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Original contribution

Evaluation of neuregulin-1's neuroprotection against ischemic injury in rats using diffusion tensor imaging

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ABSTRACT

Stroke is a devastating neurovascular disorder that results in damage to neurons and white matter tracts. It has been previously demonstrated that neuregulin-1 (NRG-1) protects neurons from ischemic injury following stroke. Here, diffusion tensor imaging (DTI) was utilized to characterize the effects of NRG-1 treatment on cererbral infarction and integrity of white matter after ischemic insult using a permanent middle celebral artery occlusion (pMCAo) rat model. In the present study, sixteen Sprague-Dawley rats underwent pMCAo surgery and received either a single intra-arterial bolus (20 μg/kg) dose of NRG-1 or saline immediately prior to pMCAo. MRI including T2-weighted imaging and DTI was performed in the first 3 h post stroke, and repeated 48 h later. It is found that the stroke infarction was significantly reduced in the NRG-1 treated group. Also, NRG-1 prevented the reduction of fractional anisotropy (FA) in white matter tracts of fornix and corpus callosum (CC), indicating its protection of CC and fornix white matter bundles from ischemia insult. As a conclusion, the present DTI results demonstrate that NRG-1 has significantly neuroprotective effects in both cerebral cortex and white matter including corpus callosum and fornix during acute stroke. In particular, NRG-1 is more effective on stroke lesion with mild ischemia. As CC and fornix white matter bundles play critical roles in transcallosal connectivity and hippocampal projections respectively in the central nervous system, the findings could provide complementary information for better understanding the biological mechanism of NRG-1's neuroprotection in ischemic tissues and neurobehavioral effects.

1. Introduction

Stroke is one of the most common causes of death and leading cause of severe adult long-term disability worldwide, and about 2% people have a stroke each year in the United States [1]. Among them, about 80% of all strokes are caused by focal cerebral ischemia due to arterial occlusion [2,3]. Ischemic stroke occurs when the blood supply to the brain is obstructed. Recombinant Tissue plasminogen activator (rt-PA) is the only FDA-approved medication for acute ischemic stroke in clinic. Unfortunately, rt-PA has a very limited time window for therapeutic use (within 3–4 h after the onset of symptoms), only 3–5% of stroke patients may qualify for the rt-PA treatment [4]. In addition, rt-PA can cause severe complications including increased risk of intracranial hemorrhage [5]. Thus, there is a critical need to develop more effective methods of treatment for stroke disease.

Neuregulin 1 (NRG-1) comprises a family of growth factors, including acetylcholine receptor inducing activities (ARIAs), glial growth factors (GGFs), heregulins and neudifferentiation factors (NDFs) [6], that performs a wide variety of functions in the normal development of the nervous system and heart [7]. NRG-1 has been shown to be neuroprotective in rat brains following cerebral ischemia [8–10]. NRG-1 administration also resulted in a significant improvement of neurological function in animals following ischemic stroke [11–14]. Most studies of the neuroprotection in rodent stroke models have focused on the protection of gray matter. However, white matter injury is observed after ischemic insult and the discrepancy between the infarct volume and functional outcome in stroke patients is suggested to be partly due to the contribution of white matter degeneration after stroke onset [15,

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16]. Recent studies suggest that structures in white matter play a critical role in stroke recovery, especially in function recovery by brain remodeling [17–19].

Conventional MRI and diffusion-weighted imaging (DWI) can provide valuable information for early detection of ischemic brain damage and identifying stroke lesion volume and territory [20–25]. Diffusion tensor imaging (DTI) allows for the non-invasive measurement of *in vivo* 3D diffusion of water molecules and has been demonstrated to be a promising noninvasive method to access the brain injury and white matter integrity [26, 27]. Quantitative analysis of DTI has shown promising to evaluate pathological changes in infarct regions and white matter bundles within stroke lesion [28–33]. Mean diffusivity (MD) or Apparent Diffusivity Coefficient (ADC) is commonly used as biomarkers to evaluate the brain injury in acute stroke [34–37]. The temporal evolution of the DTI indices in different brain regions including cortex, subcortex, and corpus callosum has been investigated systematicly in stroke rats [30], showing a different evolution pattern in corpus callosum, cortex, and subcortex after 90-min temporary ischemic stroke. The MD and fractional anasotropy (FA) changes in cortical and subcortical regions after 60-min ischemic stroke in rat brains were reported previously [38]. Also, the temporal changes of MD, FA, and transverse relaxation time (T2) in lesion areas after 3-hour transient and permanent occlusion were examined using a macaque model of stroke [28]. The prior studies suggested DTI is a novel and robust approach to evaluate the ischemic stroke injury non-invasively during clinical diagnosis and pharmaceutical discovery in stroke disease. Also, the white matter integrity changes during acute and chronic stages of stroke disease have been evaluated in recent studies of stroke patients [18,39–46], indicating white matter integrity alterations are closely associated with the functional impairment and recovery after stroke.

It has been demonstrated that neuregulin-1 (NRG-1) protects neurons from ischemic injury following stroke in previous pathological studies [12,14] and volumetric MRI analysis in which significant reduction of infarct volume and growth rate was observed in NRG-1 treated animals [47]. In the present study, DTI was used to further evaluate the neuroprotective effects of NRG-1 on ischemic infarction and white matter fibers using an experimental rat model of ischemic stroke.

