Best Practice & Research Clinical Rheumatology 28 (2014) 625–635

Contents lists available at ScienceDirect

Best Practice & Research Clinical Rheumatology

journal homepage: www.elsevierhealth.com/berh

7

Vagus nerve stimulation: A new bioelectronics approach to treat rheumatoid arthritis?

F.A. Koopman [a], P.R. Schuurman [b], M.J. Vervoordeldonk [a], P.P. Tak [a, c, d, *]

[a] *Department of Clinical Immunology and Rheumatology, Amsterdam Rheumatology and Immunology Center, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands*
[b] *Department of Neurosurgery, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands*
[c] *GlaxoSmithKline, Stevenage, United Kingdom*
[d] *University of Cambridge, Cambridge, United Kingdom*

Keywords:
Rheumatoid arthritis
Cholinergic anti-inflammatory pathway
Vagus nerve stimulation

A B S T R A C T

There has been a marked improvement in the treatment of rheumatoid arthritis (RA), but most patients do not achieve disease remission. Therefore, there is still a need for new treatments. By screening an adenoviral short hairpin RNA library, we discovered that knockdown of the nicotinic acetylcholine receptor type 7 (α7nAChR) in RA fibroblast-like synoviocytes results in an increased production of mediators of inflammation and degradation. The α7nAChR is intimately involved in the cholinergic anti-inflammatory pathway (CAP). This led us to study the effects of α7nAChR activation in an animal model of RA, and we could show that this resulted in reduced arthritis activity. Accordingly, stimulation of the CAP by vagus nerve stimulation improved experimental arthritis. Conversely, we found aggravation of arthritis activity after unilateral cervical vagotomy as well as in α7nAChR-knockout mice. Together, these data provided the basis for exploration of vagus nerve stimulation in RA patients as a novel anti-inflammatory approach.

* Corresponding author. Department of Clinical Immunology and Rheumatology, Room F4-105, Academic Medical Center, University of Amsterdam, PO Box 22700, 1100 DE Amsterdam, The Netherlands. Tel.: +31 20 566 7765; fax: +31 20 691 9658.
E-mail address: p.p.tak@amc.uva.nl (P.P. Tak).

http://dx.doi.org/10.1016/j.berh.2014.10.015

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease, which is characterized by pain, swelling, and stiffness of joints, due to synovial inflammation. During active disease, the joints are limited in motion and function, and persistence of synovial inflammation leads to the development of bone erosions and, finally, joint deformities [1]. The signs and symptoms of this condition can be reduced by treatment with synthetic and biological disease-modifying antirheumatic drugs (sDMARDs and bDMARDs, respectively). Treatment of RA is usually initiated with methotrexate, a sDMARD, and can be combined with corticosteroids and other sDMARDs. Biological DMARDs are indicated if there is insufficient response to the initial sDMARD treatment or if there are unfavorable prognostic factors present, such as very active disease, early joint damage, or presence of (high levels) of autoantibodies, immunoglobulin M (IgM) rheumatoid factor (RF), and/or anti-citrullinated protein antibodies (ACPAs) [2]. Despite the fact that there are many types of DMARDs available, there are still many RA patients who do not improve sufficiently. Besides the lack of response to therapy as a reason for the discontinuation of treatment, there are also patients who discontinue medication because of side effects, or because they do not want to take chronic medication. As a result, the need for the development of new therapeutic strategies remains.

Cholinergic anti-inflammatory pathway

Inflammation in peripheral tissues, as observed in RA, can be detected by the afferent vagus nerve and this information is signaled towards the brain. Peripheral administration of the pro-inflammatory mediators lipopolysaccharide (LPS) or interleukin-1-beta (IL1-beta) in rats normally elicits fever, but after bilateral subdiaphragmatic vagotomy the fever response is abated [3,4]. Later, there was the surprising finding that the vagus nerve could not only sense inflammation but also influence it. Activation of the vagus nerve, which is a part of the parasympathetic nervous system, was found to dampen inflammatory processes. Rats with carrageenan-induced hind paw edema (acute inflammation model) were injected intracerebroventricularly (i.c.v) with a very low dose (noneffective if given systemically [3]) of the anti-inflammatory drug CNI-1493, and there was a significant decrease in paw edema. The anti-inflammatory mechanism of action of i.c.v. CNI-1493 was, at the time, unknown, but after bilateral cervical vagotomy the drug was no longer able to reduce paw edema. In combination with the finding that an i.c.v. injection of CNI-1493, but not saline, could increase efferent vagus nerve activity, this led to the conclusion that activation of the vagus nerve by i.c.v. CNI-1493 treatment had an anti-inflammatory effect [5]. These were the first indications that efferent vagus nerve activation could inhibit inflammation in an animal model. The combination of sensing peripheral inflammation by the afferent vagus nerve and the subsequent anti-inflammatory response of the efferent vagus nerve is currently known as the cholinergic anti-inflammatory pathway (CAP) [6].

The CAP can also be activated by electrical vagus nerve stimulation (VNS) or stimulation of the nicotinic acetylcholine receptor type 7 (α7nAChR) [7,8]. The parasympathetic neurotransmitter acetylcholine is the anti-inflammatory mediator of the CAP, which activates the α7nAChR. Acetylcholine can be produced by the vagus nerve, but it can also be produced by nonneuronal cells, for instance, in the spleen. Several studies have shown that the spleen is essential for the anti-inflammatory effect of the vagus nerve, because after splenectomy VNS is no longer capable of reducing inflammation [9–12]. After VNS, the anti-inflammatory reflex appears to travel through the sympathetic splenic nerve towards the spleen. The splenic nerve produces norepinephrine, which triggers choline acetyltransferase-positive (CHAT+) T cells in the spleen to produce acetylcholine (Fig. 1) [13,14]. CHAT-positive cells are also found in the synovium of the RA joint [15,16], which suggests that acetylcholine can also be produced locally in the joint. Joints are not known to be innervated by the vagus nerve, but there appear to be sympathetic fibers in the RA synovium [17]. It is, therefore, conceivable that norepinephrine-producing sympathetic fibers in the joint activate CHAT+ T cells to produce acetylcholine, but this has not been studied yet.

