the protective effect of ultrasound, indicating a requirement for innervation of the spleen. Fourth, the protective effect of ultrasound is absent in mice lacking T and/or B cells, but could be reconstituted by adoptive transfer of CD4⁺ T cells (112). Fifth, mice lacking expression of $\alpha 7nAChR$ or with knockout of CD4-ChAT cells (CD4⁺ T cells that express ChAT) fail to respond to ultrasound (114); $\alpha 7nAChR$ and CD4-ChAT cells are the key regulators of the inflammatory reflex pathway (28, 29). Blocking of $\alpha 7nAChR$ with α -bungarotoxin abrogates the protective effect of splenic ultrasound stimulation (114). Finally, splenic ultrasound stimulation drives neurotransmitter and cytokine changes within the spleen consistent with modulation of the inflammatory reflex (114). Both norepinephrine and ACh concentrations increase in the spleen following splenic ultrasound stimulation. In addition, splenic ultrasound reduces levels of pro-inflammatory cytokines, such as TNF and IL-1 in the spleen from endotoxemic animals (114). Taken together, these studies indicate, similar to VNS, splenic

ultrasound-mediated immunomodulation is due to activation of the inflammatory reflex pathway.

Focused ultrasound modulation of neural signaling has also been evaluated for other disease models. Attenuation of post-myocardial infarction ventricular arrhythmias and inflammation can be achieved in a canine model by modulating the sympathetic neural activity (118). As focused ultrasound technologies continue to advance the ability to penetrate deeper into the body while maintaining specificity, the idea of this invasive modulation to translate to a non-invasive focused ultrasound is not a far-fetched concept. Similar to electrical VNS, a single focused ultrasound stimulation on the cervical vagus nerve was protective in endotoxemic animals in a dose-dependent manner (119). In addition, ultrasound has been explored as a therapy for inflammation induced by soft-tissue injury. Compared with placebo, ultrasound stimulation in 76 patients with lateral epicondylitis lowered inflammation and pain (120). It was shown to reduce swelling and pain, and accelerate tissue repair (121). In addition, anti-inflammatory effects of ultrasound are closely related to the decrease of inflammatory cell infiltration in the synovium and attenuation of hyperplasia (122).

Ultrasound stimulation targeted at the porta hepatis region of the liver (a region that is highly innervated by glucose-sensitive neurons (34)) provided protection against LPS-induced hyperglycemia (114). Hepatic ultrasound stimulation limited the increase in blood glucose levels. Furthermore, this protective effect was anatomically specific, as targeting the stimulation toward the right or left lobe of the liver reduced the glucose-lowering effect of hepatic ultrasound stimulation. In addition, ultrasound stimulation of the porta hepatis did not change concentrations of signaling molecules associated with hepatic glycolysis/gluconeogenesis within the liver; instead, resulted in increased insulin receptor substrate 1 and protein kinase B activation and reduced concentrations of neuropeptide Y and pro-opiomelanocortin in the hypothalamus (114). Interestingly, hypothalamic neuronal activation was accompanied by increased c-Fos expression within the NTS, suggesting ultrasound-mediated modulation via signaling through afferent pathways.

Obesity increases the risk of cardiovascular disease, type 2 diabetes and other diseases (123). Chronic low-grade inflammation mediated by immune and metabolic dysregulation is a characteristic feature in patients with obesity and is causally linked with insulin resistance and other metabolic complications (124, 125). It is increasingly recognized that the brain and the nervous system are involved in the regulation of obesity and obesity-associated complications (26). Accordingly, therapeutic strategies targeting chronic inflammation and improving autonomic function have been proposed (9, 126).

To study the effect of hepatic ultrasound stimulation on the long-term management of obesity and obesity-associated complications, our group has also performed hepatic stimulation experiments in obese mice that were fed a western diet (127). Obese mice were treated with daily ultrasound stimulation targeted to the porta hepatis for 4 weeks. At the time of the treatment initiation, mice on the western diet had already increased weight, which reached a difference of

~10 g when compared with mice fed a low-fat control diet. Ultrasound stimulation at the porta hepatis gradually attenuated the body weight gain, reaching a significant difference with the sham-stimulated group by week 12. In addition, hepatic ultrasound reduced food intake and moderated abdominal fat accumulation in obese mice. Interestingly, this reduction in weight occurred concurrently with decreases in circulating inflammatory cytokines, adipokines, lipids and hepatic leukocyte infiltration, indicating that hepatic ultrasound attenuated inflammatory responses in western-diet-fed obese mice (127). Together, these studies suggest that ultrasound stimulation focused on peripheral organs is an increasingly attractive target to develop organ-specific non-invasive therapeutic strategies for a range of inflammatory conditions.

Concluding remarks

The recent viral COVID-19 pandemic has alarmingly added to the urgent need of utilizing non-invasive therapeutic strategies for treatment of inflammation. Ongoing studies have provided mechanistic insight into neuro-immune communication, and in controlling inflammation by targeting neural circuits using bioelectronic devices. A number of pre-clinical and clinical studies have established the efficacy of non-invasive neural stimulation in the regulation of inflammatory conditions. Specifically, these studies have indicated that targeting the vagus nerve-mediated inflammatory reflex pathway by non-invasive transcutaneous VNS or focused ultrasound stimulation as promising new approaches for treating inflammatory and autoimmune conditions.

Several inter-related issues will need to be clarified for each of these potential new treatment modalities. A primary issue is to define the specificity of stimulation, i.e. to what extent are off-target tissues modulated? Because non-invasive modalities offer the potential to more selectively activate tissue-specific neural pathways and/or specific anatomical locations in contrast to invasive neuromodulation, fewer adverse and unintended effects may result. In this regard, results of clinical trials conducted in a number of diseases have not shown serious adverse effects. For example, TA-VNS treatment for drug-resistant epilepsy carried out in 10 separate trials (350 patients) has resulted in only minor adverse effects related to electrode placement: headache (9%) being most common, followed by skin irritation at the stimulation site (7%) and nasopharyngitis (5%) (85). Another pressing issue is to define specific treatment parameters (stimulation parameters such as waveform, amplitude, timing, duration, etc.) which will likely depend both upon studies of the disease as well as the device.

Pre-clinical studies have unraveled the molecular and cellular mechanisms underlying the effects of neuromodulation using non-invasive bioelectronic devices and provided a rationale for clinical translation. Non-invasive neuromodulation as a therapy for inflammatory conditions is not yet in prime time, but is gaining momentum as a novel therapeutic approach by harnessing the body's own protective neural circuits. Arguably, various modalities of non-invasive neuromodulation are at the forefront of the technological revolution and warrant multidisciplinary collaborative research

efforts to advance bioelectronic medicine and create novel therapeutic strategies.

Although bioelectricity derived from electric fish was used as immunotherapy in antiquity, an understanding of the underlying biophysics and development of therapeutic technology awaited the discoveries that electricity stimulated excitable tissue by Galvani and electromagnetic induction by Faraday, as well as the invention of a portable electricity source by Volta around the turn of the 19th century. Following a long gestational period, implanted electrical stimulators were developed to regulate cardiac rhythm (128, 129). Thereafter, the vagus-nerve stimulator was developed to control drug-resistant epilepsy (130) and activate the inflammatory reflex (22, 23). The first widespread non-invasive neuromodulation utilized TMS (131), which depends upon electromagnetic induction. Further developments in non-invasive neuromodulation have evolved into transcutaneous vagus stimulation (57, 132). Very recently, focused non-invasive mechanical nerve stimulation using ultrasound has been employed as a therapeutic method of activating the inflammatory reflex (114, 117) (please see text for details).

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