CHARACTERIZATION OF THE ISCHEMIC PENUMBRA USING DIFFUSION TENSOR MR IMAGING IN A RAT MODEL OF ISCHEMIC STROKE TREATED WITH NEUREGULIN-1

Silun Wang[1], Yonggang Li[2], Ramesh Paudyal[1], Byron D. Ford[2], and Xiaodong Zhang[1]
[1]*YERKES IMAGING CENTER, Emory University, Atlanta, GA, United States,* [2]*Department of Neurobiology, Morehouse School of Medicine, GA, United States*

Target audience: MRI scientists, Radiologists and experimental neurologists.

Purpose: As one of the most common cause of adult long-term disability and death worldwide, 2% people will have a stroke each year in the United States (1). Neuregulin 1 (NRG-1) is a growth factor with multiple potent effects, such as acetylcholine receptor inducing activities (ARIAs), glial growth factors (GGFs), heregulins and neuro differentiation factors (NDFs) (2). Recent studies have demonstrated that NRG-1 is neuroprotection in rat brains following cerebral ischemia (3-5). Diffusion tensor imaging (DTI) has been demonstrated to be a promising noninvasive method to assess the white matter integrity after stroke insult. In the present study, we hypothesized that DTI indices could be applied as imaging biomarkers to evaluate the effects of NRG-1 treatment response in a rat model with permanent middle cerebral arteries occlusion (pMCAo) as well as to detect microstructural changes in penumbra and infraction core after stroke.

Methods: A***nimal model preparation:*** Adult Sprague–Dawley rats weighing 230–270 g were used for this study. pMCAo was induced with a 40 mm 4-0 surgical monofilament nylon suture coated with rubber silicone. ***NRG-1 treatment:*** Rats were injected intra-arterially with a single bolus 50 µl dose of vehicle (1%BSA in PBS) or NRG-1β ((20ug/kg, R&D Systems, Minnesota) through a Hamilton syringe. NRG-1 (n=10) or vehicle (n=6) treated rats were administered by bolus injection into the ICA through ECA immediately before MCAo. ***MRI scanning and data analysis:*** In vivo MRI was performed using a 7T animal MRI scanner (Bruker BioSpin MRI, Billerica, MA) and a surface coil (internal diameter=2.5cm). All rats were imaged immediately after surgery from 0.5 hours (hr) to 3 hr and at 48 hr post surgery. T2-weighted imaging (T2WI) were acquired with the following parameters: field of view (FOV)=3.0 x 3.0 cm2, matrix size=256×256, repetition time (TR)=1000 ms and echo time (TE)=50 ms, slice thickness=1.0 mm. DTI were acquired with a four-shot EPI sequence. The imaging parameters were: TR=3000 ms, TE=32 ms, Δ=20 ms, δ=4 ms, field of view=3.0 x 3.0 cm2, slice thickness=1.0 mm, matrix size=128×128, image in-plane resolution=250×250 µm2, NEX=4, 30 gradient directions, b value= 1000 s/mm2, respectively. ADC and FA maps were derived for quantitatively analyze by using DTIstudio v2.4. DTI indices were analyzed by ROI drawn over ischemic lesion using Image J (NIH, U.S). Paired t-test was used to detect statistical differences of DTI indices between ipsilateral/contralateral brain tissue. ***Histopathology evaluation:*** Rats were sacrificed for histological evaluation immediately after their last MRI scanning. Brain sections were washed in PBS and incubated with Cy3 conjugated anti-NeuN (1:500, Millipore) or Cy3 conjugated anti-GFAP (1:500, Millipore) overnight at 4 °C.

