Review Article

The Cholinergic Anti-Inflammatory Pathway as a Conceptual Framework to Treat Inflammation-Mediated Renal Injury

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Keywords

Anti-inflammatory reflex · Kidney injury · Vagus · Inflammation · Neuroimmunomodulation

Abstract

Background: The cholinergic anti-inflammatory pathway, positioned at the interface of the nervous and immune systems, is the efferent limb of the “inflammatory reflex” which mainly signals through the vagus nerve. As such, the brain can modulate peripheral inflammatory responses by the activation of vagal efferent fibers. Importantly, immune cells in the spleen express most cholinergic system components such as acetylcholine (ACh), choline acetyltransferase, acetylcholinesterase, and both muscarinic and nicotinic ACh receptors, making communication between both systems possible. In general, this communication down-regulates the inflammation, achieved through different mechanisms and depending on the cells involved. **Summary:** With the awareness that the cholinergic anti-inflammatory pathway serves to prevent or limit inflammation in peripheral organs, vagus nerve stimulation has become a promising strategy in the treatment of several inflammatory conditions. Both pharmacological and non-pharmacological methods have been used in many studies to limit organ injury as a consequence of inflammation. **Key Messages:** In this review, we will highlight our current knowledge of the cholinergic anti-inflammatory pathway, with emphasis on its potential clinical use in the treatment of inflammation-triggered kidney injury.

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Introduction

The inflammatory reflex consists of sensory afferent and motor efferent branches, acting in concert to convey neuroimmunomodulation. During inflammation, afferent vagus nerve fibers become activated by inflammatory mediators and subsequently signal to the dorsal vagal complex, leading to the release of adrenocorticotropin hormone from the anterior pituitary gland [1]. The motor, neural arc of the reflex is called the anti-inflammatory pathway, which suppresses the cytokine production via the release of the primary parasympathetic neurotransmitter acetylcholine (ACh). Although the etiology of acute kidney injury (AKI) is often multifactorial, inflammation seems to play a critical role in the pathophysiology of AKI [2, 3]. One of the major causes of AKI is renal hypoperfusion [4, 5], which is common in critically ill patients. AKI is associated with a substantially increased morbidity and mortality [6, 7] and is accompanied by leucocyte recruitment in the renal parenchyma as a consequence of an increased expression of endothelial cell adhesion molecules and chemokines, vascular disintegration, and increased vascular permeability [8, 9]. Hypoperfusion or hemodynamic instability is also frequently encountered in deceased brain death donors (DBD), which apart from this, may also present hypothermia, coagulopathies, and electrolyte abnormalities [10]. It is generally believed that these changes in physiology adversely affects the quality of organ allograft and thus impact transplantation outcomes. Like in AKI, renal inflammation occurs in DBD, albeit to much smaller extent. Yet, in DBD inflammation is not restricted to the kidney but occurs in most peripheral organs [11–14]. In this article, we will briefly review the cholinergic anti-inflammatory pathway, and discuss renal inflammation in the context of AKI. We will furthermore discuss the available studies that have conceptualized the cholinergic anti-inflammatory pathway as therapeutic modality in the treatment of AKI or as a mean to improve organ allograft quality in DBD.

Cholinergic Anti-Inflammatory Pathway

The seminal finding that cholinergic neurons can inhibit acute inflammation has shaped our understanding of the cholinergic anti-inflammatory pathway and prompted early researchers to assess the therapeutic potential of vagus nerve stimulation (VNS) in attenuating systemic inflammatory responses evoked by endotoxemia [15, 16]. Since then an increasing number of studies have reported a beneficial effect of VNS in a variety of inflammation-mediated disease models [1, 17, 18–22]. Inflammatory responses require regulation to prevent excessive inflammation and to be sufficiently strong to combat the inciting pathogens. Inflammation is limited by anti-inflammatory responses, which ideally should be rapid, reversible, localized, and adaptive to changes in inflammatory input signals. As the nervous system is composed of sensory and motor systems that rapidly communicate via input signals, it also seems to be a perfect candidate platform to mediate these anti-inflammatory responses. Indeed, inflammatory input signals reflexively monitor and adjust the inflammatory response by activating neuronal sensory pathways that relay information to the nucleus tractus solitarius and dorsal motor nucleus [15, 23]. Subsequently, efferent vagus nerve fibers are activated and suppress cytokine production via the release of the primary parasympathetic neurotransmitter ACh (Fig. 1). ACh binds to both nicotinic and muscarinic cholinergic receptors. The main nicotinic cholinergic receptor found on macrophages and a central component of the cholinergic anti-inflammatory pathway is the $\alpha 7$ nicotinic ACh receptor subunit ($\alpha 7$ nAChR) [24]. This is amongst others underscored by the finding that the inhibitory effect of nicotine on tumor necrosis factor α (TNF α) production is attenuated in macrophages isolated from $\alpha 7^{-/-}$ mice. Likewise, VNS reduces TNF α serum levels in wild type,