CAP in experimental arthritis

In the past decade, the anti-inflammatory effect of the vagus nerve has been shown in animal models for sepsis, acute pancreatitis, colitis, postoperative ileus, and acute respiratory distress

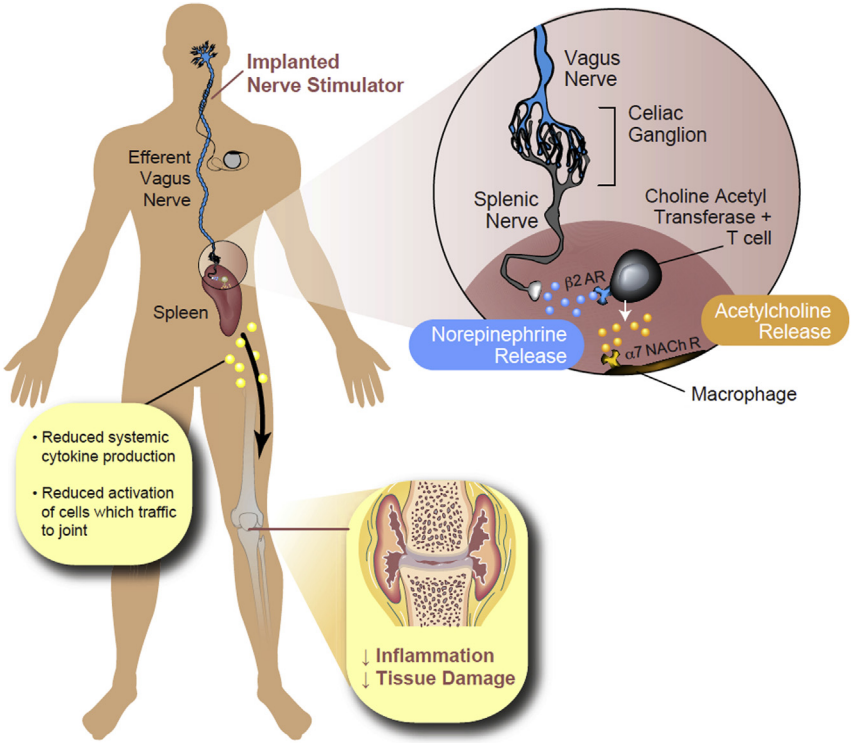

Fig. 1. Electrical stimulation of the parasympathetic vagus nerve leads to activation of the sympathetic splenic nerve which produces norepinephrine in close proximity to choline acetyltransferase positive (CHAT+) T-cells. The CHAT+ T-cells are able to produce the anti-inflammatory mediator acetylcholine, which can bind to the nicotinergic acetylcholine receptor type 7 (α7nAChR) and reduces cytokine production. This results in reduced inflammation in the joints.

syndrome [6,18–21]. We were the first to show the effect of activation of the α7nAChR using the specific agonist AR-R17779 [22] and the non-specific agonist nicotine [22,23] in an animal model of RA, and demonstrated that this approach resulted in reduced clinical signs of arthritis, synovial inflammation, serum cytokine levels, and bone erosions. These results have been confirmed by others using the partially specific agonist GTS-21 [24] and nicotine [23]. Conversely, we found a higher incidence of arthritis and more severe arthritis in mice lacking the α7nAChR [25]. The α7nAChR is present not only on many immune cell types, such as monocytes, macrophages, T lymphocytes, B lymphocytes, and dendritic cells [26] but also on fibroblast-like synoviocytes (FLSs) in the RA synovium [27,28]. FLSs of RA patients pretreated with acetylcholine, nicotine, or AR-R17779 (α7nAChR activation) reduced the production of IL-6 and IL-8 significantly [27–29], indicating that acetylcholine could also have an anti-inflammatory effect in the RA synovium. Besides FLSs, macrophages and monocytes also produce less pro-inflammatory cytokines after α7nAChR activation [6,30]. Thus, human biology studies were in line with the results obtained in animal models of RA, supporting the notion that stimulation of the CAP could have a beneficial effect in RA.

Stimulation of the CAP by electrical VNS is challenging in the experimental arthritis model, because daily electrical stimulation is needed for a prolonged period rather than single electrical stimulation in the acute mouse models. As an alternative approach, vagus suspension to the sternocleidomastoid muscle was performed, leading to activation of the vagus nerve as a result of head movement, after arthritis induction. This led to reduced arthritis activity, decreased serum levels of tumor necrosis factor (TNF), and protection against bone destruction associated with a decrease in the number of osteoclasts after 3 months of follow-up [31]. When we applied electrical stimulation of the vagus nerve for 60 s a day using an implanted vagus nerve stimulator in the collagen-induced arthritis model of RA

in rats, we observed amelioration of arthritis and decreased histological signs of synovial inflammation as well as cartilage damage after 7 days of stimulation [32]. Taken together, activation of the CAP has a consistent anti-inflammatory effect in experimental models of RA.

Effect of nicotine-containing substances in RA patients

As discussed above, nicotine can stimulate the CAP through activation of the α7nAChR. However, despite cigarette smoking being a well-defined risk factor for the development of RA [33–35], a beneficial effect of nicotine on experimental arthritis and an anti-inflammatory effect on RA FLS are observed [22,23,27,28]. How can we reconcile these data? First, it is important to note that, obviously, smoking of cigarettes results in exposure to many other compounds in addition to nicotine. In particular, in RA patients who have a specific genetic background, being positive for the shared epitope, smoking of cigarettes is associated with the development of ACPAs [35]. Indeed, several studies have shown that RA patients who smoke cigarettes have higher disease activity compared to nonsmokers [36–38], although not all data are conclusive [39–41]. The response to therapy in RA patients who are current smokers is lower in most of the longitudinal cohort studies [38,42–45]. If the negative effect of smoking of cigarettes is related to inhaling compounds other than nicotine, then lessons may be learned from the use of nicotine-containing "snuff" (smokeless tobacco) in RA patients. Interestingly, there was a lower disease activity in RA patients who used snuff compared to those who never smoke and previous smokers [46]. Consistent with our hypothesis that substances in smoked tobacco other than nicotine are associated with an increased risk of active RA in shared epitope-positive subjects, the use of snuff is not associated with the development of RA [34,47]. Obviously, RA patients who are current smokers should be advised to stop, also because of the increased risk of cardiovascular disease and cancer associated with smoking [48,49]. The use of snuff is also not harmless, because it contains carcinogenic substances and users have a higher risk of developing oral cancer and cardiovascular disease [46]. However, the observations described above do support the notion that stimulation of the CAP may result in a beneficial effect on RA.

