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Auricular vagus nerve stimulation promotes functional recovery and enhances the post-ischemic angiogenic response in an ischemia/reperfusion rat model

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ABSTRACT

Electrical stimulation of the vagus nerve, which has been used to treat epilepsy patients since 1997, also enhances long-term restoration after central nervous system (CNS) injury. Angiogenesis is a complex restorative mechanism that occurs in response to ischemic stroke, and it positively affects the recovery of neurological functions in a rat model of stroke. The aims of our study were to determine whether auricular vagus nerve stimulation (aVNS) promoted functional recovery and enhanced angiogenesis in the ischemic boundary following ischemia/reperfusion and to uncover the possible molecular mechanisms that are involved. Adult male Sprague-Dawley (SD) rats underwent transient middle cerebral artery occlusion (tMCAO) surgery and received repeated electrical stimulation of the left cavum concha starting 30 min after ischemia. For the following 21 days, we evaluated functional recovery at different time points using neurological deficit scores, the beam-walking test and the staircase test. The infarct volume was measured using TTC staining at 24 h post reperfusion, neuronal survival in the ischemic penumbra was assessed using hematoxylin and eosin (HE) staining. Microvessel density and endothelial cell proliferation in the ischemic boundary were assessed using immunofluorescence. The expression levels of brain-derived neurotrophic factor (BDNF), endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) in the ischemic penumbra were also evaluated. Our results showed that aVNS had significant neuroprotective effects and enhanced angiogenesis, which was demonstrated by improvements in the behavioral scores and brain histopathology, including increased levels of microvessel density and endothelial cell proliferation surrounding the infarct area. Furthermore, BDNF, eNOS and VEGF were expressed at higher levels in the I/R + aVNS group than in the I/R group or the I/R + sham aVNS group ($p < 0.05$). Our findings suggest that repeated aVNS promoted post-ischemic functional

Abbreviations: ANOVA, analysis of variance; CBF, cerebral blood flow; BDNF, brain-derived neurotrophic factor; CCA, common carotid artery; CNS, central nerve system; ECA, external carotid artery; EC, endothelial cell; eNOS, endothelial nitric oxide synthase; FDA, Food and Drug Administration; ICAM-1, adhesion molecule-1; I/R, ischemia/reperfusion; MCAO, middle cerebral artery occlusion; SD, Sprague-Dawley; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SEM, standard error of the mean; VEGF, vascular endothelial growth factor; aVNS, auricular vagus nerve stimulation.

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recovery and angiogenesis, possibly in conjunction with the up-regulated expression of BDNF, eNOS and VEGF in the rat brain.

1. Introduction

Ischemic stroke is a serious worldwide health problem that leads to high mortality and long-term disability, especially in the elderly (Moretti et al., 2015). However, the lack of effective therapeutic strategies that can provide a positive long-term outcome after stroke remains an urgent clinical problem. The angiogenic response induced by ischemic stroke plays an important role in the recovery of neurological functions during the chronic stage (Ma et al., 2015). Angiogenesis not only provides a sufficient supply of oxygen and nutrients but also offers a niche for the proliferation of neurons after brain damage, and these features are beneficial for salvaging cerebral tissue in the penumbra, thereby promoting long-term functional recovery (Petcu et al., 2010). Thus, enhancing post-ischemic angiogenesis should be beneficial for therapeutic interventions. Vagus nerve stimulation (VNS) was approved by the Food and Drug Administration (FDA) in 1997 for the treatment of refractory partial epileptic seizures (You et al., 2007; Morris et al., 2013) and treatment-resistant depression. Ay et al. previously demonstrated the neuroprotective effects induced by VNS in acute ischemia/reperfusion rat models. They recently reported that electric stimulation of the vagus nerve dermatome in the external ear could also exert similar neuroprotection (Ay et al., 2015). At present, the long-term neuroprotection mediated by VNS in rat stroke models is not entirely known, although several new studies have emerged (Hiraki et al., 2012; Khodaparast et al., 2013). Therefore, elucidating the possible molecular mechanisms may reveal an even greater potential for VNS.