Results: 1) ***NRG-1 treatment group showed smaller stroke lesion*** (Fig 1): In the vehicle group, the ischemic region was observed at both striatum (100%) and cortex (83%, 5/6) as early as 0.5 hr post occlusion. NRG-1 treated rats showed the ischemic lesions mainly at striatum (70%, 7/10). The stroke volumes of vehicle group were significantly larger than NRG-1 treated group. In the vehicle-treated group, the initial lesion was occupied around 7.5 % of total lesion, whereas only 3.8% in NRG-1 treatment group. At 48 hrs post occlusion, the stroke lesion in vehicle group increased to 42% of total brain volume vs. only 21% of total brain lesion in NRG-1 treatment group ($P<0.05$). The difference in infarct volume on 48 hrs between vehicle and NRG-1 treatment groups was 50 % ($P < 0.05$). These results indicated a significantly neuroprotective effects of NRG-1. 2) ***DTI index changes in penumbra and infarct core:*** ADC values in penumbra were significantly higher than those in infarct core (all $p<0.05$) but significantly lower than in contralateral cortex (all $p <0.05$). There were no significant FA changes in vehicle group until 48 hr post occlusion, where FA values in infarct regions were significantly lower than in contralateral normal cortex (0.15±0.02 vs. 0.19±0.01, p=0.03). Also, no significant FA changes were observed in NRG-1 treatment groups until 48 hr post occasion (all $p>0.05$). ***Histological evaluation:*** (Fig 2) **Penumbra, infarct core:** Distributions of GFAP positive cells were significantly decreased in the infarct core indicating the tissue infarction and necrosis. FJB positive cells widely distributed among the cortex indicating the degenerating neurons. In penumbra, the rat brain showed normal GFAP positive cells. In addition, NeuN positive neuron showed morphological changes of cell shrinked and chromatin condensed indicating the apoptosis. **White matter tracts:** There was severe tissue necrosis in the external capsule in both groups. In contrast, the white matter tracts on internal capsule, corpus callosum and Fornix seem not affected and with smaller necrotic lesion. **Cortex:** In vehicle group, FJB positive cells were widely distributed among the cortex indicating the degenerating neurons. However, only a few FJB positive cells could be observed at cortex in the treatment group. Distributions of GFAP positive cells were significantly decreased in the cortex of rats in vehicle group. The NRG-1 treated rat brain showed normal GFAP positive cells in the cortex.

Discussion: We have demonstrated that DTI indices could provide promise diagnostic information to evaluate brain injury in the animal model of ischemic stroke treated by NRG-1 as well as to characterize the penumbra evolution after stroke onset. ADC values in penumbra were significantly higher than infarct core but significantly lower than those in contralateral cortex. In addition, the neuroprotective effects could be in vivo monitored by DTI. NRG-1 seems to have significant neuroprotective effects to white matter and cortex especially at acute period post occlusion.

References : 1.Lopez, A.D., et al., Lancet, 2006. 367(9524): p. 1747-1757. 2.Falls, D.L., et al., Cell, 1993. 72(5): p. 801-815. 3.Xu, Z.F., et al., Biochemical and Biophysical Research Communications, 2004. 322(2): p. 440-446. 4. Xu, Z.F., et al., Journal of Cerebral Blood Flow and Metabolism, 2006. 26(4): p. 527-535. 5. Li, Y.G., et al., Brain Research, 2007. 1184: p. 277-283.

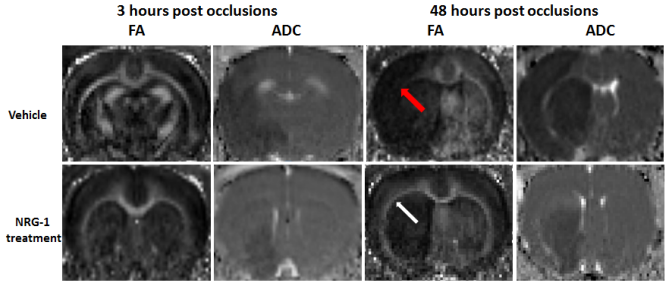

Fig 1: In vehicle group, the ischemic lesion occupied both cortex and stratum. On 48hr, the lesion expand to almost half of brain with decreased signals of EC on FA map (red arrow). In NRG-1 treatment group, the ischemic region was only observed in the striatum whereas no obvious ischemic region was found in the cortex on both 3h and 48h post occlusion without obvious signal reductions in the EC on FA map (red arrow).

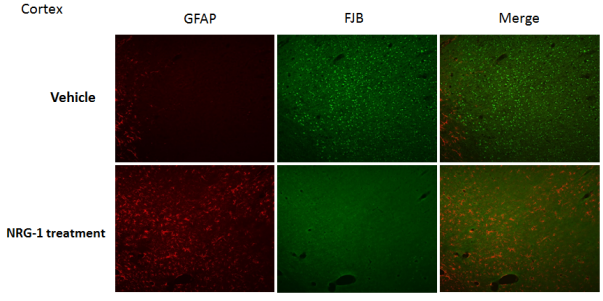

Fig 2: In vehicle rats, FJB positive cells were wildely distributed among the cortex indicating the degenerating neurons. However, only a few FJB positive cells did not show at cortex in the treatment group. Distributions of GFAP positive cells were significantly decreased. The NRG-1 treated rat brain showed normal GFAP positive cells in the cortex.