Fig. 1. Cholinergic anti-inflammatory pathway. Tissue damage results in the release of DAMPs which upon receptor engagement on immune cells induce cytokine production. These inflammatory input signals activate afferent vagus nerve fibers that relay information to the brain. Subsequently, efferent vagus nerve fibers are activated and suppress cytokine production via the release of the primary parasympathetic neurotransmitter ACh. Inhibition of cytokine production largely depends on the $\alpha 7$ nAChRs on macrophages and other cells from peripheral organs. Vagal terminals in the celiac ganglion communicate with the splenic nerve which utilizes NE as the primary neurotransmitter. Release of NE binds to $\beta 2$ ARs on CD4(+) T cells, which in turn release ACh to activate $\alpha 7$ nAChR macrophages for initiating an anti-inflammatory response. The cholinergic brain network can increase the activity of the cholinergic anti-inflammatory pathway. $\alpha 7$ nAChR, $\alpha 7$ subunit of the acetylcholine receptor; $\beta 2$ ARs, $\beta 2$ -adrenergic receptors; ACh, acetylcholine; TNF, tumor necrosis factor; NE, norepinephrine; IL, interleukin.

but not in $\alpha 7^{-/-}$ mice [24]. It is believed that cholinergic agonists through the activation of $\alpha 7$ nACh receptors inhibit NF- κ B activation and hence downregulate the production of pro-inflammatory cytokines, such as TNF α [24, 25]. TNF α activates, inter alia, macrophages to release high mobility group box 1 (HMGB1), which in turn primes resident monocytes towards an inflammatory, cytokine-producing phenotype [26]. HMGB1 is considered to be a late mediator of lethal endotoxemia and perpetuates systemic inflammation [27, 28]. Neutralizing antibodies against HMGB1 confer a dose-dependent protection against lethal endotoxemia [29] and improve the renal function in a model of ischemia-mediated AKI [30]. Because activation of $\alpha 7$ nAChR attenuates TNF α production, it also indirectly reduces serum HMGB1 levels [31] and thus conveys organ protection in systemic inflammation.

Apart from inhibiting NF- κ B, cholinergic agonists can also activate the Jak2-Stat3 signaling pathway [31–33], a negative regulator of inflammatory responses. Like macrophages, endothelial cells also express $\alpha 7$ nAChRs [34, 35], suggesting that these cells may also function as target cells in the cholinergic anti-inflammatory pathway. Indeed, Chatterjee et al. [36] showed that stimulation of endothelial cells with cholinergic agonists significantly reduces interleukin-6-mediated MCP-1 production and ICAM-1 expression in a Jak2-Stat3 dependent fashion. Also, a direct inhibitory effect of ACh and cholinergic agents on TNF α -induced endo-