Activity of the parasympathetic nervous system in RA

The vagus nerve, the main component of the parasympathetic nervous system, is most active when the body is in a resting state, and at that time it has a dominant influence on breathing, heart rate, and digestion. In a more active "flight" state, the sympathetic nervous system becomes dominant in controlling these basic body functions. The balance in the autonomic nervous system can be measured by measuring heart rate variability (HRV) [50]. Individuals with a normal HRV are able to respond adequately to changes in blood pressure and breathing, and have therefore a well-functioning autonomic nervous system. Various observational studies have demonstrated that RA patients have lower vagus nerve tone shown by reduced HRV compared to age-matched controls [51–53]. This phenomenon has also been observed in other autoimmune diseases, such as systemic lupus erythematosus [51], ankylosing spondylitis [54], and chronic inflammatory bowel diseases [55]. Parasympathetic activity can be increased by different types of exercise, like cardiac training [56], yoga [57], meditation [58], daily 5-min diaphragmatic breathing [59], and possibly acupuncture [60]. One study showed that low parasympathetic and high sympathetic activity in RA patients predicts a poor therapeutic response to anti-TNF therapy compared to RA patients with a more balanced autonomic nervous system [61]. Taken together, these data show that chronic inflammatory diseases are associated with reduced parasympathetic and increased sympathetic activity. The autonomic balance could potentially be restored by electrical stimulation of the vagus nerve.

VNS in patients with depression or epilepsy

VNS therapy has been approved as a treatment for epilepsy in Europe in 1994 and in the US in 1997 [62]; in the US, VNS has also been approved for the treatment of depression [63]. VNS can be performed after neurosurgical implantation of a vagus nerve stimulator; in 2012, 100,000 vagus nerve stimulators had been implanted [64]. The device consists of two parts: a pulse generator and a lead with electrodes.

The pulse generator contains the battery and the stimulation system, and is positioned subcutaneously below the left clavicle on the pectoral muscle. It is connected to the left vagus nerve in the neck via the lead, with three helices at the end: one positive electrode, one negative electrode, and an anchor tether. The three helices are placed around the vagus nerve to deliver the electrical pulse of the pulse generator (Fig. 2A and B). During surgery, the vagus nerve is electrically stimulated to test the impedance and functionality of the device, which can be accompanied by bradycardia and short-lasting asystole, which is a rare adverse event occurring in about 0.1% of patients; it has only been reported as a consequence of the first stimulation during surgery and is resolved when stimulation is stopped [65]. Adverse events after implantation include infections of the operated area (3–6% of patients) and vocal cord paresis (<1% of patients) [65]. After implantation, VNS therapy can be initiated starting at a low dosage of stimulation with an output current of 0.25 mA. The dosage is increased slowly with steps of 0.25 mA to a maximum output current of 3.5 mA, because toleration to the stimulation is built up with use of the VNS device. However, the final maximum tolerated dosage of the patient can be lower than 3.5 mA. Other stimulation conditions that can be selected are pulse width (130–1000 μs), frequency (10–30 Hz), and duration of stimulation (7–60 s). The VNS settings can be changed with an external programmer and information about performed stimulations (compliance) can be retrieved (Fig. 2C and D). During electrical stimulation, >10% of patients report hoarseness, sore

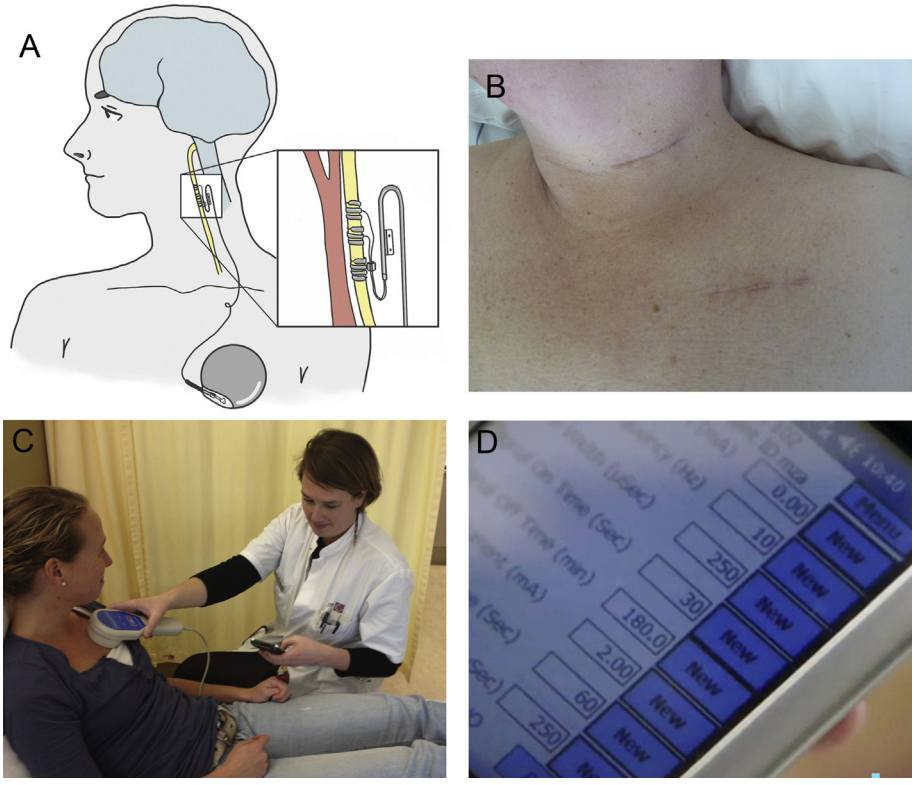

Fig. 2. Vagus nerve stimulation (VNS) from surgery to stimulation settings. A. Implantation of the three helices around on the left vagus nerve containing a positive electrode, negative electrode and an anchor tether. The electrodes are connected to the lead, which is attached to the pulse generator. B. Marks of the implantation 6 months after the operation. There are two incisions made to implant the VNS device. The first horizontal incision is made in the neck to implant the helices around the vagus nerve, the lead is tunneled under the skin and connected to the pulse generator located below the left clavicle on the pectoral muscle, which requires the second horizontal incision. C. With a wand above the pulse generator and a read-out handheld computer, the settings of the pulse generator can be changed or stimulations can be evaluated. D. Example of stimulation settings on the handheld computer.

