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Transcutaneous auricular vagus nerve stimulation regulates expression of growth differentiation factor 11 and activin-like kinase 5 in cerebral ischemia/reperfusion rats

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ABSTRACT

Growth differentiation factor 11 (GDF11), as a rejuvenation factor in heterochronic parabiosis, can increase proliferation of primary brain capillary endothelial cells (ECs). However, the angiogenic role of GDF11 in ischemia-induced brain injury is still unclear. There are no previous reports on the spatiotemporal expression of GDF11 in cerebral ischemia/reperfusion (I/R) rats. Our recent work has strongly suggested that transcutaneous auricular vagus nerve stimulation (ta-VNS) reduces infarct size and induces angiogenesis in focal cerebral I/R rats. This study focused on expression of GDF11 and activin-like kinase 5 (ALK5) and the effects of ta-VNS in a rat cerebral I/R model. For ta-VNS, electrical stimulation of the left cavum concha (1 h duration) using percutaneous needles was initiated 30 min after induction of ischemia. Expression of GDF11 was analyzed by enzyme-linked immunosorbent assay, immunohistochemistry, real-time polymerase chain reaction, and western blot 24 h, 3 d, and 7 d after reperfusion. In addition, neurobehavioral function, EC proliferation, and expression of ALK5 in ECs in the peri-infarct cortex were measured. Results showed that levels of GDF11 were significantly elevated after cerebral I/R, both in plasma and the peri-infarct cerebral cortex. Interestingly, splenic GDF11 levels decreased after ischemia. ALK5 was expressed in ECs in the peri-infarct cerebral cortex where active vessel remodeling was noted. ta-VNS improved neurobehavioral recovery, upregulated cerebral GDF11 and downregulated splenic GDF11, indicating a brain-spleen communication during stroke. ta-VNS also increased expression of ALK5 in ECs and stimulated proliferation of ECs. These results suggest that, after cerebral ischemia, GDF11 redistributes and participates in angiogenesis as an angiogenic factor that acts at least in part through ALK5. GDF11/ALK5 may represent a new potential therapy target for stroke.

1. Introduction

After focal cerebral ischemia, the newly formed collateral blood vessels can improve perfusion of the surrounding tissues and promote recovery of nervous system functions [1]. Recent studies have suggested that angiogenesis, almost in parallel to neurogenesis, participates in recovery of neurological function after ischemic stroke [2]. Developing new strategies to treat cerebral ischemia/reperfusion (I/R) injury requires a better understanding of the mechanisms that underlie angiogenesis.

Growth differentiation factor 11 (GDF11), a member of the transforming growth factor-β (TGF-β) superfamily, regulates diverse biological processes [3]. GDF11 is a circulating factor [4] in young mice that declines with age. Recently, GDF11 was identified as a rejuvenation factor in heterochronic parabiosis [5–7], which is an unusual surgical technique that merges the circulatory systems of 2 animals. GDF11 can increase proliferation of primary brain capillary endothelial cells (ECs), participate in vascular remodeling, increase the volume of blood vessels and restore age-related decline in neurogenesis [8]. Therefore, GDF11, which initially attracted attention as a factor in blood circulation, is probably one of the key angiogenic factors for improving vasculature of the neurogenic niche in the aged brain. However, the spatiotemporal expression of GDF11 in a rat cerebral I/R model still remains elusive.

Abbreviations: ANOVA, analysis of variance; ABP, arterial blood pressure; ALK5, activin-like kinase 5; cVNS, cervical vagus nerve stimulation; CCA, common carotid artery; d, day/days; ECs, endothelial cells; EPCs, endothelial progenitor cells; ELISA, enzyme linked immunosorbent assay; ECA, external carotid artery; GDF11, growth differentiation factor 11; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HR, heart rate; h, hour/hours; ICA, internal carotid artery; I/R, ischemia/reperfusion; LSD, least significant difference; MCAO/R, middle cerebral artery occlusion/reperfusion; min, minute/minutes; mNSS, modified neurological severity scores; q-PCR, quantitative real-time polymerase chain reaction; rCBF, region cerebral blood flow; s, second/seconds; TGF-β, transforming growth factor-β; ta-VNS, transcutaneous auricular vagus nerve stimulation; TTC, 2,3,5-triphenyltetrazolium chloride; VNS, vagus nerve stimulation.

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The U.S. Food and Drug Administration has approved the clinical use of vagus nerve stimulation (VNS) for treatment of medically refractory partial-onset seizures [9] and resistant depression [10]. Most recently, transcutaneous electrical stimulation of the auricular branch of the vagus nerve without surgery could partially duplicate the effect of traditional cervical vagus nerve stimulation (cVNS). It has been demonstrated that transcutaneous auricular vagus nerve stimulation (ta-VNS) reduced infarct volume, improved neurological functions, and induced angiogenesis [11] in a model of middle cerebral artery occlusion (MCAO) in rats and activated vagal pathways similar to that of the gold standard cVNS [12]. It is a novel and promising treatment that is even used for acute and chronic ischemic stroke.

The present study was mainly designed to investigate the effects of focal cerebral I/R and ta-VNS on expression of GDF11 in the spleen, plasma, and peri-infarct cerebral cortex and its receptor, activin-like kinase 5 (ALK5).

2. Experimental procedures

2.1. Animals and grouping

The experimental protocols were performed in strict accordance with the Guidelines for the Care and Use of Laboratory Animals approved by the Institutional Ethics Committee of Chongqing Medical University [Permit No. SCXK (Chongqing) 2007-0001] and the State Science and Technology Commission of China. Animal experiments were performed at the Laboratory Animal Management Committee of Chongqing Medical University. Male Sprague-Dawley rats (220–240 g) were provided by the Experimental Animal Center of Chongqing Medical University. All rats were housed and fed under specific pathogen-free and controlled conditions (12/12 h light/dark cycle with humidity of 60%, 22 °C) with free access to food and water.

