Advances in the Treatment of Cholinergic Anti-Inflammatory Pathways in Gastrointestinal Diseases by Electrical Stimulation of Vagus Nerve

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Keywords

Cholinergic anti-inflammatory pathway · Vagus nerve stimulation · $\alpha 7$ nicotinic acetylcholine receptor · Gastrointestinal tract

Abstract

Background: The cholinergic anti-inflammatory pathway (CAIP) has been proposed as a key mechanism by which the brain, through the vagus nerve (VN), modulates the immune system in the body. Recent studies of VN stimulation (VNS) in vivo systems have shown that it plays an anti-inflammatory role through CAIP. Inflammatory diseases in the gastrointestinal tract are frequent and difficult to treat. **Summary:** The mechanism of the anti-inflammatory effect of VNS through CAIP is not fully known. The current review covers anatomy, molecular mechanisms, and the application in gastrointestinal diseases of the vagal CAIP. **Key Messages:** CAIP bridges immune and nervous systems and plays pleiotropic roles in modulating inflammation in animal models by targeting different immune, proinflammatory, epithelial and endothelial cells, and signaling pathways. Numerous animal studies have shown beneficial effects of stimulation of this pathway in models of inflammatory diseases, either through (electrical) stimulation of the VN or pharmacological approaches. In this review, we focus on the anti-inflammatory

benefits of VNS as a means of providing new insights into treating inflammation-related gastrointestinal diseases, as exemplified by those described herein. © 2019 S. Karger AG, Basel

Introduction

Gastrointestinal diseases are common and many are associated with inflammation and some mainly dependent on drug treatment. In recent years, nondrug therapy has been highly praised for its fewer side effects. Vagus nerve (VN) stimulation (VNS) is one of the focuses, but the mechanism is not very clear. Cholinergic anti-inflammatory pathway (CAIP) has a restrictive effect on inflammation of the central nervous system (CNS). CNS pathways respond to injury or inflammation signals and reflexively inhibit inflammation cascades, which may occur in local and/or systemic inflammation [1, 2]. In the inflammatory response, the peripheral and CNSs perceive inflammatory mediators and modulate downwardly through the typical efferent arm reflex. CAIP signals originate in the brainstem nucleus of the VN and pass through the VN to form synapses in the abdominal cavity and other peripheral ganglia [3]. According to the anatomical basis of the effect of the CAIP on the effector organs, the $\alpha 7$

nicotinic acetylcholine receptor ($\alpha 7$ nAChR) on the surface of macrophages in the vagal-innervated organs, such as liver, lung, and intestine, is activated by acetylcholine (ACh) released from the cholinergic nerve endings. The $\alpha 7$ nAChR on macrophages, monocytes, and mast cells has been shown to mediate cholinergic anti-inflammatory output [4, 5]. Some researchers have found that VNS achieves the anti-inflammatory effect by inhibiting macrophage production of proinflammatory cytokines with an $\alpha 7$ nAChR-dependent manner [6]. The present paper will review the recent progress of CAIP in the treatment of gastrointestinal diseases by VNS.

As a treatment, VNS has attracted extensive attention, and its application in gastrointestinal diseases is gradually expanding. The VNS includes electrical stimulation (EVNS), chemical stimulation, acupuncture points, and so on. In the treatment of VNS, the anti-inflammatory effect is the most important. The anti-inflammatory pathway of VN not only takes a role in brain-gut-liver axis but also mediates the regulation of neuroimmunity. The further study of this neuroregulatory mechanism will provide more perspectives for the treatment of gastrointestinal diseases and lay a foundation for the finding of new therapeutic targets for intestinal immune diseases.

Cholinergic Anti-Inflammatory Pathway

Anatomy

As a bidirectional connection between the brain and the immune system, VN can reduce the inflammatory deterioration process outside the CNS [7]. Cytokines and/or endotoxins can be detected in circulation when systemic inflammation occurs. Animal researches have clearly shown that the brain can perceive the presence of cytokines and/or endotoxins in the area postrema, the organum vasculum of the lamina terminalis, and the subfornical organ [8]. Under the condition of local peripheral inflammation, circumventricular organs cannot be activated because of the lack or too low level of cytokines. In this environment, the brain senses inflammation by introducing cytokine (IL-1) receptors on vagal afferents and glomus cells adjacent to the VN. In this “inflammatory reflex” mechanism, the increased release of local inflammatory cytokines activates vagal nerve fibers, which transmit signals from the vagal nerve fibers to the nucleus tractus solitarius (NTS) of the medulla oblongata. And NTS then projected to the efferent neurons of the dorsal motor nucleus of the VN, further to the innate ganglion of the viscera [9].