throat, shortness of breath, and coughing, but in general the stimulation is well tolerated and these symptoms do not require adjustments of VNS device settings [65]. Patients normally only experience side effects if the VNS device is activated. Epilepsy patients receive stimulation several times per hour (the standard setting is 30 s on/5 min off) and have the option to give an additional stimulation with a magnet activation to prevent an upcoming seizure [66].

Early experience with VNS in RA patients

In 2011, we initiated an open pilot study on the safety and efficacy of VNS in RA patients. The hypothesis based on preclinical studies is that VNS will have a beneficial effect on the clinical signs and symptoms of RA. Patients were eligible for inclusion in the study if they were diagnosed with RA >6 months ago according to the American College of Radiology (ACR)/The European League Against Rheumatism (EULAR) 2010 criteria and presented with active disease, defined by the presence of at least four swollen joints and C-reactive protein (CRP) levels ≥7 mg/L. Patients needed to be on stable methotrexate treatment for at least 3 months and were not allowed to have failed bDMARD treatment. The exclusion criteria of the study were mainly general contraindications for VNS therapy (Table 1).

At the time of study initiation, we expected that it would be difficult to include RA patients in a study evaluating a new therapy requiring surgery. However, an overwhelming number of patients responded and wanted to participate in the clinical trial: we received around 1000 responses after the publication of an article in a national newspaper in the Netherlands. Many patients indicated that they were interested in participating in the study as they preferred a minor surgical procedure over chronic drug therapy. They also liked the concept of restoring the natural balance in the body and the feeling of having more control over their disease themselves. Three of these patients fulfilled all inclusion and exclusion criteria, had sufficiently active disease (at least four swollen joints and CRP level ≥7 mg/L), and were included in the pilot study at the Academic Medical Center (AMC) in Amsterdam. Other patients were included in the following centers: University Clinical Hospital, Mostar; Clinical Hospital Center Sestre Milosrdnice, Zagreb; and Sarajevo University Clinical Center, Sarajevo. In total, eight patients were included.

The design of the study is depicted in Fig. 3. Two weeks after implantation of the VNS device, VNS was initiated at 0.5 mA once daily (QD), and this was increased upon toleration by the patient up to a maximum of 2.00 mA during weekly visits. Patients triggered the 60-s stimulation themselves by moving a strong magnet across the pulse generator. In the first week at day 0, patients received their first stimulation and responses were evaluated 1, 2, 4, and 24 h and 4 days after stimulation. Daily home stimulation was initiated at day 7, and until day 21 the stimulation intensity (output current) was

Table 1
In- and exclusion criteria of the vagus nerve stimulation study in rheumatoid arthritis (RA). The exclusion criteria of the study are based on recommendations of Cyberonics™ for use of a Vagus Nerve Stimulation device.

Inclusion criteria
Rheumatoid arthritis of a duration of at least 6 months as defined by the ACR/EULAR classification criteria
Active disease, defined by at least four swollen joints and CRP >7 mg/L
Active disease, despite treatment with methotrexate at a stable dose for at least 3 months
Previous failure to respond to bDMARD therapy is not allowed, but discontinuation of bDMARD for a different reason is allowed
Exclusion criteria
Known history of cardiac rhythm disturbances, atrioventricular block > first degree, or cardiac conduction pathway abnormalities
Significant pharyngeal dysfunction or swallowing difficulties
Preexisting clinically significant vocal cord damage or hoarseness
Asthma or chronic obstructive pulmonary disease not controlled by medication, or any other disease causing significant dyspnea
Previously implanted electrically active medical devices
History of recurrent vasovagal syncope episodes
History of obstructive sleep apnea
Active peptic ulcer disease

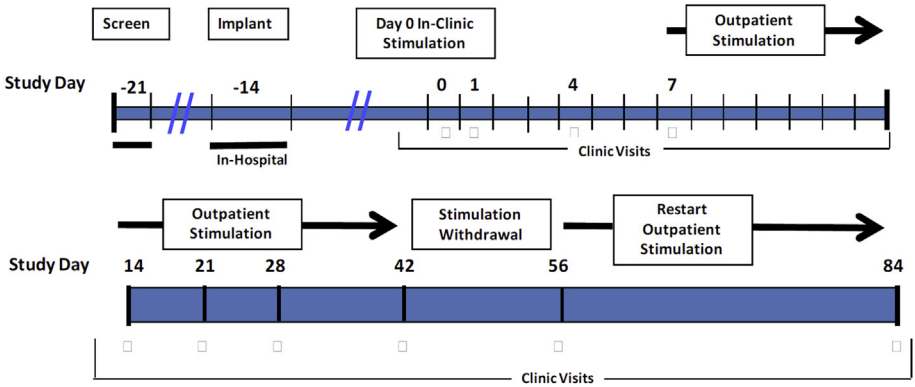

Fig. 3. Overview of set-up of the open pilot study to evaluate the safety and initial efficacy of VNS therapy in RA patients.

increased upon toleration by the patient. At day 28, we evaluated the clinical response according to the EULAR criteria [67]; if the patient did not fulfill the response criteria, then the stimulation frequency was increased from QD to four times daily (QID). Stimulations were continued until day 42 (primary end point) and discontinued from day 42 until day 56 for 14 days. At day 56, stimulation was started again at the previously set stimulation level (mA) and frequency (QD or QID) until day 84. After day 84, patients were enrolled in an extension study, during which they continued VNS therapy. The primary end point of the study was the change in DAS28 (CRP) at day 42 from baseline. Fulfillment of the ACR20 response criteria was one of the secondary end points [68]. There were no serious adverse events in the first eight patients; there were 21 adverse events in seven patients, which were all mild or moderate. Examples of adverse events were dry throat, hoarseness, dyspnea, numbness of skin on neck under the jaw, and tingling in the scar area [69]. The clinical data in a larger group of patients are currently being analyzed.

