ASSESSMENT OF NEUROPROTECTIVE EFFECTS OF NEUREGULIN-1 ON IN ACUTE STROKE USING DIFFUSION MRI

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Target audience: MRI scientists, Radiologists and experimental neurologists.

Purpose: Diffusion tensor imaging (DTI) allows for the non-invasive measurement of in vivo 3D diffusion of water molecules in brain tissue and has been demonstrated to be a robust tool to access the integrity of myelin and axons. Quantitative analysis of DTI indices has shown promise to evaluate microstructural changes in brain tissue with stroke lesion[1]. Neuregulin-1 (NRG-1) is a growth factor with multiple potent effects including acetylcholine receptor inducing activities (ARIAs), glial growth factors (GGFs), neuro differentiation factors (NDFs)[2]. In the present study, we hypothesized that DTI indices could be applied as imaging biomarkers to access the response of NRG-1 treatment in stroke disease.

Methods: ***Animal model preparation:*** Adult Sprague–Dawley rats weighing 230–270 g were used for this study. Permanent MCA occlusion (pMCAo) was induced with a 40 mm 4-0 surgical monofilament nylon suture coated with rubber silicone (1). ***CBF monitor:*** Laser Doppler flowmetry (LDF) (wavelength, Sweden) was used to continuously monitor relative changes in CBF prior to, during, and 10 minutes following vessel occlusion to confirm appropriate MCA occlusion. ***NRG-1 treatment:*** To determine the effects 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 ((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. T2WI were acquired with the following parameters: FOV=3.0 x 3.0 cm2, matrix size=256×256, TR=1000 ms and TE=50 ms. DTI was acquired with a four-shot EPI sequence. The imaging parameters were: TR=3000 ms, TE=32 ms, Δ=20 ms, δ=4 ms, FOV=3.0 x 3.0 cm2, image in-plane resolution=250×250 µm2, NEX=4, 30 gradient directions, b = 0 and 1000 s/mm2, respectively. ADC, FA, radial and axial diffusivity ($\lambda_{//}$ and $\lambda_{\perp}$) 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). ***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. All sections were examined with fluorescence microscopy in three random MCA served areas in the inner border of the infarct in the ischemic fronto-parietal cortex of each rat.

Results: ***Comparison of stroke volume between treatment and control groups*** (Fig 1): The infarct volumes of vehicle group were significantly larger than NRG-1 treated group at 0.5 hrs (85.0 ± 50.0 mm^3 vs. 44.4±21.3 mm^3), 1 hr (118.6 ± 70.0 mm^3 vs. 56.5± 27.1 mm^3) , 2 hrs (147.2 ± 74.5 mm^3 vs. 75.6± 41.1 mm^3), 3 hrs (211.1 ± 127.0 mm^3 vs. 83.0± 45.6 mm^3) and 48 hrs (533.4 ± 175.5 mm^3 vs. 264.8 ± 192.0 mm^3) post occlusion (all $p<0.05$). The stroke volumes of vehicle group were significantly larger than those of mild ischemia group (<70% CBF reduction) at 1 hr, 2 hr, 3 hr and 48 hr post occlusion ($p<0.05$ at any time points). Overall, there were significant negative correlations between the mean stroke volume at 48 hr and CBF reduction during the surgery ($p=0.003$, $r=0.326$). ***Quantitative DTI analysis of ischemic lesions***: At 48 hrs post occlusion, FA values in treatment groups were increased significantly compared to vehicle group (all $p<0.05$). At 0.5 hrs post occlusion, the ADC values in severe ischemia group were significantly higher than those in vehicle group (0.71±0.11 μm^2/ms vs. 0.57±0.05μm^2/ms, $p<0.05$) but no significant differences of DTI indices were seen at other time points. Longitudinally, FA values decreased from Day 1 to 48 hr post occlusion. However, increased ADC and $\lambda_{\perp}$ were found in vehicle and severe ischemia group. There were decreased $\lambda_{//}$ and increase $\lambda_{\perp}$ values on 48 hr post surgery. However, the differences did not reach significance. ***Histological evaluation:*** The immunohistological results of NRG-1 treated and vehicle rats at 48 hr post occlusion are shown in Figure 2. FJB labeling of brain tissues collected 48 hr after vehicle treament revealed numerous FJB-positive cells in the ischemic cortex (Fig. 2A). NRG-1 pretreatment effectively abolished FJB labeling in a similar regional pattern as illustrated in representative photomicrographs of the cortex (Figure 2A versus Fig 2B). The ischemic areas showed high numbers of FJB labeling, which co-localized with the low or no NeuN expressing cells (Fig 2C). Neighboring neurons that were not injured showed relatively higher levels of NeuN immunoreactivity. NRG-1 treatment rescued NeuN immunoreactivity (Fig 2D). The distribution of GFAP positive cells was dramatically reduced in the cortex of vehicle treated rats following stroke. The NRG-1 treated rat brain showed normal GFAP positive cells in the cortex (Fig 2F).