Although brain-derived neurotrophic factor (BDNF) is closely associated with synaptic plasticity and neuronal cell survival, it is also involved in promoting angiogenesis (Liu et al., 2006). Recent studies indicated a significant correlation between BDNF and vascular endothelial growth factor (VEGF) expression (You et al., 2015). The participation of BDNF in the angiogenic processes of endothelial cells from human umbilical vessels indicated that BDNF mediated an increase in the expression of angiogenic markers (Kallmann et al., 2002).

As noted, endothelial nitric oxide synthase (eNOS) activation appears to play an important role in vascular relaxation, suppression of platelet aggregation, and increased proliferation of vascular smooth muscle (Lähteenvuo and Rosenzweig, 2012). Interestingly, its production could promote neovascularization and increase the number of circulating endothelial progenitor cells (EPC) in adults, and EPC could increase neovascularization after stroke. In addition, eNOS could be regulated by many up-stream mediators, and the PI3K/Akt signaling pathway was involved in activating eNOS (Balakumar et al., 2012).

VEGF is commonly accepted to be an angiogenic polypeptide growth factor, but it also exerts a neuroprotective effect on the central nervous system (CNS). Emerging evidence has demonstrated that the silencing of VEGF expression leads to impaired vascularization and neuronal expansion during brain development (Kuo et al., 2001). In contrast, the exogenous administration of VEGF promotes angiogenesis in the CNS of adult rats. The role of VEGF in the proliferation and survival of endothelial cells (ECs) and the formation of fenestrations has been confirmed in tumor studies. Although ECs are the main target of VEGF, VEGF is generally secreted by tissues in a paracrine manner and is widely expressed in adult rat brains (Maharaj, 2007). Interestingly, it has been found that acute VNS could upregulate the expression of BDNF and fibroblast growth factors (Mravec, 2010). Additionally, we found that acute VNS could increase Akt phosphorylation and inhibit apoptosis (Jiang et al., 2014). Taken together, we hypothesized that aVNS could promote functional recovery and neovascularization. In the present study, we tested this hypothesis in a middle cerebral artery occlusion model of ischemic stroke in rats. We also investigated whether aVNS affected the expression of some pro-angiogenic mediators in the recovery phase, which may partially explain the long-term neuroprotective effect induced by aVNS.

2. Materials and methods

2.1. Ethics statement

All of the procedures were approved by and conformed to the guidelines of the Institutional Ethics Committee of Chongqing Medical University (Permit No. SCXK (Chongqing) 2007-0001) and the State Science and Technology Commission of China. The animal experiments were approved by the Laboratory Animal Management Committee of Chongqing Medical University.

2.2. Animals

Male Sprague–Dawley (SD) rats (250–350 g) were purchased from the experimental animal center of Chongqing Medical University and were housed with free access to water and food and under an inverted 12-h light/dark cycle prior to the experiments. The rats used in the experimental study were randomly assigned to one of the four following groups: the sham + aVNS group, the ischemia/reperfusion group (I/R group), the ischemia/reperfusion + sham aVNS group (I/R + sham aVNS group) and the ischemia/reperfusion + aVNS group (I/R + aVNS group).

A total of 368 rats were included in the study; however, only 224 rats met the requirements for the experiment. There were 8 rats in each group for each test. In total, 90 rats were excluded from our study because of death during the experiment, 32 rats were excluded because of improvement in experiment method, and 22 rats were excluded because they did not meet the reduced CBF criterion.

2.3. Focal cerebral ischemia/reperfusion surgery

Focal ischemia was induced by occlusion of the right middle cerebral artery, as previously described (Koizumi et al., 1986). Briefly, rats were anesthetized with 10% chloral hydrate (3.5 mg/kg). After the right common carotid artery was exposed, the external carotid artery was ligated. A nylon monofilament (diameter 0.25–0.28 mm) coated with silicone resin was inserted into the right internal carotid artery to obstruct the middle cerebral artery for 2 h. During this 2-h period, cerebral blood flow fluctuation was measured by laser Doppler flowmetry (PeriFlux 4001; PeriMed) to ensure that the model was successfully established. Rats in which the blood flow did not decrease to 60–70% of the baseline level immediately after middle cerebral artery occlusion (MCAO), and