Advantages and disadvantages of VNS therapy of RA: could there be a place in the treatment of RA?

As described above, there is over 15 years of safety information available about VNS therapy in epilepsy and depression patients. The consistent preclinical data in experimental models of arthritis and the wide experience with the implantation of the device and known side effects of VNS therapy paved the way to initiate an exploratory clinical trial in RA patients, and for patients to participate. With side effects occurring primarily during stimulation and a frequency of stimulation much lower compared to that used in epilepsy, the burden of stimulation in RA was expected to be low. Stimulation was tolerated better every week and became part of the patients' daily routine. A downside of this approach is that it requires ~1-h surgery, which entails certain risks during and after the operation as described above in “VNS in patients.” It should also be noted that complete removal of the VNS system is challenging, because of the formation of fibrosis around the helices attached to the vagus nerve. As not all patients will respond to therapy, it is important for patients to consider the risk of placement and possible removal of the VNS device. It is, however, not necessary to have the VNS device removed if the therapy failed, as it can also be deactivated. It is also worth mentioning that, when a VNS device has been implanted, magnetic resonance imaging (MRI) can only be performed on some areas of the body with specific settings. Areas that can be imaged include the knee joints, ankles, and the head, but MRI of the chest is not possible when the device is in place as there is a risk of overheating the lead. This can also occur with the use of therapeutic diathermy. MRI and diathermy restrictions are lifted after complete removal of the VNS device, including the three helices around the vagus nerve [70]. The positioning of VNS therapy in the treatment of RA will obviously depend on its effectiveness which is currently unknown. If effective, many RA patients may prefer this approach as it is appealing based on

the concept of restoration of the natural balance in the body without the need for long term immunosuppressive treatment associated with possible side effects. It is conceivable that VNS therapy would be more cost-effective than bDMARD therapy. The CyberonicsTM (Houston, Texas) VNS device used in our clinical trial currently costs around € 9500 [71] and there are of course additional costs for surgery. The VNS pulse generator (battery) has a life-span of around 7–10 years in epilepsy treatment, but because of the lower frequency of stimulation it may have a longer life-span in RA patients. RA therapy with bDMARD currently costs around € 15,000 per patient per year [72]. If VNS therapy can prevent the need for bDMARD treatment, it starts to be cost-effective after around 1–1.5 year. Taken together, VNS therapy has the potential to be a low-cost therapy compared to bDMARD therapy for RA patients if the therapy has a long-term beneficial effect.

Summary and future directions

Innovative therapies have been developed for the treatment of RA over the past decade, which have greatly improved the disease outcome [73]. However, many patients continue to be unresponsive to current treatment; therefore, there remains a need to develop new therapies. We have shown in experimental models of RA that stimulation of the vagus nerve or activation of the CAP consistently has an anti-inflammatory effect, leading to amelioration of arthritis, reduction in serum cytokine levels, and protection against joint destruction. Electrical VNS therapy has been applied in epilepsy and depression patients for >15 years. The preclinical studies and the experience with VNS in other indications have paved the way for an experimental clinical trial using this approach in RA patients, which is currently ongoing. If successful, the advantage of this approach could be that it is a safe, and well-tolerated therapy, appealing to patients as it aims to restore the natural balance by targeting an intrinsic anti-inflammatory pathway. VNS therapy should not lead to immunosuppression and can therefore be combined with both sDMARDs and bDMARDs, nor does it have the problem of causing development of anti-drug antibodies. The long life-span (7–10 years) of the VNS pulse generator will make this approach cost-effective, if there is a prolonged therapeutic effect.

Practice points

- The extensive knowledge about vagus nerve stimulation in epilepsy and depression patients shows that it is generally a safe and well-tolerated therapy.
- VNS therapy is an appealing concept for patients, as it aims to restore the natural balance in the body without the need for long-term immunosuppressive treatment associated with possible side effects.
- The disadvantage of implantation of the VNS device is that it requires an ~1-h surgery and that after implantation there are restrictions in performing MRI and therapeutic diathermy.

Research points

- Preclinical studies show that the cholinergic anti-inflammatory pathway, via activation of the nicotinic acetylcholine receptor type 7 (α7nAChR) or by vagus nerve stimulation, has a beneficial effect on synovial inflammation and protects against progressive joint damage. Lack of the α7nAChR or abrogation of the vagus nerve has a detrimental effect on arthritis activity in experimental models of RA.
- An open pilot study has been initiated to evaluate the short- and long-term safety and initial efficacy of VNS therapy in RA patients

References

[1] McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011;365:2205–19. http://dx.doi.org/10.7748/phc2011.11.21.9.29.c8797.

[2] Smolen JS, Landewe R, Breedveld FC, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. Ann Rheum Dis 2014;73:492–509. http://dx.doi.org/10.1136/annrheumdis-2013-204573. annrheumdis-2013-204573 [pii].

[3] Bianchi M, Ulrich P, Bloom O, et al. An inhibitor of macrophage arginine transport and nitric oxide production (CNI-1493) prevents acute inflammation and endotoxin lethality. Mol Med 1995;1:254–66.

*[4] Watkins LR, Goehler LE, Relton JK, et al. Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. Neurosci Lett 1995;183:27–31.

*[5] Borovikova LV, Ivanova S, Nardi D, et al. Role of vagus nerve signaling in CNI-1493-mediated suppression of acute inflammation. Auton Neurosci 2000;85:141–7. http://dx.doi.org/10.1016/S1566-0702(00)00233-2. S1566–0702(00)00233-2 [pii].