Discussion and conlusion: The DTI results demonstrate NRG-1's neuroprotection effect after ischemic insult indicated by reducing infarct volume and microstructural damage, delaying the injury of neurons following ischemic insult. In addition, NRG-1 shows better neuroprotective effects in rats with lower CBF reduction (less than 70% CBF reduction) during the surgery. Finally, the quantitative changes of DTI indices reflect the evolution of ischemic tissues as validated by histology. Our results suggest that NRG-1 has better neuroprotective effects with mild ischemic insult than severe insult. More studies are needed to fully understand the mechanisms of NRG-1 neuroprotective effects. In vivo multiparametric MRI could serve as a valuable monitor tool in this endeavor.

References : 1. Xu et al., JCBF 2006 ; 26 : 527-535. 2. Wang et al., Stroke 2008; 39: 2348-2353. 3. Falls et al, Cell, 1993;72:801-815;

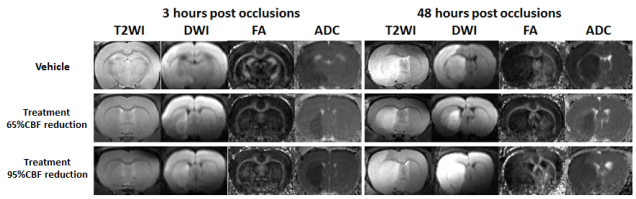

Fig 1: The stroke lesions in the vehicle and treatment group at 3 hrs and 48 hrs post occlusion. In a rat in the vehicle group (65% CBF reduction during surgery), the stroke volumes are 327.3 mm^3 and 660.3 mm^3, respectively. In NRG-1 treatment group, the ischemic region was only found in the striatum whereas no obvious ischemic region was seen in the cortex at both 3 hr and 48 hr post occlusion. In a rat with 95% CBF reduction, the ischemic region was only found in the striatum on 3 hr post occlusion. However, the ischemic regions expand to most of ipsilateral brain including cortex.

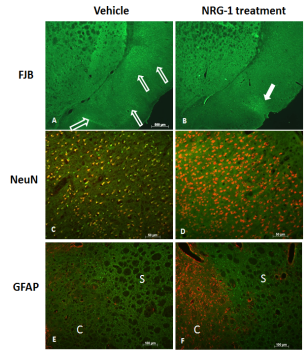

Fig 2: FJB stain. In vehicle group (A), FJB positive cells widely distributed among the cortex where MCA supplied (open arrows). However fewer FJB positive cells could be observed at cortex in NRG-1 treated group (B). **NeuN Stain**. In vehicle group(C), NeuN positive neuron showed obvious morphological changes of cell shrinkage and chromatin condenses. Relative normal neuron were found in the cortex in the NRG-1 treated rats (D). **GFAP Stain.** Distribution of GFAP positive cells were observed in the cortex of NRG-1 treated group(F). Whereas, the rats in the vehicle group showed no GFAP positive cells in both cortex and striatum (E) indicating the necrosis. C=cortex, S=Striatum