thelial cell activation has been described [23]. The spleen, as a major source of inflammatory cytokines, is critically involved in systemic inflammation. Efferent vagus nerve fibers terminate in the celiac ganglion, where also neural cell bodies are localized that project axons in the splenic nerve [37]. Since the vagus nerve does not appear to innervate the spleen directly [35, 36], the vagal terminals should be regarded as parasympathetic junctions where communication occurs with the sympathetic nervous system. Stimulation of either the efferent vagus nerve fibers or the splenic nerve itself significantly inhibits TNF α production by splenic macrophages [22, 38]. However, nerve fibers in the spleen that originate in the celiac ganglion are adrenergic and utilize norepinephrine (NE) as the primary neurotransmitter [39]. While inhibition of systemic inflammation by cholinergic stimulation depends on the activation of the splenic nerve, VNS fails to inhibit TNF α production in splenic neurectomized or splenectomized animals in the course of endotoxemia [38, 40]. In line with this, the effect of vagal stimulation on TNF α production is abolished by vagotomy of the ventral vagus nerve or the celiac branches. Hence, the spleen, the splenic nerve, and the vagus nerve all contribute to the cholinergic anti-inflammatory pathway. Although the spleen is mainly innervated by adrenergic fibers, ACh has been successfully isolated from the spleen of animals [41]. The paradox between the presence of ACh in the spleen and general consensus that the spleen does not receive innervation by parasympathetic cholinergic neurons was ultimately understood by the finding of ACh in the blood and its localization to lymphocytes [42, 43]. Within this fraction, rat T cells were found to contain about 3 times the amount of ACh as compared to B cells, and CD4(+) cells showed significantly higher levels as compared to CD8(+) cells [42]. Moreover, it was shown that immunological activation of T cells upregulates ACh synthesis, leading to the modulation of immune function [42, 44, 45]. Besides this main fraction of ACh-producing lymphocytes, macrophages and dendritic cells (DCs) also express choline acetyltransferase (ChAT), which is, as the enzyme responsible for the synthesis of ACh, an indicator for ACh production [46–48]. It is described that MyD88-dependent Toll-like receptors are involved in LPS-induced ChAT expression in DCs and macrophages and might affect the upregulation of cholinergic activity in macrophages and DCs [44, 46]. However, compared to the ChAT(+) T and B cells, the amount of quantifiable ChAT is marginal and information on the physiological significance of ACh synthesis in DCs and macrophages needs further elucidations [49]. Within the ChAT(+) immune cells, a study of Rosas-Ballina et al. [50] identified an ACh-producing T cell population (CD4(+) ChAT(+) T cells) as the integral component of the cholinergic anti-inflammatory pathway. In an endotoxemia model in mice, the vagus impulse was transmitted to the noradrenergic splenic nerve in the celiac ganglion, after which the activated splenic nerve releases NE in the spleen. NE acts on β 2-adrenoceptors on ChAT(+) CD4(+) T cells, which facilitates ACh synthesis and release. ACh, in turn, acts at α 7nAChRs on macrophages, suppressing the synthesis and release of TNF α . By contrast, VNS failed to attenuate serum TNF α in nude mice, which are devoid of functional T cells, indicating that T cell deficiency impairs the inflammatory reflex [50]. Unlike the previously described CD4(+) ChAT(+) T cell population, ChAT(+) B cell release of ACh is independent of NE [46]. In the study of Reardon et al. [46], ChAT(+) B cell release of ACh does not have an impact on pro-inflammatory cytokine or chemokine production during endotoxemia in mice, but reduces the local neutrophil recruitment, even after vagotomy. Therefore, ChAT(+) lymphocytes fulfill different roles in the control of immune responses. While ChAT(+) T cells provide the terminal neurotransmitter in the cholinergic anti-inflammatory pathway, ChAT(+) B cells limit local neutrophil recruitment independent of vagal signaling. In addition, Jiang et al. [51] were able to show that inflammation-induced production of ACh originating from ChAT(+) NK cells reduced brain and spinal cord damage and decreased the number of infiltrating monocytes after induction of autoimmune encephalomyelitis in a preclinical model. Apart from immune cells, ACh and ChAT activity as well as α 7nAChR expression have been described in various non-

neuronal epithelial and endothelial cells as well as mesenchymal cells [52]. This non-neuronal cholinergic system is thought to play a pivotal role in cellular homeostasis and regeneration via auto- and paracrine activation. However, its involvement in the regulation of inflammation is unclear and needs further evaluation [53]. Hence, the cholinergic anti-inflammatory pathway is hardwired to the spleen via the splenic nerve receiving ACh input signals from efferent vagus nerve fibers and relaying these to ACh-producing memory CD4(+) T cells via the release of NE. The $\alpha 7$ nAChR, therefore, represents the molecular link between the parasympathetic and sympathetic system to control inflammation (Fig. 1).