The VN covers almost the entire gastrointestinal tract. The nerve fibers of the nucleus ambiguus of the brainstem and the dorsal motor nucleus of the VN innervate the upper and middle digestive tracts, respectively, while the sacral nerve tract innervates the distal colon, rectum, and anal canal [10–14]. The efferent fibers from the dorsal vagal nucleus connect with the splenic nerve in the celiac mesenteric ganglion and transmit anti-inflammatory signals to the spleen. The splenic nerve endings release norepinephrine activates the adrenergic receptors of specific T lymphocytes (ChAT + T cells), which express choline acetyltransferase and synthesize ACh. The norepinephrine-activated CHAT + T cells enter splenic macrophages and release ACh. ACh released by ChAT + T cells activates $\alpha 7$ nAChR in macrophages, resulting in downregulation of proinflammatory cytokines production and release, while the level of anti-inflammatory cytokines remains unchanged [15]. In other words, instead of reaching the lamina propria directly, the vagus efferent fibers synapses act on intestinal neurons innervating the lamina propria, and then release ACh on the nicotinic or muscarinic receptors.

$\alpha 7$ Nicotinic ACh Receptor

Nicotine receptors are considered to be ligand-gated ion channel complexes. It is currently believed that ACh plays an anti-inflammatory role by binding to nicotine receptors to a large extent [16]. The muscarinic receptors and nicotine receptors both play an important role in lowering tumor necrosis factor (TNF) levels in endotoxemia, but the anti-inflammatory effect of VN on peripheral immune cells is unrelated to muscarinic receptors [17]. Therefore, the anti-inflammatory effects mediated by nicotinic receptors are mainly discussed.

The $\alpha 7$ nAChR is a homologous oligomer expressed extensively in the CNS and in addition to their ionic activity; these membrane proteins regulate several downstream signal cascades [18]. It transmits action electric potentials between cholinergic synapses by binding to the neurotransmitter ACh. $\alpha 7$ nAChR is essential for the cholinergic anti-inflammatory response and is expressed on the surface of many inflammatory cells such as macrophages, dendritic cells, B and T lymphocytes, polymorphonuclear neutrophils, endothelial cells, microglia, and mast cells [19–21].

The $\alpha 7$ nAChR-mediated CAIP is associated with many diseases. Recently, Kong et al. [22] explored the protective effect of $\alpha 7$ nAChR on lipopolysaccharide (LPS)-induced cardiomyopathy by CAIP which reverses the pathological process of cardiomyopathy and reduces

cardiomyocyte apoptosis. Yamada and Ichinose [23] found that $\alpha 7$ nAChR also regulates lung inflammation and its activation inhibits cytokine synthesis in different immune cells, including respiratory cells. In addition, Hayashi et al. [24, 25] also found that $\alpha 7$ nAChR not only plays a role in lung inflammation but also may downregulate the innate immune system, regulate the defensive function of cancer cells, and affect the growth and secretion function of cancer cells. Ibrahim et al. [26] found that galantamine ($\alpha 7$ nAChR agonist) can improve the survival rate of mice with acute kidney injury and improve the histopathological conditions. After $\alpha 7$ nAChR is inhibited, this protective effect disappears, suggesting that this receptor also plays a significant role in the protection of renal inflammation. Moreover, $\alpha 7$ nAChR has anti-inflammatory effects in neurological diseases, such as ischemic encephalopathy and arthritis [27].

Among the gastrointestinal diseases, some scholars have demonstrated that macrophages are located near the nerve endings of gastric circular muscle and ileal plexus by double-labeled immunohistochemistry using vesicular ACh transporter-positive vagal efferent fibers and macrophages [28]. For example, Mihara et al. [29] found that the activation of $\alpha 7$ nAChR expressed in intestinal mesothelial cells reduced the expression of inflammatory factor IL-1 β . In the experimental colitis model, Salaga et al. [30] and Tasaka et al. [31] both indicated that $\alpha 7$ nAChR could be activated to change the number or activation of intestinal immune cells to alleviate colitis. In terms of the improvement of postoperative intestinal obstruction (POI), Hong et al. [32] showed that the activation of $\alpha 7$ nAChR on macrophages could inhibit intestinal wall inflammation and the development of POI.

