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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 cerebral infarction and integrity of white matter after ischemic insult using a permanent middle cerebral 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 neuregulin-1 (NRG-1) [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 systematically 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 anisotropy (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 O₂ (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 Digi-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, pCO₂, PO₂, hematocrit, Na⁺, K⁺, Ca²⁺, 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/O₂ (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 cm², 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 cm², slice thickness = 1.0 mm, matrix size = 128 × 128, in-plane image resolution = 250 × 250 µm², NEX = 4, 30 gradient directions, b values = 0, 1000 s/mm². 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 identify 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):

Fig. 1. Regions of interests (ROIs) are illustrated on FA maps of a rat brain. 1) external capsule (EC), 2) corpus callosum, 3) fornix, 4) internal capsule (IC).

Fig. 2. Stroke infarction in the vehicle and NRG-1 treatment groups are demonstrated on the T2WI, FA, and MD maps of representative rat brains 3 h and 48 h post occlusion. In vehicle group, the ischemic lesion occupied both cortex and striatum and the lesion expanded into almost entire hemisphere with dramatic FA reduction in external capsule (EC) 48 h post occlusion (arrow). In NRG-1 treatment group, the ischemic infarct was always observed in the striatum area whereas no obvious cortical infarction was only observed in those with mild CBF. External capsule is seen in each group 3 h post stroke. At 48 h post stroke, it is only seen in NRG-1 treated rats with mild ischemia but not in other groups. (For interpretation of the references to colour in this figure legend, the reader is referred to

the web version of this article.)

$$FA = \frac{\sum_i (FA_i \times Area_i)}{\sum_i Area_i}$$

We calculated the weighted average because this is mathematically identical to calculating the average of FA and MD values directly from all voxels within the ROI for an infarction lesion.

To evaluate the white matter injury under the ischemic insults, we assessed the multiple white matter tracts in the ipsilateral side, including corpus callosum (CC), external capsule (EC), fornix and internal capsule (IC) (Fig. 1) and compared with those in the contralateral side using the DTI data at 3 and 48 h post stroke. The region-of-interest (ROIs) were identified by referencing FA maps and T2-weighted structural images. Then the ROIs were placed on the corresponding slices of MD maps.

2.5. Histopathology evaluation

Rats were euthanized immediately after their last MRI scans (48 h following ischemia). The brains were fixed in 4% formaldehyde overnight and then dehydrated in 30% sucrose in 1xPBS. Coronal brain sections (20 μm) were used for GFAP and Fluoro JadeB (FJB; Chemicon) labeling according to the manufacturer's protocols. Also, in order to validate stroke infarction in whole brains, the 1.0 mm-thick brain

sections were stained with 2% triphenyltetrazolium chloride (TTC) solution and then transferred into a 4% formaldehyde solution for fixation.

2.6. Statistical analysis

All results were expressed as mean ± standard deviation (SD). The paired *t*-test was applied to evaluate differences between the ipsilateral and contralateral DTI indices in vehicle and treatment groups. A one-way analysis of variance (ANOVA), followed by the Tukey test, was applied to analyze the statistical differences in DTI values of vehicle and NRG-1 treated animals. All statistical analysis procedures were performed using the SPSS for Windows statistical package (Version 18, SPSS, Chicago, IL). A *p*-value < 0.05 was considered statistically significant.

3. Results

3.1. Stroke volume outcome after NRG-1 treatment

MCAo produced a large infarct in the ipsilateral brain following ischemia and the lesion size was significantly reduced in the NRG-1 treated group. The protective effect is more evident in the treatment group with mild ischemia (Fig. 2). The spatio-temporal volumetric

Table 1

Temporal evolution of fractional anisotropy (FA) and mean diffusivity (MD) in the infarction regions of rat brains with/without neuregulin-1 (NRG-1) treatment.