Renal Inflammation in Critical Care Patients

A recently conducted prospective study (Assessment of Worldwide AKI, Renal Angina, and Epidemiology) concluded that severe AKI is associated with increased use of mechanical ventilation and renal replacement therapy in critically ill children and young adults [54]. AKI is also common in adult critically ill patients and is generally associated with poor outcomes including increased mortality [6, 7]. It is a complex disorder with a wide variety of etiologies and risk factors [55–58] and is characterized by a rapid loss of kidney function accompanied by inflammation and tissue damage. The most common causes for AKI include renal ischemia, systemic inflammation or sepsis, hemodynamic insufficiency, and nephrotoxicity. Among these, ischemia-reperfusion injury (IRI) has been studied most extensively and is associated with endothelial cell activation, vascular disintegration and increased vascular permeability. The IRI components of damaged cells or extracellular matrix, also known as damage-associated molecular patterns (DAMPs), are released and recognized by pattern recognition receptors on immune cells. Renal ischemia also releases lipid antigens, which activate CD1d-restricted NK cells to release large amounts of IFN γ . This, in turn, amplifies the innate inflammatory response by facilitating neutrophil infiltration leading to tissue damage [59, 60]. Complement activation further supports leucocyte recruitment, ultimately perpetuating the inflammatory cascade through the production of cytokines and chemokines [8, 9, 61]. Within the inflammatory milieu, increased amounts of reactive oxygen radicals are produced and contribute to tubular apoptosis and necrosis, thereby rapidly accelerating renal function decline [62]. Although B cells migrate into the post-ischemic kidney during the repair phase, there are conflicting data as to whether this contributes or limits tissue repair. While studies in B cell deficient mice have shown that adoptive transfer of B cells reduced tubular proliferation and increased tubular atrophy in the repair phase of AKI [63], other studies have shown that pre-emptive infusion of natural IgM-treated B cells protects mice from ischemia-induced AKI via regulation of in vivo NK cells [64]. Like in critically ill patients, DBD donors in the intensive care unit display a deregulated physiology. Hypoperfusion, hypothermia, coagulopathies, and electrolyte abnormalities are commonly seen in DBD donors. This requires active and appropriate donor management to avoid or limit deterioration of organ allograft quality [10, 65, 66]. Brain death also leads to systemic and local immune activation, reflected by increased plasma cytokine levels [67, 68] and upregulation of adhesion molecules in peripheral organs [13], thereby increasing the amount of donor-derived mononuclear cells in potential allografts [11–14]. It seems that renal allografts obtained from extended criteria donors have a heavier inflammatory burden, as compared to those of standard criteria donors [69]. Although the cause of brain death induced immune activation is not completely understood, traumatic brain injury can evoke a systemic inflammatory response via the release of DAMPs. Elevated levels of mitochondria-derived DAMPs (mtDAMPs) have also been detected in the plasma of patients following trauma [70, 71], in patients with myocardial injury [72, 73] and in the setting of sepsis [74, 75]. The role of mtDAMPs has recently been

investigated in DBD donors [76] and found to correlate with elevated plasma levels of inflammatory cytokines and chemokines. Moreover, a correlation between donor plasma mtDAMPs levels and early allograft dysfunction was demonstrated in liver transplant recipients [76]. Apart from brain injury other studies have suggested increased intestinal permeability [77] and temporal changes in organ perfusion per se [78] as a major cause for brain death associated inflammation. For brain death induction, animal models make use of increased intracranial pressure by inflating a balloon catheter placed in the intracranial cavity. This leads to an immediate systemic release of (nor) epinephrine and consequently to tachycardia and hypertension followed by vasoplegia and hypotension [79]. Although the changes in mean arterial pressure depend on the speed of balloon inflation, in both the explosive and gradual model of increasing intracranial pressure, animals will suffer from a hypotensive episode [80]. Due to the impairment of microcirculation, hemodynamic instability in BD may cause tissue hypoperfusion and ischemia [78, 81]. Mesenteric hypoperfusion can lead to intestinal necrosis and loss of barrier function and is associated with elevated expression of endothelial cell adhesion molecules and increased leukocyte migration to the perivascular tissues [82–84]. We have previously suggested that failure of the cholinergic anti-inflammatory pathway in DBD donors may also underlie inflammation in peripheral organs [85]. Irrespective of its cause, the awareness that immune activation occurs in DBD donors provides a conceivable rationale for intervening strategies in these donors prior to organ procurement [86, 87].