$\alpha 7$ nAChR Intracellular Signaling Mechanism

After macrophage $\alpha 7$ nAChR activation, a series of intracellular signal changes may occur, leading to a decrease of inflammatory cytokines such as high-mobility group protein 1 (HMGB1) and TNF- α . The current research focuses on 3 possible signal mechanisms (Fig. 1): First, by regulating the transcription factor B (NF- κ B), $\alpha 7$ nAChR activating the expression of proinflammatory cytokines participates in the regulation of inflammatory processes. Second, activate the JAK2/STAT3 signaling cascade to regulate inflammatory responses. Finally, Ca²⁺-dependent mechanism activates classic PKC leading to increased production of reactive oxygen species and the activation of PI3K/Akt/Nrf-2 pathway, which induces the expression of heme oxygenase (HO-1) in macrophages to regulate the inflammatory response.

NF- κ B Pathways

Other results showed that the activation of $\alpha 7$ nAChR in macrophages inhibited inflammation by inhibiting the nuclear translocation of NF- κ B [22]. The regulation of NF- κ B transcription factor on the inflammatory process is involved by activating the expression of proinflammatory cytokines [33]. Normally, NF- κ B is inactivated in the cytoplasm and does not need to be stimulated, thus responding quickly to proinflammatory stimuli. NF- κ B subunits P50 and p65 are inactivated by binding to I- κ B [34]. When ligands such as proinflammatory cytokines and LPSs activate toll-like receptors 4, I- κ B is phosphorylated by I- κ B kinase and degraded by the signal. With the phosphorylation of I- κ B, P50 and P65 subunits of NF- κ B can be freely transported to the nucleus and activate the transcription of proinflammatory cytokines, thereby enlarging the body's inflammatory response to proinflammatory stimuli [35, 36]. Therefore, inflammation can be regulated by regulating nuclear translocation of NF- κ B. In fact, the CAIP induced by activation of $\alpha 7$ nAChR prevents the nuclear translocation of NF- κ B in macrophages, thus inhibiting the secretion of HMGB1, an important proinflammatory cytokine, which is a late inflammatory mediator in sepsis [37]. Besides, nicotine, as an agonist of $\alpha 7$ nAChR, decreased serum HMGB1 level and improved the survival rate in sepsis experimental model. Nicotine also reduced TNF- α -induced nuclear translocation of macrophage NF- κ B, increased the cytoplasmic levels of I- κ B and ϵ , and the nicotine stimulation blocks cell activation and leukocyte recruitment during inflammation [38]. However, the exact mechanism of blocking nuclear translocation of NF- κ B by activating $\alpha 7$ nAChR in macrophages remains unclear. Nevertheless, studies have shown that nicotine can induce IKK to inhibit the phosphorylation of I- κ B and the transcriptional activity of NF- κ B [32]. Nicotine treatment has been also shown to increase STAT3 phosphorylation and reduce LPS-induced p65 translocation [39, 40].

JAK2/STAT3

Activating $\alpha 7$ nAChR in macrophages can regulate inflammation by activating the JAK2/STAT3 signaling cascade. Anti-inflammatory cytokine IL-10 can activate the JAK2-STAT3 pathway [41]. The researchers found that nicotine had anti-inflammatory effects on peritoneal macrophages mediated by $\alpha 7$ nAChR and JAK2/STAT3 activation in vitro and in vivo. To clarify the role of the JAK2/STAT3 pathway in CAIP, 2 alternative models are proposed.

Fig. 1. The cholinergic anti-inflammatory reflex. (1) When ligands such as proinflammatory cytokines and lipopolysaccharides activate TLR4, IK-kappa B is phosphorylated by IKK and degraded by the signal. With the phosphorylation of I-kappa B, P50, and P65 subunits of NF-kappa B can be freely transported to the nucleus and activate the transcription of pro-inflammatory cytokines. The CAIP triggered by the activation of alpha7nAChR prevented the nuclear translocation of NF-kappa B in macrophages and inhibited its transcriptional activity. (2) It has been reported that the activation of alpha7nAChR can directly inhibit the transcriptional activity of NF-kappa B. (3) JAK2 is autophosphorylated, then JAK2 aggregates and phosphorylates STAT3, and finally p-STAT3 forms dimer and transfers to the nucleus, p-STAT3 dimers have been found to block the production and release of proinflammatory cytokines, and another study