*[6] Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 2000;405:458–62. http://dx.doi.org/10.1038/35013070.

[7] Parrish WR, Rosas-Ballina M, Gallowitsch-Puerta M, et al. Modulation of TNF release by choline requires alpha7 subunit nicotinic acetylcholine receptor-mediated signaling. Mol Med 2008;14:567–74. http://dx.doi.org/10.2119/2008-00079.Parrish.

*[8] Wang H, Yu M, Ochani M, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 2003;421:384–8. http://dx.doi.org/10.1038/nature01339. nature01339 [pii].

[9] Gigliotti JC, Huang L, Ye H, et al. Ultrasound prevents renal ischemia-reperfusion injury by stimulating the splenic cholinergic anti-inflammatory pathway. J Am Soc Nephrol 2013;24:1451–60. http://dx.doi.org/10.1681/ASN.2013010084. ASN.2013010084 [pii].

[10] Huston JM, Ochani M, Rosas-Ballina M, et al. Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. J Exp Med 2006;203:1623–8. http://dx.doi.org/10.1084/jem.20052362. jem.20052362 [pii].

[11] Huston JM, Rosas-Ballina M, Xue X, et al. Cholinergic neural signals to the spleen down-regulate leukocyte trafficking via CD11b. J Immunol 2009;183:552–9. http://dx.doi.org/10.4049/jimmunol.0802684. 183/1/552 [pii].

[12] Ji H, Rabbi MF, Labis B, et al. Central cholinergic activation of a vagus nerve-to-spleen circuit alleviates experimental colitis. Mucosal Immunol 2014;7:335–47. http://dx.doi.org/10.1038/mi.2013.52. mi201352 [pii].

[13] Olofsson PS, Katz DA, Rosas-Ballina M, et al. alpha7 nicotinic acetylcholine receptor (alpha7nAChR) expression in bone marrow-derived non-T cells is required for the inflammatory reflex. Mol Med 2012;18:539–43. http://dx.doi.org/10.2119/molmed.2011.00405. molmed.2011.00405 [pii].

*[14] Rosas-Ballina M, Olofsson PS, Ochani M, et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science 2011;334:98–101. http://dx.doi.org/10.1126/science.1209985. science.1209985 [pii].

[15] Grimsholm O, Rantapaa-Dahlqvist S, Dalen T, et al. Unexpected finding of a marked non-neuronal cholinergic system in human knee joint synovial tissue. Neurosci Lett 2008;442:128–33. http://dx.doi.org/10.1016/j.neulet.2008.06.082. S0304–3940(08)00890-2 [pii].

[16] Schubert J, Beckmann J, Hartmann S, et al. Expression of the non-neuronal cholinergic system in human knee synovial tissue from patients with rheumatoid arthritis and osteoarthritis. Life Sci 2012;91:1048–52. http://dx.doi.org/10.1016/j.lfs.2012.04.032. S0024–3205(12)00222-6 [pii].

[17] Koopman FA, Stoof SP, Straub RH, et al. Restoring the balance of the autonomic nervous system as an innovative approach to the treatment of rheumatoid arthritis. Mol Med 2011;17:937–48. http://dx.doi.org/10.2119/molmed.2011.00065. molmed.2011.00065 [pii].

[18] Bai A, Guo Y, Lu N. The effect of the cholinergic anti-inflammatory pathway on experimental colitis. Scand J Immunol 2007;66:538–45. http://dx.doi.org/10.1111/j.1365-3083.2007.02011.x. SJI2011 [pii].

[19] Mabley J, Gordon S, Pacher P. Nicotine exerts an anti-inflammatory effect in a murine model of acute lung injury. Inflammation 2011;34:231–7. http://dx.doi.org/10.1007/s10753-010-9228-x.

[20] The F, Cailotto C, van d V, et al. Central activation of the cholinergic anti-inflammatory pathway reduces surgical inflammation in experimental post-operative ileus. Br J Pharmacol 2011;163:1007–16. http://dx.doi.org/10.1111/j.1476-5381.2011.01296.x.

[21] van Westerloo DJ, Giebelen IA, Florquin S, et al. The vagus nerve and nicotinic receptors modulate experimental pancreatitis severity in mice. Gastroenterology 2006;130:1822–30. http://dx.doi.org/10.1053/j.gastro.2006.02.022. S0016–5085(06)00285-X [pii].

*[22] Van Maanen MA, Lebre MC, van der Poll T, et al. Stimulation of nicotinic acetylcholine receptors attenuates collagen-induced arthritis in mice. Arthritis Rheum 2009;60:114–22. http://dx.doi.org/10.1002/art.24177.

[23] Li T, Zuo X, Zhou Y, et al. The vagus nerve and nicotinic receptors involve inhibition of HMGB1 release and early pro-inflammatory cytokines function in collagen-induced arthritis. J Clin Immunol 2010;30:213–20. http://dx.doi.org/10.1007/s10875-009-9346-0.

[24] Hu Y, Liu R, Li J, et al. Attenuation of collagen-induced arthritis in rat by nicotinic Alpha7 receptor partial agonist GTS-21. Biomed Res Int 2014. http://dx.doi.org/10.1155/2014/325875. 325875.

*[25] Van Maanen MA, Stoof SP, LaRosa GJ, et al. Role of the cholinergic nervous system in rheumatoid arthritis: aggravation of arthritis in nicotinic acetylcholine receptor alpha7 subunit gene knockout mice. Ann Rheum Dis 2010;69:1717–23. http://dx.doi.org/10.1136/ard.2009.118554. ard.2009.118554 [pii].

[26] de Jonge WJ, Ulloa L. The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. Br J Pharmacol 2007;151:915–29. http://dx.doi.org/10.1038/sj.bjp.0707264. 0707264 [pii].

[27] Van Maanen MA, Stoof SP, van der Zanden EP, et al. The alpha7 nicotinic acetylcholine receptor on fibroblast-like synoviocytes and in synovial tissue from rheumatoid arthritis patients: a possible role for a key neurotransmitter in synovial inflammation. Arthritis Rheum 2009;60:1272–81. http://dx.doi.org/10.1002/art.24470.