Renal Inflammation in Chronic Kidney Disease

Many of the diseases that fall under the description of glomerulonephritis are characterized by inflammation either of the glomeruli or of the small blood vessels in the kidney. This may lead to tissue scarring in the glomeruli, chronic loss of renal function, and ultimately to end stage renal disease. Autoimmune disorders related to chronic kidney diseases and inflammation include amongst others lupus nephritis, Goodpasture's syndrome, and ANCA-associated vasculitis. Also in diabetic nephropathy, renal inflammation may contribute to the progression to end stage renal disease [88–92]. Different immune cells, cytokines, and chemokines participate in the pathogenesis of these diseases. In autoimmune kidney diseases, T cells are critical drivers of autoimmunity by supporting B cell differentiation and antibody production or by directly promoting renal inflammation. Monocyte/macrophages play roles in many aspects of renal disease and are implicated in the induction of injury and fibrosis as well as renal repair [92]. Moreover, macrophages are found in both acute and chronic diseases of kidney transplantation. These cells express the $\alpha 7$ nAChR and are the key components of the cholinergic anti-inflammatory pathway.

Activation of the Cholinergic Anti-Inflammatory Pathway for Treating AKI

Initial studies performed in a model of aortic occlusion demonstrated the protective effect of VNS to attenuate systemic TNF α release and shock in IRI [93]. The first study which suggested activation of the cholinergic anti-inflammatory pathway as treatment modality for AKI was published by Yeboah et al. [21]. They applied cholinergic agonists prior to or after the induction of renal ischemia in rats and showed that this treatment significantly attenuated renal dysfunction and tubular necrosis. Renal inflammation was likewise diminished, revealing a reduced renal TNF α expression and leukocyte infiltration. Cholinergic agonists also significantly decreased tubular necrosis in vagotomized rats, implicating that an intact vagus nerve is not required for renoprotection in the AKI model. Their study also revealed

constitutive expression of many nAChR subunits in the rat kidney, whereby the $\alpha 7$ nAChR was localized predominantly in the endothelium of cortical peritubular capillaries [94]. Although the precise physiological function of renal nAChR remains to be elucidated, their presence provides an intrarenal target for cholinergic stimulation and is consistent with ACh being an autocrine/paracrine hormone in the kidney [95, 96]. It may also explain ACh-induced vasodilation in perfused kidneys [97] and thus may regulate renal vascular tone in vivo. In a recent study, Abe et al. [98] showed that restraint stress or optogenetic C1 neuron (C1) stimulation protects mice from renal IRI. This protection was absent in $\alpha 7$ nAChR^{-/-} mice and was greatly reduced by destroying or transiently inhibiting C1, which implies that C1 neurons mediate a stress-induced anti-inflammatory reflex. Protection conferred by C1 stimulation was abrogated in splenectomized mice, but restored after injection of splenic T cells that had been preincubated with noradrenaline or splenocytes harvested from stressed mice. These data are in concordance with the study of Inoue et al. [99] demonstrating that the protective effect of VNS in AKI largely depends on $\alpha 7$ nAChR-positive splenocytes. Data from both studies indicate that T cells stimulated with adrenergic agonists communicate with $\alpha 7$ nAChR-positive splenocytes to downregulate the inflammation in AKI and underscores the importance of cross-talk between the cholinergic pathway and the spleen. It is not completely understood how the kidneys are ultimately protected from injury after the spleen is activated. Yet, a macrophage switch to a M2 phenotype might be one mechanism of protection. Like electric VNS, Gigliotti et al. [100, 101] reported that ultrasound also prevents renal IRI and suppressed systemic and renal inflammation via the splenic cholinergic anti-inflammatory pathway. In particular, the accumulation of neutrophils and myeloid cells in kidney tissue was reduced. Furthermore, splenectomy and adoptive transfer studies revealed that the spleen and CD4⁺ T cells mediated the protective effects of ultrasound, while chemical blockade or genetic deficiency of the $\alpha 7$ nicotinic ACh receptor abrogated the protective effect of ultrasound. Ghrelin, an endogenous ligand for the growth hormone secretagogue receptor [102], has received much scientific interest as it has been shown that it ameliorates IRI in various organ systems [103–105]. Since growth hormone secretagogue receptor are expressed at a high density in the dorsal vagal complex of the brain stem, ghrelin signals might be transmitted to the brain by the vagal afferent nerves. Therefore, it has been postulated that the anti-inflammatory property of ghrelin is mediated through VNS [106]. Compatible with this is the finding that vagotomy prior to ghrelin treatment abolishes the protective effect in renal IRI [103].