showed that TTP is induced by activated STAT3, which may have anti-inflammatory effects. However, STAT3 may also interfere with LPS-induced proinflammatory response by binding to NF-kappa B and form u-STAT3-NF-kappa B complex by replacing I-kappa B. The u-STAT3-NF-kappa B complex can inhibit the activation of NF-kappa B and produce anti-inflammatory effect. (4) Another the possible mechanism regulating cholinergic anti-inflammation is the activation of PI3K/Akt/Nrf-2 pathway, which leads to the upregulation of HO-1. LPS, lipopolysaccharide; TLR4, toll-like receptors 4; ACh, acetylcholine; alpha7nAChR, alpha7 nicotinic acetylcholine receptor; NF-kappa B, transcription factor kappa B; TTP, tristetraprolin; p-STAT3, phosphorylates STAT3; HO-1, heme oxygenase; NADPH, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species.

The first model suggests that the combination of cholinergic agonists with alpha7nAChR leads to the recruitment of JAK2 to alpha7nAChR. JAK2 is autophosphorylated, and then JAK2 aggregates and phosphorylates STAT3 (p-STAT3), and finally p-STAT3 forms dimer and transfers to the nucleus [42–44]. Although STAT3 acts as a negative regulator of proinflammatory response, the mechanism of p-STAT3 dimer inhibiting the production and release of proinflammatory cytokines is not fully understood. A recent study has revealed that the mechanism of nicotine-activating STAT3 may be related to anti-inflammatory effects. In experiments using U937 cell-monocyte lines, nicotine-activated STAT3 has been

shown to induce tristetraprolin, an Au-rich-binding protein. The tristetraprolin induces cholinergic anti-inflammatory effects by destroying inflammatory transcripts containing Au-richs that are present in the 3'-untranslated region [45].

Peña et al. [46] proposed a second model for cholinergic regulation of inflammation associated with the JAK2/STAT3 signaling cascade. They believe that unphosphorylated STAT3 (u-STAT3) has anti-inflammatory effects rather than p-STAT3. The results suggested that the anti-inflammatory response of cholinergic is due to the inhibition of STAT3 tyrosine phosphorylation. The results showed that the inhibition of STAT3 tyrosine phosphor-

ylation was the main mechanism of the cholinergic anti-inflammatory reaction. It is worth noting that nicotine was used as an agonist in previous articles that demonstrated the role of p-STAT3 in CAIP, which is different from Pena's experiment in which choline was used as an agonist. They believed that u-STAT3 replaced I-kappa B by combining with NF- κ B to form a u-STAT3-NF- κ B complex to alleviate LPS-induced inflammatory response. They hypothesize that the anti-inflammatory effect of cholinergic agonists produced by uSTAT3-NF- κ B complex may be created through inhibiting the activation of NF- κ B.

In addition to the above 2 models, another study showed that nonphosphorylated STAT3 might compete with NF- κ B. The inhibition of STAT3 protein expression could enhance cytokine production and eliminate α 7nAChR signaling [40]. Furthermore, other studies have found that nicotine increases the level of IL-1 receptor-related kinase M in macrophages, which depends on JAK2, STAT3, and PI3K [47].

PI3K/Akt/Nrf-2

Another mechanism of cholinergic regulation of pro-inflammatory response is mediated by the upregulation of HO-1. In neurosurgery and LPS administration models, the activation and oxidative stress of reduced nicotinamide adenine dinucleotide phosphate on monocytes are associated with neuroinflammation and memory dysfunction. The activation of NF-kappa B can reduce neuroinflammation and improve cognitive deficits, which activates the PI3K/Akt/Nrf-2 pathway [48, 49]. Oxidative stress and neuroinflammation are currently considered to be 2 of the most important pathological mechanisms in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's chorea. It is also found that HO-1 expression is not related to the density of α 7nAChR in Huntington's chorea. Increased expression of HO-1 may induce neuroprotective effects related to the activation of α 7nAChR in early-stage Huntington's chorea by CAIP [50].