[28] Waldburger JM, Boyle DL, Pavlov VA, et al. Acetylcholine regulation of synoviocyte cytokine expression by the alpha7 nicotinic receptor. Arthritis Rheum 2008;58:3439–49. http://dx.doi.org/10.1002/art.23987.
[29] Zhou Y, Zuo X, Li Y, et al. Nicotine inhibits tumor necrosis factor-alpha induced IL-6 and IL-8 secretion in fibroblast-like synoviocytes from patients with rheumatoid arthritis. Rheumatol Int 2012;32:97–104. http://dx.doi.org/10.1007/s00296-010-1549-4.
[30] Rosas-Ballina M, Goldstein RS, Gallowitsch-Puerta M, et al. The selective alpha7 agonist GTS-21 attenuates cytokine production in human whole blood and human monocytes activated by ligands for TLR2, TLR3, TLR4, TLR9, and RAGE. Mol Med 2009;15:195–202. http://dx.doi.org/10.2119/molmed.2009.00039.
[31] Zhang P, Han D, Tang T, et al. Inhibition of the development of collagen-induced arthritis in Wistar rats through vagus nerve suspension: a 3-month observation. Inflamm Res 2008;57:322–8. http://dx.doi.org/10.1007/s00011-008-8070-1.
*[32] Levine YA, Koopman FA, Faltys M, et al. Neurostimulation of the cholinergic anti-inflammatory pathway ameliorates disease in rat collagen-induced arthritis. PLoS One 2014;9:e104530. http://dx.doi.org/10.1371/journal.pone.0104530. PONE-D-14–09205 [pii].
[33] de Hair MJ, Landewe RB, van de Sande MG, et al. Smoking and overweight determine the likelihood of developing rheumatoid arthritis. Ann Rheum Dis 2013;72:1654–8. http://dx.doi.org/10.1136/annrheumdis-2012-202254. annrheumdis-2012-202254 [pii].
[34] Carlens C, Hergens MP, Grunewald J, et al. Smoking, use of moist snuff, and risk of chronic inflammatory diseases. Am J Respir Crit Care Med 2010;181:1217–22. http://dx.doi.org/10.1164/rccm.200909-1338OC. 200909–1338OC [pii].
[35] Klareskog L, Malmstrom V, Lundberg K, et al. Smoking, citrullination and genetic variability in the immunopathogenesis of rheumatoid arthritis. Semin Immunol 2011;23:92–8. http://dx.doi.org/10.1016/j.smim.2011.01.014. S1044–5323(11)00015-7 [pii].
[36] Fisher MC, Hochberg MC, El-Taha M, et al. Smoking, smoking cessation, and disease activity in a large cohort of patients with rheumatoid arthritis. J Rheumatol 2012;39:904–9. http://dx.doi.org/10.3899/jrheum.110852. jrheum.110852 [pii].
[37] Papadopoulos NG, Alamanos Y, Voulgari PV, et al. Does cigarette smoking influence disease expression, activity and severity in early rheumatoid arthritis patients? Clin Exp Rheumatol 2005;23:861–6. 1724 [pii].
[38] Soderlin MK, Petersson IF, Bergman S, et al. Smoking at onset of rheumatoid arthritis (RA) and its effect on disease activity and functional status: experiences from BARFOT, a long-term observational study on early RA. Scand J Rheumatol 2011;40:249–55. http://dx.doi.org/10.3109/03009742.2010.541495.
[39] Harrison BJ, Silman AJ, Wiles NJ, et al. The association of cigarette smoking with disease outcome in patients with early inflammatory polyarthritis. Arthritis Rheum 2001;44:323–30. http://dx.doi.org/10.1002/1529-0131(200102)44. 2<323::AID-ANR49>3.0.CO;2-C.
[40] Lu B, Rho YH, Cui J, et al. Associations of smoking and alcohol consumption with disease activity and functional status in rheumatoid arthritis. J Rheumatol 2014;41:24–30. http://dx.doi.org/10.3899/jrheum.130074. jrheum.130074 [pii].
[41] Vesperini V, Lukas C, Fautrel B, et al. Association of tobacco exposure and reduction of radiographic progression in early rheumatoid arthritis: results from a French multicenter cohort. Arthritis Care Res (Hoboken) 2013;65:1899–906. http://dx.doi.org/10.1002/acr.22057.
[42] Westhoff G, Rau R, Zink A. Rheumatoid arthritis patients who smoke have a higher need for DMARDs and feel worse, but they do not have more joint damage than non-smokers of the same serological group. Rheumatology (Oxford) 2008;47:849–54. http://dx.doi.org/10.1093/rheumatology/ken057. ken057 [pii].
[43] Canhao H, Rodrigues AM, Mourao AF, et al. Comparative effectiveness and predictors of response to tumour necrosis factor inhibitor therapies in rheumatoid arthritis. Rheumatology (Oxford) 2012;51:2020–6. http://dx.doi.org/10.1093/rheumatology/kes184. kes184 [pii].
[44] Rojas-Serrano J, Perez LL, Garcia CG, et al. Current smoking status is associated to a non-ACR 50 response in early rheumatoid arthritis. A cohort study. Clin Rheumatol 2011;30:1589–93. http://dx.doi.org/10.1007/s10067-011-1775-5.
[45] Soderlin MK, Petersson IF, Geborek P. The effect of smoking on response and drug survival in rheumatoid arthritis patients treated with their first anti-TNF drug. Scand J Rheumatol 2012;41:1–9. http://dx.doi.org/10.3109/03009742.2011.599073.
[46] Andersson ML, Bergman S, Soderlin MK. The effect of snuff (smokeless tobacco) on disease activity and function in rheumatoid arthritis: experiences from the Better Anti-Rheumatic FarmacOTherapy, a longitudinal multicenter study on early rheumatoid arthritis. J Clin Rheumatol 2013;19:14–8. http://dx.doi.org/10.1097/RHU.0b013e31828214ed. 00124743-201301000-00003 [pii].
[47] Jiang X, Alfredsson L, Klareskog L, et al. Smokeless tobacco (moist snuff) use and the risk of developing rheumatoid arthritis: results from the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) case-control study. Arthritis Care Res (Hoboken) 2014. http://dx.doi.org/10.1002/acr.22325.
[48] Martin-Martinez MA, Gonzalez-Juanatey C, Castaneda S, et al. Recommendations for the management of cardiovascular risk in patients with rheumatoid arthritis: scientific evidence and expert opinion. Semin Arthritis Rheum 2014. http://dx.doi.org/10.1016/j.semarthrit.2014.01.002. S0049–0172(14)00003-1 [pii].
[49] Albano SA, Santana-Sahagun E, Weisman MH. Cigarette smoking and rheumatoid arthritis. Semin Arthritis Rheum 2001;31:146–59. http://dx.doi.org/10.1053/sarh.2001.27719. S0049–0172(01)56332-5 [pii].
[50] Camm AJ, Malik M. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996;93:1043–65.
*[51] Aydemir M, Yazisiz V, Basarici I, et al. Cardiac autonomic profile in rheumatoid arthritis and systemic lupus erythematosus. Lupus 2010;19:255–61. http://dx.doi.org/10.1177/0961203309351540. 0961203309351540 [pii].
[52] Bruchfeld A, Goldstein RS, Chavan S, et al. Whole blood cytokine attenuation by cholinergic agonists ex vivo and relationship to vagus nerve activity in rheumatoid arthritis. J Intern Med 2010;268:94–101. http://dx.doi.org/10.1111/j.1365-2796.2010.02226.x. JIM2226 [pii].