Though these studies are still in a preclinical phase, the results support the concept that vagal stimulation prevents AKI through the cholinergic anti-inflammatory pathway, which thus may serve as a novel therapeutic target.

Activation of the Cholinergic Anti-Inflammatory Pathway in DBD Donors

Sympathetic nervous system activity is increased after cardiopulmonary arrest and brain death. Activation of the perivascular sympathetic nerves can induce the release of vasoactive mediators, that is, NE and neuropeptide Y, causing vasoconstriction, while stimulation of the parasympathetic nerves releases vasodilators (i.e., ACh and nitric oxide) [107]. Biswas et al. [108] suggested that an intracranial pressure >30 mm Hg or a cerebral perfusion pressure <40 mm Hg may be associated with marked autonomic dysfunction and poor outcome. Global cerebral ischemia, often seen during cardiac arrest or in brain-dead patients, leads to a reduction of central cholinergic signaling. This is reflected by a reduction in ChAT enzymatic activity and the number of ChAT-positive neurons [109] and may hamper the cholinergic anti-inflammatory pathway to control tissue inflammation. Accordingly, pharmacological activation of $\alpha 7$ nAChR provides significant protection against ischemia-related cell death and

inflammation in the brain of mice, which undergo cardiac arrest [09]. As reported by Su et al. [110], sympathetic and parasympathetic activities can be evaluated by heart-rate variability (HRV), in which a decrease in high frequency power indicates augmented sympathetic and attenuated parasympathetic activity. Changes in HRV relate to the severity of brain-stem damage. In brain dead patients, both low frequency power and high frequency power are nearly abolished [110]. The CARDIA study not only demonstrates that HRV is a useful tool for evaluating autonomic functions in patients with brain damage, but also suggested an inverse relation with inflammatory markers [111]. This is in line with the assumption that a diminished vagal activity may support inflammation in humans. The role of the cholinergic anti-inflammatory pathway in brain death has been poorly studied. We have recently reported that HRV is decreased in brain-dead rats, suggesting a decreased parasympathetic activity. Moreover, VNS in BD rats decreased serum TNF α concentrations, reduced the expression of pro-inflammatory cytokines in different donor organs, and improved renal function in recipients that received a graft from a vagus nerve-stimulated BD donor rat [85]. This translated long-term in improved recipient survival, superior renal function, and significantly reduced vasculopathy and tubulopathy in the transplanted grafts [112]. Hence, collectively current studies demonstrate a marked autonomic dysfunction in BD donors, accompanied by a decreased parasympathetic activity. A decreased parasympathetic activity might result in a decreased activity of the cholinergic anti-inflammatory pathway. This in turn may hamper negative regulation of inflammatory processes that are initiated by the release of DAMPs as is the case in BD donors [76].

Pharmacological and Non-Pharmacological Stimulation of the Cholinergic Anti-Inflammatory Pathway

There are 2 different approaches to activate the cholinergic anti-inflammatory pathway, pharmacological, for example, through nicotinic receptor agonists, and non-pharmacological, for example, through electrical stimulation or ultrasound stimulation.