In experiments performed in RAW264.7 cells – a mouse mononuclear macrophage cell line – nicotine dose-dependent increased the expression of Ho-1 through α 7nAChR, and when α 7nAChR was antagonized by megaramine or silenced by RNA interference, the nicotine-induced Ho-1 upregulation was eliminated [51]. This study [51] confirms the necessity of upregulation of HO-1 induced by Ca^{2+} influx activation of α 7nAChR because the presence of Ca^{2+} chelators decreases the upregulation of HO-1 induced by nicotine, and the effect is dose-de-

pendent. The activation of α 7nAChR is accompanied by Ca^{2+} influx and the termination of the anti-inflammatory cascade. Then the influx of Ca^{2+} activates the classical PKC pathway through Ca^{2+} -dependent mechanism. The oxidation of nicotinamide adenine dinucleotide phosphate oxidase is accompanied by the production of a large number of reactive oxygen species, and PI3K/Akt/Nrf-2 pathway of phosphoinositol-3 kinase was activated [52], which HO-1 expression in macrophages was further induced [53]. The nicotine-induced upregulation of HO-1 may play a necessary role in the anti-inflammatory effect of nicotine-induced macrophages.

Vagus Nerve Electrical Stimulation

In 1990, EVNS was first used in humans to treat drug-resistant epilepsy [54]. Several years later, the US Food and Drug Administration approved EVNS for this indication and in 2005 for drug-resistant depression [55, 56]. EVNS is a treatment that stimulates the VN with an implantable device, that is, spiral electrodes were wound on the VN in the neck through surgical operation, the stimulator is buried in the chest, the parameters and modes of the device are adjusted, so that the stimulator can automatically stimulate the VN for therapeutic purposes [57].

With the further study of VN, the application of EVNS involves more subjects, for example, EVNS can reduce the area of cerebral infarction and improve the neurological function score after cerebral ischemia [58, 59] and can also be used to treat heart failure, thereby increasing left ventricular ejection fraction and NYHA score [60].

Electroacupuncture (EA) is an improvement on the traditional EVNS, which improves the safety and tolerance of EVNS because it does not require surgical implantation. The traditional EVNS needs to implant a small electrode piece into the body, while EA only needs to needle specific parts or stick the electrode piece on specific parts. The traditional Chinese medicine acupuncture has a history of >2000 years and is becoming an epidemic therapy for various diseases worldwide [61, 62]. Acupuncture is a medical intervention in the process of which slender needles are penetrated into muscles or other subcutaneous tissues are acted on acupoints, specific parts of the body. The traditional medical theory holds that the stimulation of acupuncture promotes the flow of "Qi" in the body, thus promoting the circulation of meridians and collaterals and finally promoting blood circulation [57–58]. Acupoints are considered to be pathophysiologically related to visceral and systemic conditions and may reflect

the state of visceral organs and the state of the whole body. Therefore, the stimulation of specific acupoints can cause reactivity, thereby controlling the imbalance of the internal environment and improving physical symptoms [63, 64]. Acupuncture stimulation is the direct treatment of local symptoms at or near acupoints, such as knee pain or muscle stiffness, at acupoints or adjacent affected areas, whereas acupuncture stimulation at distant affected areas is used to treat visceral diseases and systemic abnormalities. In EA treatment, a pair of needles was applied with a small current, and studies have shown that the therapeutic effect of EA can be regulated by changing the frequency, intensity, and duration of electrical stimulation [65, 66]. For example, EA can activate different opioid receptors at low and high frequencies to achieve different analgesic effects [67, 68]. Besides, it has the function of bidirectionally regulating the neuroendocrine-immune system, which can antagonize systemic inflammation without side effects.

However, the mechanism of EA treatment on gastrointestinal diseases is not completely clear and needs further study. EA has been reported to induce the VN to activate the aromatic amino acid decarboxylase, which leads to the production of dopamine in the adrenal medulla, thus controlling systemic inflammatory response [69, 70]. At present, in gastrointestinal diseases, the most relevant point is Zusanli (ST36), Zusanli point belongs to the stomach meridian of Zuyangming. It is also the combination point of the stomach meridian, one of the 9 points of the lower stomach and Huiyang, the key point of strength. Zusanli is named for its ability to treat upper, middle, and lower abdominal diseases. It is found that acupuncture at ST36 is related to the parasympathetic nervous system, which can alleviate inflammation and promote gastrointestinal function [71, 72]. Since the effect of acupuncture on ST36 is similar to that of activating the VN, it is possible to have a positive effect on gastrointestinal diseases by stimulating the anti-inflammatory pathway of the VN.

