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Vagus nerve stimulation mediates microglia M1/2 polarization via inhibition of TLR4 pathway after ischemic stroke

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Abstract

Ischemic stroke is the leading cause of death and disability. Microglia are polarized toward the proinflammatory M1 phenotype and neuroprotective M2 phenotype after stroke and play an important role in the pathological process of ischemic stroke. Emerging research suggests that vagus nerve stimulation (VNS) can mediate microglia polarization after ischemic stroke and may serve as a potential treatment for ischemic stroke. However, the mechanism by which VNS mediates microglia polarization remains unclear. In this study, we aimed to investigate the underlying mechanism. Sprague-Dawley rats were randomly divided into the sham, ischemic stroke, ischemic stroke+VNS, ischemic stroke+VNS+lentivirus (LV)-TLR4 and ischemic stroke+VNS+LV-CON groups. LV was injected into the lateral ventricles of the rats 14 days before ischemic stroke surgery, and VNS was administered after 30min of occlusion. We assessed the infarct volume, neurological scores, the TLR4/MyD88/NF-κB protein level and microglia polarization after 3 days of reperfusion. Our results revealed that VNS can promote M2 microglia polarization and inhibit M1 microglia polarization to alleviate brain injury via inhibition of the TLR4/MyD88/NF-κB pathway in microglia in the acute stage of stroke.

Introduction

Ischemic stroke has become the second leading cause of death and the most disabling disease [1]. Immunity and inflammation are two important elements of the pathophysiology of stroke [2]; an immune response occurs in the ischemic brain parenchyma, and inflammatory mediators produced in situ in the brain parenchyma might further exacerbate brain injury [3]. As the key immune cells in the central nervous system (CNS), resident microglia, the first cells to respond to ischemic stroke, rapidly move toward the periphery of the ischemic lesion within an hour after stroke [[4], [5], [6], [7]]. Microglia play an dual role in the progression of stroke; microglia can be polarized toward different phenotypes, which can be roughly divided into the “classically activated” M1 phenotype and the “alternatively activated” M2 phenotype, and M1 and M2 microglia engage in different functions in response to specific stimulation. M1 microglia release proinflammatory cytokines, such as interleukin (IL)-1β, IL-6 and express specific surface markers, such as CD86 and inducible nitric oxide synthase (iNOS). In contrast, M2 microglia release anti-inflammatory cytokines, such as transforming growth factor-β (TGF-β), IL-4 and IL-10, and express the specific surface markers CD206 and Arginase 1 (Arg1), to protect injured neurons [[8], [9], [10]]. Thus, promoting microglia polarization toward the M2 phenotype is a potential therapeutic strategy for ischemic stroke.

Toll-like receptors (TLRs) are pattern recognition receptors that recognize pathogen-associated molecular patterns (PAMPs) and endogenous-derived danger-associated molecular patterns (DAMPs) [11]. Among mammalian TLRs in the CNS, TLR4 has been found to be mainly expressed in microglia and to mediate neuroinflammatory diseases [12]. Previous studies have demonstrated the role of the TLR4 pathway in the polarization of microglia in neurodegenerative diseases, such as Alzheimer's disease (AD) [13] and Parkinson's disease (PD) [14], as well as CNS trauma, such as traumatic brain injury (TBI) [15], intracerebral hemorrhage (ICH) [16] and ischemic stroke [17,18]. An *in vitro* study revealed that activation of the TLR4/NF-κB pathway promoted microglia polarization toward the M1 phenotype under ischemic stroke conditions [18]. Pharmacological modulation of TLR4, such as by resatorvid (TAK-242) and naloxone, can significantly decrease the infarct volume and alleviate neurological deficits in an animal model of ischemic stroke, but clinical evidence for the application of such drugs is lacking [11,19]. Vagus nerve stimulation (VNS), which is approved by the U.S. Food and Drug Administration (FDA), is applied as an alternative therapy for several diseases, such as refractory epilepsy, depression, and migraine [20], and recently, a growing number of clinical trials have reported that VNS is a potential therapy for ischemia [[21], [22], [23], [24]]. VNS can inhibit microglia activation induced by lipopolysaccharide (LPS), an agonist of TLRs [25]. Therefore, we hypothesized that VNS might inhibit the expression of TLR4 in microglia and then mediate the polarization of microglia after ischemic stroke. The aim of our study was to investigate whether VNS mediates microglia polarization via inhibition of the TLR4 pathway in microglia to relieve ischemic stroke injury.

Section snippets

Experimental animals and groups

Adult male Sprague-Dawley rats (weight, 200–230g; age, 8 weeks) fed a healthy diet and housed under specific pathogen-free conditions were used in our study. The animals were kept at a controlled temperature (25±1°C) and humidity (50%) on a 12h light/dark cycle. The animal study was reviewed and approved by the Institutional Ethics Committee of Chongqing Medical University. The rats were divided into the following groups: (1) the sham group (n=30), (2) ischemic stroke group (n=30), (3) …

VNS

VNS was administered after 30 min of occlusion. The left vagus nerve was isolated during MCAO surgery and stimulated at 0.5 mA and 20 Hz for 0.5 ms for a total of 1 h after occlusion for 30 min, as described in a previous study [27].…

Stereotaxic injection of LV vectors

An LV vector expressing TLR4 (LV-GFP-TLR4) was obtained from Tsingke Biological Technology. An LV vector expressing GFP was used as a control. Fourteen days before MCAO surgery, 10 μl LV-GFP-TLR4 or negative control was injected into the ipsilateral lateral ventricles. The rats were anesthetized with 1% pentobarbital sodium (45 mg/kg, intraperitoneally) and placed in an animal stereotaxic apparatus (RWB Life Science Co, Ltd., China). The coordinates for LV injection relative to bregma, which…

VNS reduced the expression of TLR4 in microglia after MCAO

We first assessed the protein level of TLR4 in the ischemic stroke group and VNS-treated group at different time points (1, 3, and 7 days) after stroke. The results showed that the expression of TLR4 was highest at 3 days after stroke (Fig. 1A), however VNS significantly reduced the level of TLR4, especially at 3 days after stroke ($P<0.0001$) (Fig. 1A). Interestingly, a significant increase in the number of M1 microglia was also observed at 3 days after stroke in a previous study [6].…

Discussion

Our study revealed that VNS mediated the microglia polarization balance by inhibiting the TLR4/MyD88/NF-κB pathway in microglia, promoted microglia polarization toward the M2 phenotype, reduced the release of the proinflammatory cytokines IL-1β and IL-6, thus reducing the infarct volume and improving sensorimotor function in the acute stage of stroke.

A large number of clinical trials have demonstrated the benefits of VNS in the recovery of motor function after ischemic stroke, but almost all of …

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Declaration of competing interest

The authors claim no conflict of interest.…

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Citation Excerpt :

…This finding suggests that nVNS, when applied as described in the present study, does not pose a significant risk of raising arterial embolism. Several mechanisms by which VNS can exert ischemic neuroprotection have been proposed; nVNS augments GABAergic inhibitory pathways [27], reduces blood–brain barrier permeability [13], and suppresses ischemia-induced microglial activation, particularly of the pro-inflammatory M1 phenotype, which is associated with reduced levels of inflammatory cytokines in preclinical models of stroke [10,11,28]. In addition, activation of vagal afferents by nVNS reduces cortical spreading depression and peri-infarction depolarizing waves [12,29,30]….

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