DTI indices	Groups	0.5 h	1 h	2 h	3 h	48 h
FA	Vehicle	0.35 ± 0.06 ^a	0.33 ± 0.05 ^a	0.37 ± 0.06 ^a	0.34 ± 0.07 ^a	<u>0.17 ± 0.03^b</u>
	NRG-1(severe ischemia)	0.33 ± 0.08 ^a	0.36 ± 0.08 ^a	0.37 ± 0.10 ^a	0.35 ± 0.12 ^a	0.26 ± 0.05^b
	NRG-1(mild ischemia)	0.43 ± 0.03 ^a	0.42 ± 0.06 ^a	0.43 ± 0.09 ^a	0.45 ± 0.09 ^a	<u>0.27 ± 0.05^b</u>
	Vehicle	0.57 ± 0.05 ^a	0.54 ± 0.03 ^a	0.50 ± 0.03 ^a	0.58 ± 0.05 ^a	<u>0.73 ± 0.11^b</u>
MD (μm ² /ms)	NRG-1(severe ischemia)	0.60 ± 0.08	0.58 ± 0.08	0.58 ± 0.08	0.53 ± 0.09 ^a	<u>0.69 ± 0.08^b</u>
	NRG-1(mild ischemia)	0.71 ± 0.11	0.62 ± 0.12	0.61 ± 0.13	0.56 ± 0.13	0.66 ± 0.09

Values in **bold**: significant differences ($p < 0.05$) between NRG-1 treated group and vehicle group.Underlined values: significant differences ($p < 0.05$) between the time points of a and b.

Values are shown as mean ± SD.

changes of infarction in those rats during the first 3 h and at 48 h has been reported previously [47]. In the vehicle-treated group, the initial lesion accounted for $7.5 \pm 2.8\%$ of total brain volume. In contrast, it was only $3.8 \pm 1.0\%$ in NRG-1 treatment group. At 48 h post occlusion, the stroke lesion in vehicle group increased to 42% of total brain volume vs. 21% in NRG-1 treatment group ($p < 0.05$). The infarct volume difference on 48 h post stroke between vehicle and NRG-1 treatment groups was 50% ($p < 0.05$), indicating significant neuroprotective effects of NRG-1 on stroke injury.

3.2. Quantitative DTI of ischemic infarct

The temporal evolution of FA and MD at infarction areas of rat brains in vehicle and treatment groups were illustrated respectively in Table 1. As seen in Table 1, no evident difference was seen between the temporal changes of FA in vehicle and treatment groups (with severe and mild ischemia) during the first 3 h post stroke. At 48 h post occlusion, substantial FA reduction was seen in every group, compared to that at 3 h post stroke. However, FA value in the treatment groups was significantly higher than that in vehicle group (0.26 or 0.27 vs. 0.17, $p < 0.05$) at 48 h post stroke.

Significantly MD increase or pseudo-normalization was observed in vehicle and severe ischemia groups at 48 h post stroke by comparing to that at 3 h time points, but not in the NRG-1 treatment group of rats with mild ischemia (Table 1). In addition, the tendency of mild MD decrease during 0.5–3 h post stroke was observed in each treatment group while MD recovery was seen at 3 h time point in vehicle group.

3.3. Quantitative DTI of white matter bundles after NRG-1 treatment

FA and MD changes of white matter bundles after MCAo in the NRG-1 treatment and vehicle groups are illustrated in Figs. 3 and 4, respectively. No significant FA reduction was observed in 0.5–3 h following ischemia in EC, CC, fornix, and IC (all $p < 0.05$) in the vehicle and NRG-1 treatment groups. At 48 h post occlusion, significantly reduced FA values were seen in those ROIs in the vehicle group (all $p < 0.05$). NRG-1 prevented the ischemia-induced reduction of FA seen in the CC and fornix at 48 h treated group.

In the vehicle group, no significant MD changes were seen in CC and fornix at any time points in all groups. Significant MD reduction at IC was observed only at 48 h. Interestingly, MD at EC started declining as early as 1 h post stroke, and kept decreasing until 48 h (the entire observation period) post stroke.

In contrast, significant MD reduction in the NRG-1 treatment group was only seen in EC at 48 h post stroke, but not in other ROIs of white matter fibers. This indicates that NRG-1 prevents ischemia-induced MD reduction in the IC and at early stages in the EC.

3.4. Histological evaluation

The stroke lesions in treatment and vehicle rats on 48 h post occlusion were demonstrated with the TTC (Fig. 5) and

immunohistological results (Fig. 6). Significant infarct reduction is observed in cortex and partial subcortex areas (Fig. 5). The representative TTC staining and corresponding T2-weighted images exhibit the EC bundles are well delineated in NRG-1 treated rats but degenerated in the vehicle treated animals (Fig. 5).

Also, distributions of GFAP positive cells were decreased in the cortex after MCAo and vehicle treatment (Fig. 6A). NRG-1 treatment prevented the reduction of GFAP-positive cells in the cortex by ischemia (Fig. 6B). In vehicle group, FJB positive cells were widely distributed among the cortex indicating the degenerating neurons (Fig. 6C). However, only a few FJB positive cells could be observed at the cortex in the NRG-1 treatment group (Fig. 6D).