EVNS and Gastrointestinal Diseases

Postoperative Intestinal Obstruction

Every patient undergoing abdominal surgery suffers from temporary impaired gastrointestinal motility or POI. TNF- α released from inflammatory muscular lamina propria and activated permanent macrophages following abdominal surgery [73]. Exhaustion and inactivation of macrophages in the muscular layer can prevent POI.

During operation, EVNS can reduce the inflammatory response to intestinal operation [74]. This anti-inflammatory effect mediated by macrophage activation and cytokine production reduction is driven by CAIP [75]. EVNS may be a potential therapy for POI prevention because CAIP as a therapeutic target can improve POI through its anti-inflammatory effect.

Stakenborg et al. [76] showed in preclinical models that preoperative VNS could reduce inflammation caused by surgery and prevent POI. It was also confirmed that this anti-inflammatory effect was caused by ACh acting on $\alpha 7$ nAChR located in macrophages. Stakenborg et al. [77] stimulated abdominal VN in POI mice and found that LPS-induced TNF- α levels decreased, while significantly improved intestinal transport. They also conducted clinical trials in humans and found that abdominal VNS significantly reduced the production of whole blood IL-8 and IL-6 induced by LPS.

EA has also been shown to improve symptoms of POI. Hong et al. [78] tested whether noninvasive auricular electrical percutaneous vagus stimulation affects inflammation in POI models. The results showed that EA activated NTS and DMV, decreased the expression of intestinal cytokines, and reduced the recruitment of white blood cells to the intestinal segment of the operation, which improved the gastrointestinal transport after an operation. Clinical prospective studies were also conducted, to provide evidence for EA activation of the efferent visceral VN fibers. It is proved that EA is a low risk, simple operation and can effectively prevent POI after an operation. Zhang et al. [79] also recruited 42 patients who were about to undergo abdominal surgery in clinical trials and conducted ST36 electrical stimulation, which further showed that the main symptoms after surgery could be improved by enhancing vagal activity and inhibiting sympathetic activity.

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a chronic inflammatory disease with an increasing incidence worldwide. IBD is generally divided into 2 types, namely, Crohn's disease and ulcerative colitis. Low vagal activity has been observed in IBD disease, and thus the relationship between IBD and VN has also been gradually recognized. Experimental evidence in mouse models suggests that the reflex control of intestinal inflammation is mediated by efferent vagal nerve signals. This signal is transmitted to macrophages located in the intestinal tract and mediated by an inflammatory reflex, through a nerve-immune interaction in the spleen [80, 81].

Animal experiments have shown that the use of oxazolidone in the colon can seriously damage the colon mucosa, resulting in a mortality rate of 65% on the fifth day [82]. Compared with sham-treated mice, EVNS significantly increased the survival rate of model mice, which was related to the decrease of HMGB1 and the decrease of cytokine levels in colon and serum. After EVNS treatment in the TNBS-induced colitis model, colitis was alleviated compared with the control group by evaluating physiological (such as weight, temperature, exercise activity), macroscopic (lesion area), histological, and biological characteristics (such as cytokine and cytokine-related mRNAs) [83]. In the experiment with EVNS applied by Meregnani et al. [84] in the rat model, the course of colitis induced by TNBS was relieved, which was related to the inhibition of nuclear translocation of NF-kappa B and the upregulation of mitogen-activated protein kinase, both of which were important factors of inflammation. Sun et al. [85] also used clinical, histological, and biochemical parameters to assess the chronic EVNS effect and recorded the heart rate variability in rats with colitis using EVNS. They also observed a significant decrease in colon inflammation and IL-6 and TNF- α cytokines stimulated by the VN. Subsequently, Jin et al. [66] used a similar method and the same model of TNBS colitis, suggesting that chronic VNS can improve colonic inflammation by inhibiting proinflammatory cytokines through an autonomous mechanism.

In IBD, clinical studies using EVNS are limited. Two clinical studies have shown preliminary clinical outcomes of EVNS in patients with active CD. Bonaz et al. [86] reported a 6-month follow-up study of 7 CD patients, 2 of whom were receiving azathioprine treatment, and the remaining 5 of whom were not receiving treatment at the same time. Before treatment, the endoscopic inflammation score (CDEIS) of Crohn's disease was >7 in all patients. The Crohn's disease activity index and CDEIS were evaluated after 6 months of continuous treatment. Two patients withdrew from the study in advance because of the deterioration of their condition. The activity index of Crohn's disease was improved in the remaining 5 patients, and clinical remission was achieved in 4 of them at the end of the study (Crohn's disease activity index <150). Within 6 months, all patients achieved endoscopic remission (CDEIS <6). All 5 patients reported reduced pain after 6 months. EVNS can also be used to maintain drug-induced remission. As a slow-acting treatment, EVNS is an effective means to prevent recurrence after CD surgery.