Rats were randomly assigned to each group. The experiment included 4 groups: (1) sham I/R group (control group), (2) I/R group, (3) I/R with ta-VNS (I/R + ta-VNS group), (4) I/R with sham stimulation (I/R + SS group). To minimize interference of age, we limited the experiment to rats 42–48 days of age. There were no significant differences in weight and age among the groups.

A total of 266 rats were included in the study; however, only 218 rats met the requirements for the experiment. Twenty-two rats were excluded because they did not meet the reduced rCBF criterion. In all, 26 rats were excluded because of death during the experiment; 8 rats in the I/R group (5 rats in 24 h, 3 rats in 3 d), 9 rats in the I/R + ta-VNS group (7 rats in 24 h, 2 rats in 3 d), and 9 rats in the I/R + SS group (5 rats in 24 h, 3 rats in 3 d, 1 rat in 7 d).

2.2. Right middle cerebral artery occlusion/reperfusion (MCAO/R) model

Right MCAO was induced with an intraluminal suture occlusion as previously described [13,14]. Anesthesia was induced with 3.5% chloral hydrate (350 mg/kg). The right common carotid artery (CCA) and internal carotid artery (ICA) were exposed via a midline incision in the neck. After the stump of the external carotid artery (ECA) was pulled down to obtain a straight line with the ICA, the ICA and CCA were clipped temporarily with a microvascular clamp. A small incision was made on the ECA stump near the bifurcation, and a 40 mm-length nylon filament suture, which was blunted at the tip (diameter = 0.31–0.32 mm) by a flame and coated with melted paraffin wax [15], was inserted from the right ECA through the CCA and up to the ICA for a distance of about 12 mm. The rats were then placed in a stereotaxic frame, and a laser Doppler flowmeter probe (PeriFlux System 5000; Perimed, Stockholm, Sweden) monitored changes in regional cerebral blood flow (rCBF) in the right MCA region [16] to confirm successful MCAO. When the nylon filament was inserted approximately 18–22 mm to block the origin of the MCA, a silk suture was then tightened around the ECA stump and nylon filament. After 2 h of occlusion, the animals were anesthetized again and the nylon suture was removed to allow reperfusion. The standard I/R model was defined as a decrease in cortical rCBF to 70–80% of baseline during the first 30 min and >70% flow recovery within the initial 10 min of reperfusion. Rats that did not meet these requirements or those without neurological deficits were excluded from the study. In the sham I/R group, the embolus was inserted at a distance of 12 mm and removed immediately.

Throughout the duration of the experiment, animals were anesthetized and body temperature was maintained at 37 ± 0.5 °C with a heating pad. In addition, 24-gauge catheters (Becton Dickinson and Company, Franklin Lakes, NJ, USA) were inserted into the caudal ventral artery of the tail and connected to a Datex AS3 physiological monitoring instrument (Communications Specialties, Inc., NY, USA) through heparin-filled pressure transducers and sampled for blood gas analysis (ABL700, Radiometer, Bronshoi, Denmark) at 3 time points: before, during, and after ta-VNS. Invasive arterial blood pressure (ABP) and heart rate (HR) were measured at 5 time points: the 3 time points listed above, before ischemia (baseline), and after reperfusion as in our previous study [17] (n = 12 for each group).

2.3. ta-VNS

Rats received the first ta-VNS 30 min after MCAO with 2 acupuncture needles (38-gauge, stainless steel with silver handle, Cloud Dragon, Hangzhou, China) connected to a Grass Model S48 stimulator (Grass Technologies, Warwick, RI, USA) and constant current as previously described [12]. The needles were inserted 0.5–1 mm under the skin over the left cavum concha 15 min after MCAO. A 30 s train of stimulation consisting of 0.5 ms square pulses (0.5 mA) delivered at 20 Hz was initiated 30 min after MCAO. Stimulation was repeated every 5 min for 1 h. The animals received ta-VNS treatment twice daily until euthanasia on the scheduled day. All of the procedures including electrode implantation, were replicated in the I/R + SS group except delivery of electrical stimulation.

2.4. Neurobehavioral evaluation

A battery of neurobehavioral tests, which included the modified neurological severity scores (mNSS) test and the adhesive-removal somatosensory test [18], were performed before MCAO (baseline) as well as 24 h, 3 d, and 7 d after reperfusion by an investigator who was blinded to the experimental groups (n = 12 for each subgroup).

2.4.1. mNSS test

The mNSS test [19,20] is a composite of motor, sensory, balance, and reflex tests. Neurological function was graded on a scale of 0 to 18 (normal score: 0, maximal deficit score: 18). In the severity scores of injury, 1 score point was awarded for a specific abnormal behavior or for the lack of a tested reflex. Thus, a higher score reflected more severe injury. Before induction of ischemia, rats were pre-trained in the beam-walking test for 3 d until every rat could readily undergo the beam-traversing task without any slipping [21]. Their performances were video recorded and 3 trials were recorded for analysis.

2.4.2. Adhesive removal somatosensory test

The adhesive removal somatosensory test [20,22] is a sensitive method to assess sensorimotor deficits [23]. All rats were familiarized with the testing environment before surgery. Two small pieces of circular adhesive-backed paper patch (diameter: 10 mm) were used gently as bilateral tactile stimuli occupying the distal-radial region on the wrist of each forelimb. The rat was then returned to its home cage. The time to remove each stimulus was recorded (with a maximum limit of 120 s) during 3 trials per day. Individual trials were separated