MONITORING RESPONSE TO NEUREGULIN-1 IN A RAT MODEL OF STROKE USING PERFUSION- AND DIFFUSION WEIGHTED MRI

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Introduction: Following ischemic stroke, cerebral blood flow (CBF) is substantially disrupted in the affected vascular territories[1, 2]. The general aim of acute stroke treatment is to preserve tissue at risk of infarction in the ischemic penumbra[1, 2], where neurons are mostly dysfunctional but still viable. The ischemic penumbra is being used as an indicator to identify therapeutic targets for early interventions and to assess the effects of interventions on the charactertics of ischemic region[3]. The MRI perfusion and diffusion mismatch approximates the penumbra, where CBF is decreased substantially but the tissue is still salvageable, in both animals and human acute strokes [3, 4]. Recombinant tissue plasminogen activator (rtPA) is currently used in patients within 3-4 hours after ischemic insult but its clinic application is highly limited within a very narrow window time[4]. Administering neuroprotectants could serve as an alternative therapeutic strategy to preserve the neurons in ischemic brain injury[5, 6]. Neuregulin-1 (NRG-1) has shown the potential to reduce the cerebral cortical infarct volume in animal models of ischemic stroke [5, 6]. The present study aimed to evaluate the neuroprotective effect of NRG-1 against cerebral ischemia in a rat model of permanent middle cerebral artery occlusion (MCAo).

Materials and Methods: Permanent MCAo was induced by the intraluminal suture MCAO method as described elsewhere[5, 6]. Laser Doppler flowmetry (LDF) (wavelength, 780 nm; probe 407, Perimed, Stockholm, Sweden) was used to continuously monitor relative changes in CBF prior to, during, and following vessel occlusion to confirm appropriate occlusion[5, 6]. Fourteen Male Sprague–Dawley rats (n=14, 250-300 g) were anesthetized with 2% isoflurane during the surgery. Heart rate, respiratory rate, SpO2, and body temperature were continuously monitored during surgery and MRI scanning. NRG-1 was intra-arterially injected with a single bolus of 10 µl dose of vehicle (1% BSA in PBS) and NRG-1β (10 nmol/L NRG-1 (EGF-like domain, R&D Systems, Minneapolis, Minnesota) in 1% BSA in PBS). This resulted in the administration of 2–3 ng of NRG-1/kg body weight, respectively, through a Hamilton syringe as described elsewhere [5, 6]. Vehicle (N=7) and NRG-1 treated (N=7) rats were administered by a bolus injection into the ICA through ECA about 3 minutes before MCAO5, 6. MR data were acquired on day 0 and day 2 with T2 weighted imaging (i.e.,TR/TE=2500/11ms, slices=15, NEX=2, FOV=30x30 mm, thickness=1mm, and matrix size=128×128) and diffusion tensor imaging with a four-shot EPI sequence(TR/TE=3000/32 ms, FOV=30.0mmx30.0mm, slices=15, thickness=1.0 mm, matrix size=128×128, NEX=2, gradient directions=30, b=0 and 1000 s/mm2), and perfusion weighted MRI with a single-shot gradient-echo EPI (i.e., TR/TE/FA=600/10 ms/10 matrix size=128x 128, thickness =1.0 mm, dynamics images= 400 slices=5, total scan time of 86s) at 7T. Gadoversetamide (Optimark, Mallinckrodt Inc., St. Louis, U.S.A., 0.2mM/kg) was injected via a tail vein. Data analyses were performed using Image J plugin (https://dblab.duhs.duke, NIH.gov/ij/) [8, 9]. Regions of interest (ROIs) on ADC and perfusion maps were defined by using threshold of a 35% and 65% reduction[8] of ADC and CBF in contralateral regions, respectively.