Pharmacological Stimulation

Cholinergic agonists that have been frequently used in this context are nicotine, ACh, and GST-21. Also, the use of acetylcholinesterase inhibitors (AChEIs) to increase both the level and duration of action of ACh have proven their benefit in the treatment of systemic inflammation resulting from sepsis [113] or endotoxemia [114], and localized inflammatory processes such as rheumatoid arthritis (RA) [115]. It has been reported that brain acetylcholinesterase activity controls systemic and organ-specific TNF production during endotoxemia. While peripheral administration of the AChEI galantamine significantly reduced serum TNF levels and lethality during murine endotoxemia, a centrally acting muscarinic receptor antagonist abolished the suppressive effect of galantamine. Thus, suppression of acetylcholinesterase activity, coupled with central muscarinic receptors, controls peripheral cytokine responses and ameliorates both central and peripheral inflammation [114]. The AChEI physostigmine is currently tested in a small, randomized, double-blind, placebo-controlled, monocentric trial in 20 patients with perioperative sepsis and septic shock as a result of intra-abdominal infection [116]. Intracerebroventricular application of CNI-1493, a tetravalent guanyl hydrazone molecule that directly stimulates vagal activity, likewise suppressed endotoxin-induced TNF α release and shock [117] but not in vagotomized animals. In ulcerative colitis patients, smoking seems to reduce the disease severity, diminishing flares and hospitalization rates [118]. In addition, many ulcerative colitis patients noted disease exacerbation upon cessation of smoking, whereas symptom relief is reported when smoking is resumed

[119]. Based on the beneficial effect of smoking, therapeutic trials have been initiated evaluating the effect of nicotine enemas in patients with ulcerative colitis, however, with controversial results [120–122].

Non-Pharmacological Stimulation

Non-pharmacological strategies are also widely used and were shown to be efficacious in a number of models. VNS in clinical practice pertains to neuromodulation in the treatment of drug-resistant epilepsy and depression [123–126]. Being safe and highly effective, VNS may also offer new treatment modalities for (auto-) immune-mediated inflammatory diseases (IMID) where autonomic dysfunction may occur [127]. VNS on cytokine production in patients with a chronic IMID has been tested in a 1b/2a clinical trial in 17 patients with RA [128]. VNS (up to 4 times daily) in RA patients significantly inhibited TNF production for up to 84 days. In addition, RA disease severity, as measured by standardized clinical composite scores, improved significantly. The use of VNS in other IMIDs, for example, inflammatory bowel diseases [129–131], seems obvious as the vagus efferent fibers innervate the intestine directly. In a current clinical study, Kibleur et al. [132] aimed to quantify the acute and chronic effects of VNS on brain activity in the Crohn's disease patients. Apart from activation of vagal efferent fibers that regulate the autonomic nervous system, their data suggest that chronic VNS has a regulatory action via afferent vagal fibers on anxio-depressive symptomatology associated Crohn's disease.

Conclusion

Although preclinical and small clinical studies have provided evidence that electronic VNS is highly effective in the treatment of IMIDs, the optimal site of VNS, and exploration of the effects of bioelectronic treatment in a wide variety of chronic IMIDs in human are still warranted. Data from other non-invasive and non-pharmacologic modalities to activate the cholinergic anti-inflammatory pathway, for example, by ultrasound [100, 101] or transcutaneous mechanical VNS [133], as treatment option for IMIDs are still limited. Even though experimental evidence has unambiguously demonstrated that VNS protects the kidney from acute ischemic injury and diminishes renal inflammation in the course of brain death, the efficacy of stimulating the cholinergic anti-inflammatory pathway as treatment modality in human is underexplored [128, 132, 134]. A more profound understanding of the roles of neural circuits and the mechanisms that instigate protective responses to acute and chronic kidney injuries may provide new therapeutic approaches through non-pharmacological, and possibly non-invasive, techniques. VNS seems to be a more promising therapy with no or only mild side-effects, which tend to diminish with time [135].

Disclosure Statement

The authors declare no conflict of interest in this study.

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