EVNS and Intestinal Barrier

The intestinal barrier consists of the mechanical barrier, chemical barrier, microbial barrier, and immune barrier. Intestinal mucosal barrier injury involves many fields such as microecology, immunity, and molecular biology. Intestinal epithelial dysfunction is associated with the development of various gastrointestinal diseases, such as Bauer's syndrome, IBD, abdominal diseases, and mucosal diseases [87–89].

Studies have shown that cutting the abdominal VN at the gastroesophageal junction before EVNS prevents the protective effect of EVNS, thus confirming the efferent VN signal could regulate the integrity of intestinal barrier after injury [90]. The intestinal permeability of animals with abdominal vagotomy before EVNS is comparable to that of animals with burns. Abdominal vagotomy eliminates the protective effect of EVNS. They also showed that the protective effect of EVNS on the intestinal tract was not due to the regulation of TNF- α production in splenic circulation. It is well known that intestinal glia can improve intestinal barrier function by increasing the expression of tight junction protein, thus improving the integrity of the intestinal barrier. The barrier induction of enteric glial activation can prevent or limit the disruption of the intestinal barrier, which may be a therapeutic target for diseases that cause intestinal inflammation. Costantini et al. [91] further demonstrated that $\alpha 7$ nAChR is a necessary receptor for the vagal nerve to regulate inflammation and mediate the protective effect of the intestinal barrier in the burn model.

The role of EA in intestinal barrier protection has also been discussed. Hu et al. [92] studied the protective effect and mechanism of vagal nerve stimulation on intestinal barrier dysfunction and distal organ injury after intestinal ischemia-reperfusion injury in rats utilizing integrated traditional Chinese and Western medicine. They compared the effects of EA at ST36 and non-acupoint points, as well as intraperitoneal injection of cholinergic agonists. The levels of cytokines in plasma, intestinal tissue, lung tissue, and liver tissue were determined. Intestinal barrier damage was detected by histology, intestinal injury score, the permeability of 4 kDa-fic dextran, and changes of tight junction protein ZO-1 detected by immunofluorescence and Western blot. The results showed that EA can significantly reduce the levels of TNF and IL-8 in plasma and organ tissues, decrease intestinal permeability of fic-dextran, and prevent the expression and localization of ZO-1 protein. However, abdominal vagot-

omy or intraperitoneal injection of $\alpha 7$ nAChR inhibitors reversed these effects.

All these results suggest that in the presence of intact VN, EVNS can protect the integrity of the intestinal barrier and reduce the systemic inflammatory response and distant organ damage.

Conclusion

VN plays an anti-inflammatory role through the CAIP, targeting the VN, which opens up a new therapeutic approach for gastrointestinal inflammatory diseases (such as IBD, POI, IBS) and other TNF- α -mediated diseases (such as RA or psoriasis). Although the exact mechanism and neural circuits of intestinal CAIP have not been fully clarified, many experiments have shown that vagal nerve stimulation by electrical or chemical stimulation can alleviate inflammation. We gradually recognized how the VN is activated by certain inflammatory conditions and inhibits the production of proinflammatory cytokines through the activation of macrophage $\alpha 7$ nAChR. However, the existing research results still do not fully explain how the VN affects and controls inflammation in the gastrointestinal tract. From the perspective of anatomy, abdominal VN does not directly contact the intestinal im-

mune cells, but the macrophages are located near the nerve endings of the gastric circular muscle and ileal plexus. The brain-gut axis from nerve to intestine needs further exploration and research.

Artificial modulation of peripheral nerve signals by VNS (neuromodulation) is an innovative therapy with the potential to develop alternative or complementary drug therapy. More clinical studies are needed to confirm its feasibility, which will lay the foundation for the long-term use of nondrug and noninvasive therapies to treat diseases and may become an important treatment for inflammation-related diseases shortly.

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Author Contributions

W.L. wrote the paper. All authors read and gave approval of the final version of the article to be published.

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