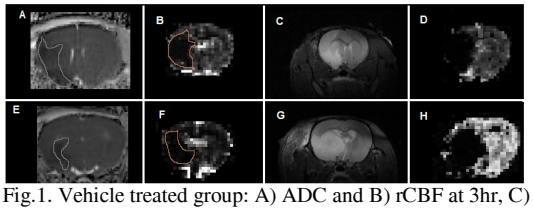

Fig.1. Vehicle treated group: A) ADC and B) rCBF at 3hr, C) T_2WI and D) rCBF at 48 hr. NRG-1 treated group: E) ADC and F) rCBF at 3hr, G) T_2WI and H) rCBF at 48 hr. Solid lines represent ROI

Results: ADC (A and E at 3hr), T_2W images (C and G at 48 hr) and rCBF maps (B and F at 3hr and D and H at 48 hr) of a typical vehicle treated group and NRG-1 treated groups is demonstrated (Fig 1). A large ischemic lesion in rCBF map (compared to the lesion in the ADC map) at 3 hr post occlusion is observed, suggesting the existence of the mismatch. Fig.2A shows the ischemic lesion volumes at 3 hr and 48 hr, respectively in vehicle treated and NRG-1 treated groups. Fig. 2B shows mismatch volume at 3 hr in vehicle and NRG-1 treated groups, respectively. The mean total ischemic lesion volume of vehicle group differed significantly (145.430±3.963 mm3 vs. 120.857±3.058 mm3, p<0.05) from that of NRG-1 treated group at 3 hr. There was a significant difference in mismatch volume between the vehicle treated group and NRG-1 treated group (22.857±0.738 mm3vs 33.286 mm3, p<0.05) at 3 hr post occlusion. The ADC and rCBF values in infarct core of vehicle and NRG-1 treated groups were significantly different (p<0.05) from those in the mismatch area at 3 hr. The rCBF values of mismatch area were not significantly different (p>0.05) between the groups (p<0.05) whereas ADC values showed a trend towards different but not significant. The lesion volumes assed by rCBF grew by about 60% and 66% from day 0 to day 2 in vehicle and NRG-1 treated group, respectively. The infarct volume represented about 33% and 44% of the ipsilateral volume in day 0 and day 2 in vehicle group, respectively. In contrast, the infarct volume was about 27% and 38% of the ipsilateral volume in NRG-1 treated group. Fig. 3 shows the TUNEL staining of vehicle and NRG-1 treated groups (a and b: cortical core in vehicle and NRG-1 treated group, c and d: cortical mismatch area in vehicle and NRG-1 treated groups) and CV (e and f: cortical mismatch area in vehicle and NRG-1 treated groups). TUNEL and CV staining showed the injured neurons undergoing apoptosis and presence of viable neurons in the cortical brain sections of vehicle and NRG-1 treated groups.

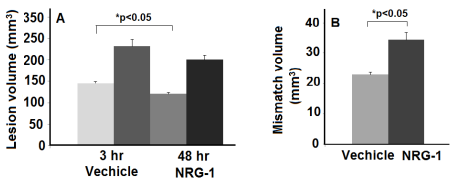

Fig.2. Plot of the vehicle and NRG-1 treated group's after pMCAo. A. ischemic lesion volume at 3 hr and 48 hr. B. mismatch volume at 3 hr

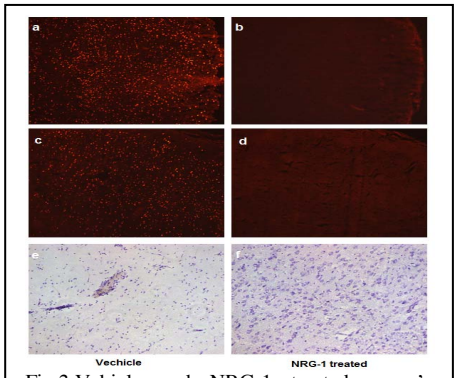

Fig.3.Vehicle and NRG-1 treated group's cortical region TUNEL (a-d) and cresyl violet staining (e-f) at 48 hr after pMCAo.

Discussion and Conclusion: The ADC deceases in the ischemic region in the initial hours after stroke onset obviously indicates the severity of the perfusion deficit. NRG-1 treatment significantly reduced infarct volume (p<0.05) compared to the vehicle treated group within 3 hr of ischemic insult. TUNEL and CV staining showed that NRG-1 treatment reduces the number of positive cells and diminishes neuronal density in the ischemic lesion of cerebral cortex, in agreement with the imaging findings. Selective protection of NRG-1 in cortex but not striatum might be related to the severity of local blood flow reduction due to the lack of collateral blood supply. Moreover, NRG-1 is a widely expressed signaling molecule that is involved in cell differentiation, proliferation, growth, survival, and apoptosis. Therefore, NRG-1 treatment suppressed apoptosis and provided neuroprotection against ischemic brain injury. O*ur* results emphasize that NRG-1 is a promising neuroprotective candidate for stroke therapy.

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