[53] Evrengul H, Dursunoglu D, Cobankara V, et al. Heart rate variability in patients with rheumatoid arthritis. Rheumatol Int 2004;24:198–202. http://dx.doi.org/10.1007/s00296-003-0357-5.
[54] Borman P, Gokoglu F, Kocaoglu S, et al. The autonomic dysfunction in patients with ankylosing spondylitis: a clinical and electrophysiological study. Clin Rheumatol 2008;27:1267–73. http://dx.doi.org/10.1007/s10067-008-0906-0.
[55] Sharma P, Makharia GK, Ahuja V, et al. Autonomic dysfunctions in patients with inflammatory bowel disease in clinical remission. Dig Dis Sci 2009;54:853–61. http://dx.doi.org/10.1007/s10620-008-0424-6.
[56] Routledge FS, Campbell TS, McFetridge-Durdle JA, et al. Improvements in heart rate variability with exercise therapy. Can J Cardiol 2010;26:303–12.
[57] Khattab K, Khattab AA, Ortak J, et al. Iyengar yoga increases cardiac parasympathetic nervous modulation among healthy yoga practitioners. Evid Based Complement Altern Med 2007;4:511–7. http://dx.doi.org/10.1093/ecam/nem087.
[58] Nesvold A, Fagerland MW, Davanger S, et al. Increased heart rate variability during nondirective meditation. Eur J Prev Cardiol 2012;19:773–80. http://dx.doi.org/10.1177/1741826711414625. 1741826711414625 [pii].
[59] Kulur AB, Haleagrahara N, Adhikary P, et al. Effect of diaphragmatic breathing on heart rate variability in ischemic heart disease with diabetes. Arq Bras Cardiol 2009;92:423–7. 457. S0066-782X2009000600008 [pii].
[60] Chung JW, Yan VC, Zhang H. Effect of acupuncture on heart rate variability: a systematic review. Evid Based Complement Altern Med 2014. http://dx.doi.org/10.1155/2014/819871. 819871.
[61] Holman AJ, Ng E. Heart rate variability predicts anti-tumor necrosis factor therapy response for inflammatory arthritis. Auton Neurosci 2008;143:58–67. http://dx.doi.org/10.1016/j.autneu.2008.05.005. S1566–0702(08)00071-4 [pii].
[62] Scherrmann J, Hoppe C, Kral T, et al. Vagus nerve stimulation: clinical experience in a large patient series. J Clin Neurophysiol 2001;18:408–14.
[63] O'Reardon JP, Cristancho P, Peshek AD. Vagus nerve stimulation (VNS) and treatment of depression: to the Brainstem and beyond. Psychiatry (Edgmont) 2006;3:54–63.
[64] Greg Browne. Cyberonics announces 100,000th patient implant of VNS Therapy®. 2012.
*[65] Ben-Menachem E. Vagus nerve stimulation, side effects, and long-term safety. J Clin Neurophysiol 2001;18:415–8.
[66] DeGiorgio CM, Thompson J, Lewis P, et al. Vagus nerve stimulation: analysis of device parameters in 154 patients during the long-term XE5 study. Epilepsia 2001;42:1017–20. epi08101 [pii].
[67] Felson DT, Anderson JJ, Boers M, et al. American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995;38:727–35.
[68] NCT01552941. Clinical trial registration – safety and efficacy vagal nerve stimulation in patients with rheumatoid arthritis. 2013.
[69] Koopman FA, Miljko S, Grazio S, et al. Pilot study of stimulation of the cholinergic anti-inflammatory pathway with an implantable vagus nerve stimulation device in patients with rheumatoid arthritis. Arthritis Rheum 2012;64(Suppl.).
[70] Anonymous. VNS Therapy® System Physician's Manual – chapter: MRI with the VNS Therapy® System. 2014.
[71] Sperling W, Reulbach U, Kornhuber J. Clinical benefits and cost effectiveness of vagus nerve stimulation in a long-term treatment of patients with major depression. Pharmacopsychiatry 2009;42:85–8. http://dx.doi.org/10.1055/s-0028-1103294.
[72] Schabert VF, Watson C, Joseph GJ, et al. Costs of tumor necrosis factor blockers per treated patient using real-world drug data in a managed care population. J Manag Care Pharm 2013;19:621–30. 2013(19)8: 621–630 [pii].
[73] Tak PP, Kalden JR. Advances in rheumatology: new targeted therapeutics. Arthritis Res Ther 2011;13(Suppl. 1):S5. http://dx.doi.org/10.1186/1478-6354-13-S1-S5. 1478-6354-13-S1-S5 [pii].