4. Discussion

The neuroprotective effect of NRG-1 in both brain grey matter and white matter with ischemic injury was evaluated longitudinally in the present study. NRG-1 treated rats have significant reduced stroke lesion compared to those in vehicle group. Meanwhile, NRG-1 prevented the degeneration of white matter tracts of corpus callosum and fornix after stroke insult.

NRG-1 demonstrates evident neuroprotective effects mostly because it may involve the inhibition of apoptotic and pro-inflammatory responses [8,14], and prevent mononuclear infiltration, astrocyte activation and cytokine production, which are known to be associated with delayed neuronal death following ischemia [8,14]. In addition, the therapeutic window for NRG-1 to treat stroke can be > 12 h as observed in rat MCAo models [9].

MD reduction is usually seen immediately after stroke insult and continues during hyper acute and acute phases, and then starts recovery during subacute phase and MD pseudonormalization is seen thereafter [28,49]. As demonstrated in the present study, NRG-1 prevented the MD reduction during acute stroke and temporally delayed the MD pseudonormalization process in the subacute stage.

Dynamic change in MD reflects the water distribution between extra- and intracellular space [50]. Decreased MD in ischemic brain is commonly explained as water influx from extra cellular to intra cellular space due to failure of $\text{Na}^+ - \text{K}^+$ ATP pump, namely cytotoxic edema [51]. In particular, MD values may be used to characterize the border of penumbra which can be useful to monitor severity of ischemic lesion and assess the treatment response of neuroprotective approaches [52,53]. Our previous reports have demonstrated NRG-1 could reduce the infarct volume substantially after stroke insult. However, the present MD findings provide complementary biological information of the ischemic tissues during the NRG-1 treatment.

Water diffusion in white matter is highly sensitive to its microstructural architecture with components associated to the myelin sheath, axonal transportation, and direction of neural fibers. There are contradictory results with regards to FA changes during acute stroke. Yang et al. analyzed patient data of acute ischemic stroke, and found FA values could increase or decrease [54]. Reduction in FA has been also found in animal studies with ischemic stroke [38,50]. However, initial

Fig. 3. Progressive FA changes at multiple white matter tracts including corpus callasum (CC), Fornix, internal capsule (IC), external capsule (EC) in NRG-1 treatment and vehicle groups during the acute phase (0.5–3, and 48 h post stroke). Blue bar: ipsilateral ischemic white matter. Red bar: contralateral white matter. * $p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

elevation of FA values has been observed during the first few hours of ischemic insults in a pMCAO animal model, then the FA values began to decline. Such initial increase of FA may reflect sudden cessation of motion/flow or myelin swelling [54].

Decreased FA is usually seen during subacute and chronic stroke due to the disruption of white matter microstructure. As shown in the present study, significantly decreased FA at 48 h post occlusion was observed in the CC, fornix, IC, and EC in vehicle group, whereas no such FA changes were observed in CC and fornix in NRG-1 group, indicating NRG-1 protected CC and fornix white matter bundles from ischemia but not IC and EC. The CC is the largest commissural fiber system in the brain and places an essential role in interhemispheric communication [55,56]. Also, its status may be associated with the degree of motor function and neurological deficit in stroke patients [57] and the gait function in the elderly [58]. In addition, the hippocampus plays a critical role in memory function [59]. The fornix consists of fibers arising from the hippocampus. Fornical microstructural alteration has been reported in subjects with hippocampal lesion [60] or Alzheimer's disease [61] and is associated with impaired memory.

Therefore, the integrity status of CC and fornix fiber bundles may provide complementary information for understanding the biological mechanism of NRG-1 neuroprotection following stroke. In particular, the finding may be more useful for interpreting functional and neuro-behavior deficits in non-human primate stroke models or clinical trials in which subjects have more abundant white matter bundles in the brain and more advanced neurological examination is performed usually, compared to rodent studies.

Interestingly, progressive MD decrease was seen from 1 to 3 h and 48 h post stroke only in the EC of the vehicle group but not other fiber bundles. MD reduction has been observed in acute white matter injury [62]. Currently most studies focus on grey matter injury in stroke. There are few investigations on white matter bundles during hyper-acute and acute phase. The present result suggests each bundle may be affected differently in the ischemic regions during stroke evolution. As white matter plays critical role in information transfer in the central nervous system and brain remodeling, more studies are needed to evaluate the situation.