2. Materials and methods

2.1. Animal model preparation

All experimental procedures followed the protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Emory University in accordance with the NIH Guide for Care and Use of Laboratory Animals. Male adult Sprague–Dawley rats (230–270 g) were used in this study and left intraluminal permanent middle cerebral artery (MCA) occlusion (pMCAo) was induced. Briefly, rats were anesthetized using isoflurane (3% for induction and 2% for maintenance) mixed with O_2 (100%) and administrated with a nose cone. The left common carotid artery (CCA) was exposed through a midline incision and was carefully dissected free from surrounding nerves and fascia. The internal carotid artery (ICA) was isolated and carefully separated from the adjacent vagus nerve, and the pterygopalatine artery was ligated close to its origin with a 6–0 silk suture. Then, a 40 mm 4–0 surgical monofilament nylon suture coated with a rubber silicone tip (diameter 0.37 mm, length 2.3–2.5 mm) was inserted from the external carotid artery (ECA) into the internal carotid artery (ICA) and then into the circle of Willis to occlude the left MCA [10,11].

Heart rate, respiratory rate, EtCo2, SpO2 were monitored using a SurgiVet capnometer (with oximeter) (Smiths Medical PM, Inc., Norwell, MA) during surgery, and with the Mouse-Ox system (Starr Life Science, Oakmont, PA) and a SurgiVet capnometer during MRI scans. The rectal temperature was monitored with a Diqi-Sense thermometer and maintained at 36.7–37.3 °C with a heated circulating water bath (Homeothermic Blanket Control Unit, Harvard Apparatus, Hollister, MA) during the surgery and MRI scans. Regional cerebral blood flow (CBF) was measured by continuous laser Doppler flowmetry with a laser Doppler probe (Perimed, Ardmore, PA) placed in the ipsilateral skull close to MCA from the beginning of the MCAo surgery.

2.2. NRG-1 administration

To determine the neuroprotective efficacy of NRG-1 on ischemic stroke, rats were injected intra-arterially with a single bolus 50 μl dose of vehicle (1%BSA in PBS) or NRG-1β (20 μg/kg, EGF-like domain, R&D Systems, Minneapolis, Minnesota) through a Hamilton syringe as previously described [12]. NRG-1 ($n = 10$) or vehicle ($n = 6$) was administered to rats by bolus injection into the ICA through ECA immediately before MCAo. It has been previously shown that NRG-1 did not significantly affect physiological parameters, including pH, pCO_2, PO_2, hematocrit, Na^+, K^+, Ca^{2+}, heart rate, mean arterial pressure and blood pressure following MCAo [9]. All NRG-1 and vehicle treatment studies were performed in a blinded manner.

2.3. In vivo MRI data acquisition

MRI was performed using a 7T animal MRI scanner (Bruker BioSpin, Billerica, MA) and a home-made surface coil (ID = 3 cm) for transmission and receiving. The rats were placed in the prone position on a custom-made head holder with ear bars and teeth bars to minimize head motion while under spontaneous respiration. Rats were anesthetized using isoflurane/O_2 (3% for induction and 1.5% for maintenance). All rats were imaged immediately after surgery from 0.5 h to 4 h and rescanned 48 h post-surgery. Coronal MRI sections were collected from 2 mm anterior to the corpus callosum and the end of the cerebrum. T2-weighted imaging (T2WI) images were acquired with the following parameters: field of view (FOV) = 3.0 × 3.0 cm^2, matrix size = 256 × 256, repetition time (TR) = 1000 ms, echo time (TE) = 50 ms, slice thickness = 1.0 mm. DTI images were acquired with a four-shot EPI sequence at 0.5 h, and repeated at 1, 2, 3, and 48 h post stroke. The DTI parameters were: TR = 3000 ms, TE = 32 ms, Δ = 20 ms, δ = 4 ms, field of view = 3.0 × 3.0 cm^2, slice thickness = 1.0 mm, matrix size = 128 × 128, in-plane image resolution = 250 × 250 μm^2, NEX = 4, 30 gradient directions, b values = 0, 1000 s/mm^2. The MRI scan lasted 3.5 to 4 h in surgery day and up to 2 h at 48-hour scan.

2.4. MRI data processing

DTI images were processed using DTI Studio v2.4 [48] (Johns Hopkins University, Baltimore, MD). T2-weighted images were used to idenfity the infarction and assist the definition of ROIs during data analysis. Furthermore, the NRG-1 treated rats were divided into mild and severe subgroups in order to evaluate the NRG-1's effects on the ischemic severity which was based upon the CBF reduction during MCAo surgery (mean CBF reduction = 76%; mild: < 70% CBF reduction; severe: > 70% CBF reduction) as suggested by previous report [47].

To determine the stroke lesion area, the ROI was identified by referencing DWI and MD maps firstly. Then the ROIs were duplicated on the FA and MD maps. The stroke lesion and total brain area in every slice was manually measured by ImageJ 1.34 (National Institutes of Health, Bethesda, Md). The total stroke volume was calculated as the sum of the lesion area across all slices, multiplied by the total slice thickness.

FA and MD of the infarction lesions were calculated as the weighted average in each infarction using the following eq. (FA is used as an example):