Brain Stimulation 9 (2016) 166–173

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: www.brainstimjrnl.com

Transcutaneous Cervical Vagus Nerve Stimulation Ameliorates Acute Ischemic Injury in Rats

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ARTICLE INFO

Article history:
Received 22 June 2015
Received in revised form 12 October 2015
Accepted 23 November 2015
Available online 1 December 2015

Keywords:
Vagus nerve
Electrical stimulation
Cerebral ischemia
Neuroprotection
Inflammation

ABSTRACT

Background: Direct stimulation of the vagus nerve in the neck via surgically implanted electrodes is protective in animal models of stroke. We sought to determine the safety and efficacy of a non-invasive cervical VNS (nVNS) method using surface electrodes applied to the skin overlying the vagus nerve in the neck in a model of middle cerebral artery occlusion (MCAO).
Methods: nVNS was initiated variable times after MCAO in rats (n = 33). Control animals received sham stimulation (n = 33). Infarct volume and functional outcome were assessed on day 7. Brains were processed by immunohistochemistry for microglial activation and cytokine levels. The ability of nVNS to activate the nucleus tractus solitarius (NTS) was assessed using c-Fos immunohistochemistry.
Results: Infarct volume was 43.15 ± 3.36 percent of the contralateral hemisphere (PCH) in control and 28.75 ± 4.22 PCH in nVNS-treated animals ($p < 0.05$). The effect of nVNS on infarct size was consistent when stimulation was initiated up to 4 hours after MCAO. There was no difference in heart rate and blood pressure between control and nVNS-treated animals. The number of c-Fos positive cells was 32.4 ± 10.6 and 6.2 ± 6.3 in the ipsilateral NTS ($p < 0.05$) and 30.4 ± 11.2 and 5.8 ± 4.3 in the contralateral NTS ($p < 0.05$) in nVNS-treated and control animals, respectively. nVNS reduced the number of Iba-1, CD68, and TNF-α positive cells and increased the number of HMGB1 positive cells.
Conclusions: nVNS inhibits ischemia-induced immune activation and reduces the extent of tissue injury and functional deficit in rats without causing cardiac or hemodynamic adverse effects when initiated up to 4 hours after MCAO.

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Introduction

There is a surge of interest in brain stimulation to reduce tissue injury and to restore the lost function in a wide variety of neurological disorders. Experimental evidence indicates that stimulation of a group of anatomically connected areas such as the fastigial nucleus [1], periaqueductal gray matter [2], subthalamic vasodilator area [3], sphenopalatine ganglion [4], and cervical vagus nerve [5–8] leads to a reduction in infarct volume by up to 50% in animal models of cerebral ischemia. Unlike intracranial structures that require craniotomy for access, the vagus nerve is accessible in the neck and hence can be stimulated using surgically implanted electrodes following a small incision in the overlying skin. Neural impulses following cervical vagus nerve stimulation (cVNS) project to a wide variety of cortical and subcortical structures via the nucleus tractus solitarius (NTS) [9,10] and can activate circuits that inhibit neuronal excitability [11] and block microglial response to ischemia-induced inflammation [12].

Although cVNS is protective in experimental models of cerebral ischemia, it is not feasible for human application in the setting of acute ischemic stroke because direct nerve stimulation requires a surgical procedure. This urges a search for less invasive or non-invasive techniques for cVNS. In this study, we explored the safety and efficacy of a non-invasive transcutaneous cervical vagus nerve stimulation (nVNS) approach using surface electrodes applied to the skin overlying the vagus nerve in the neck in a model of middle cerebral artery occlusion (MCAO) in rats.

Abbreviations: MCAO, middle cerebral artery occlusion; cVNS, cervical vagus nerve stimulation; nVNS, non-invasive cervical vagus nerve stimulation; SHR, spontaneously hypertensive rat; PCH, percent of the contralateral hemisphere; NTS, nucleus tractus solitarius; ABP, arterial blood pressure; HR, heart rate; rCBF, regional cerebral blood flow; α7nAChR, α7 nicotinic acetylcholine receptor.

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http://dx.doi.org/10.1016/j.brs.2015.11.008

Materials and methods

All experiments were performed in accordance with the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals and were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee. Adult male spontaneously hypertensive rats (SHR, 326–420 g, n = 66; Charles River Laboratories, Wilmington, MA) were used. We used SHR in compliance with the Stroke Therapy Academic Industry Roundtable (STAIR) criteria which explicitly require the use of animals with co-morbid conditions in order to increase the quality of translational stroke research [13].

Experimental protocols

We studied the following four hypotheses:

1. *nVNS reduces infarct volume and improves neurological outcome after MCAO*: Animals were randomly allocated into two experimental groups: treatment (351.00 ± 14.41 g; n = 7) and control (336.17 ± 16.46 g; n = 6). Electrical stimulation of the right cervical vagus nerve was initiated 30 minutes after occlusion of the right MCA and repeated every 10 minutes for a period of 1 hour. Control animals received sham stimulation delivered to the right quadriceps femoris muscle. Twenty minutes after the last stimulation, animals were returned to their home cages. Functional assessments were performed daily after surgery for 7 days. Infarct volume was assessed on day 7. The following safety parameters were recorded in each animal: arterial blood pressure (ABP), heart rate (HR), arterial blood gases (ABGs) and pH, symptomatic brain hemorrhage, and mortality or development of any of the euthanasia criteria described by AVMA Guidelines for the Euthanasia of Animals 2013.
2. *nVNS activates NTS*: We explored whether nVNS caused activation in the NTS after MCAO in rats. We performed the same surgical and stimulation procedures in Experiment-1, but animals were euthanized 3 hours after MCAO and brains were processed for c-Fos immunohistochemistry. There were two experimental groups: treatment (390.25 ± 5.32 g; n = 4) and control (394.00 ± 4.83 g; n = 4).
3. *nVNS leads to inhibition of MCAO-induced immune response in the brain.* cVNS causes marked inhibition of the immune response to various stimuli both in the periphery and the central nervous system [12,14]. We explored whether nVNS was able to inhibit microglia activation and normalize altered cytokine levels after MCAO. We performed the same experimental protocol as in Experiment 1 but euthanized animals 3 or 24 hours after MCAO and processed the brains for immunohistochemistry for TNF-α, high mobility group box protein 1 (HMGB1), IL-1β, IL-6, and microglial markers Iba1 and CD68. There were four experimental groups: treatment at 3 hour euthanasia (377.25 ± 31.61 g; n = 4), control at 3 hour euthanasia (356.25 ± 28.45 g; n = 4), treatment at 24 hour euthanasia (390.75 ± 2.63 g; n = 4), control at 24 hour euthanasia (378.00 ± 12.19 g; n = 4). We have chosen bright-field immunohistochemistry to detect inflammation markers because it allows examination of the morphology of tissue samples and hence provides spatial information on the effect of nVNS on cytokine protein levels.
4. *nVNS is effective when initiated late after MCAO:* First, we tested the effect of nVNS initiated 4 hours after MCAO on functional and tissue outcome. There were two experimental groups: treatment (398.67 ± 16.12 g; n = 9) and control (391.38 ± 6.48 g; n = 8). Depending on the observed effect size at 4 hours, we planned to repeat experiments by changing the therapeutic window up and down by 1 hour each time until a comparable effect size with 30 minute stimulation was achieved (i.e., at 3 hours after ischemia if 4 hour treatment window failed to show a neuroprotective effect or at 5 hours after ischemia if 4 hour treatment was as effective as the treatment at 30 minutes after ischemia).

Surgical preparation

Animals were anesthetized using isoflurane (induction: 4–5% in 30% oxygen–70% nitrous oxide, maintenance: 1–2% in room air). Buprenorphine HCl (0.05 mg/kg; sc) and bupivacaine (8 mg/kg; intraincisional) were injected to alleviate pain. Rectal temperature was monitored continuously during the anesthesia period and maintained at 37.0 °C by a homeothermic blanket system (Harvard Apparatus, Holliston, MA). Temporalis muscle temperature was monitored continuously using a T-type thermocouple probe and pod (ADInstruments, Colorado Springs, CO) and kept at 36.0 °C by a heating lamp. The right femoral artery was cannulated for continuous ABP and HR monitoring (ADInstruments) as well as intermittent ABGs and pH measurements (Rapidpoint 300 blood gas system, Siemens Healthcare Diagnostics Inc., Tarrytown, NY). Regional cerebral blood flow (rCBF) was recorded over the right parietal cortex (5 mm lateral and 1 mm posterior to bregma) using laser Doppler flowmeter (ADInstruments) to verify induction of MCAO, as described before [6,15].

MCA occlusion

A midline neck incision was performed to expose the common, external, and internal carotid arteries. All the visible sections of the external and internal carotid arteries and a 2–3 mm segment of the common carotid artery before the bifurcation were isolated from the surrounding tissues. Caution was applied to avoid injury to the superior cervical ganglion, sympathetic trunk, and vagus nerve during dissection of the carotid arteries. Ischemia was induced by intraluminal filament occlusion of the right MCA for 2 hours by a silicone-coated nylon monofilament (diameter: 0.39 ± 0.02 mm or 0.41 ± 0.02 mm; Doccol Corporation, Redlands CA) as reported before [16]. The filament was introduced from the carotid bifurcation. Occlusion was confirmed when ≥60% drop in baseline rCBF was detected. The skin incision was sutured after the occlusion to allow transcutaneous stimulation.

nVNS treatment

We used an experimental non-invasive stimulator developed exclusively for cervical vagus nerve stimulation (gammaCore; electroCore, LLC). The stimulator consisted of two surface disc electrodes (6 mm in diameter) separated by 6 mm. Skin was shaved prior to stimulation, and a conducting gel (Signa gel, Parker Laboratories, Fairfield, NJ) was applied to the electrodes to allow for good electrical contact with the skin. Electrodes were placed on the skin overlying the right cervical vagus nerve (between mid to lower portions of the neck, parallel to the anterior margin of the sternocleidomastoid muscle) without applying any extra mechanical pressure. Electrical stimulation (1 msec duration, 5 kHz, 7.2V sine waves repeated at 25 Hz; impedance: 350 ohm) was delivered in the form of 2-minute trains, every 10 minutes, for a period of 1 hour [17]. In control animals, electrical stimulation was delivered into the skin overlying the right quadriceps femoris muscle using the same stimulation parameters. We selected the quadriceps femoris muscle because it provided sufficient contact area for the surface electrodes to achieve selective muscle stimulation in rats. In a pilot study, we demonstrated that electrical stimulation of the skin overlying the ipsilateral quadriceps femoris was not associated with any neuroprotection/increased cerebral blood flow in rats. The infarct

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