NRG-1 (also known as Neucardin) has been shown to be efficacious

Fig. 4. Progressive MD changes at multiple white matter tracts including corpus callasum (CC), Fornix, internal capsule (IC), external capsule (EC) in NRG-1 treatment and vehicle group during the acute phase (0.5–3, and 48 h post stroke). Blue bar: ipsilateral ischemic white matter. Red bar: contralateral white matter. * $p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 5. Illustration of stroke infarction in rats with/without NRG-1 treatment and stained by 2,3,5-triphenyltetrazolium chloride (TTC) matching the regions of infarction on T2-weighted images at 48 h after pMCAo.

Fig. 6. Histological evaluation of stroke rat brain cortex. Distributions of GFAP positive cells were decreased in the cortex of vehicle treated rats after MCAo (A). The NRG-1 treatment prevented the reduction of GFAP positive cells in the cortex after ischemia (B). After MCAo and vehicle treatment, FJB positive cells were widely distributed among the cortex indicating the degenerating neurons (C). However, only a few FJB positive cells could be observed at the cortex in the NRG-1 treatment group (D).

in treating human patients with congestive heart failure (<http://zensunusa.com/research/index2.aspx>). Zensun USA performed phase I and phase II clinical trials in China (Chinese Clinical Trial: ChiCTR-TRC-00000414), Australian and New Zealand (Clinical Trials Registry: ACTRN12607000330448) and the U.S. (ClinicalTrials.gov: NCT01251406), demonstrating that NRG-1 is safe, tolerable and significantly improved cardiac function in patients with chronic heart failure [63]. Three additional clinical trials to determine the ability of NRG-1 to improve cardiac function after heart failure and cardiac arrest have been initiated in the U.S. (ClinicalTrials.gov: NCT01541202; NCT01258387; NCT01944683). The doses of NRG-1/Neucardin used for heart failure are similar to those used for stroke, suggesting the feasibility for developing clinical trials for stroke in the near future.

There are some limitations in the present report. Firstly, a permanent MCAO model was used and NRG-1 was administrated prior stroke. The major aim of the study is to investigate the protective effects of NRG-1 on white matter damage post stroke. In comparison to the conventional transient model of stroke, the permanent MCAO model allows the infarcted core expanded into peripheral regions of the MCA territory to reach its maximal infarct size quickly during acute stroke, resulting in substantial white matter damage. Therefore, the permanent MCAO model was utilized to examine the neuroprotective mechanism of NRG-1 on stroke-induced white matter damage in the present study. As NRG-1 must be delivered intra-arterially (i.a.) for its maximal efficacy, it was given prior pMCAo in this study accordingly. The neuroprotective effect of NRG-1 on stroke lesion has been demonstrated

previously using a transient MCAO model of rat, in which the cortical infarct volume could be reduced by 90% when administered immediately following stroke insult and extended therapeutic window (> 13 h) was seen [8,9]. Even through a permanent MCAO model was used in the present report, it still provides additional information for understanding NRG-1's protective effects on white matter injury in future clinical trials. Secondly, DTI data was acquired with a regular single-shell protocol and the typical DTI indices (FA and MD) were assessed to evaluate the integrity of the white matter post stroke. In comparison to conventional DTI, multi-shell DTI with neurite orientation dispersion and density imaging (NODDI) strategy can provide additional parameters such as neurite density index (NDI), orientation dispersion index (ODI) which can explicitly estimates orientation dispersion and neurite density [64]. Recent studies indicate that NODDI is superior than traditional DTI to detect white matter changes in brain maturation and multiple sclerosis patients [65,66], and should be explored in future stroke studies.

5. Conclusions

We have demonstrated the neuroprotective effects of NRG-1 for ischemic brain injury in cerebral cortex and white matter bundles using DTI. These results could provide important information for understanding the biological mechanism and neurobehavior effects of the NRG-1's protective effects in ischemic brains and clinical practice in regarding to optimizing treatment strategy. As neuregulins have been

implicated in normal brain function, as well as in neuroprotection following cerebral ischemia, NRG-1 represents a novel neuroprotective treatment that has potential therapeutic value in treating individuals after acute ischemic stroke.

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Disclosure

Byron Ford owns patents that are held by Brain-Gen, LLC without direct corporate involvement at the time.

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