PROFILING WALLERIAN DEGENERATION IN IPSILATERAL PYRAMIDAL TRACT AFTER EXPERIMENTAL INTRACEREBRAL HEMORRHAGE

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INTRODUCTION: Intracerebral hemorrhage (ICH) is a common type of stroke. It often produces severe motor function deficits in survivors, which is closely related with secondary corticospinal tract (CST) injury[1,2]. Wallerian degeneration (WD) of CST after stroke has been characterized in humans by diffusion tensor imaging (DTI)[3,4]. However, such observations were largely preliminary due to large inter-sbuject variations. Rodent models of ICH have greatly promoted the understanding of histopathology underlying brain injury after ICH[5] and have been employed widely for exploring therapeutic strategies. This study aims to observe WD of pyramidal tract (PY) after ICH, an important part of CST, longitudinally in a well-controlled rodent model by T_2-weighted imaging and DTI as well as histological evaluations.

METHODS: ***Animal Preparation:*** Fourteen female Sprague-Dawley (SD) rats (~15wks; 320g~340g) were infused with 0.28U collagenase (Type IV, Sigma) in 1.4 μL heparinized saline (0.125μL/min) into the right striatum[6]. Three rats were sacrificed for histology at day 3, day 7 and day 42 and day 120 after surgery. Six animals served as normal control. ***MRI Protocols:*** All MRI experiments were performed on a 7T Bruker MRI scanner at 3 to 4 hours (D0), day 1, 3, 7, 14, 28, 42, 90 and 120 (D1, 3, 7, 14, 28, 42, 90, 120) after ICH. The animals were kept warm with circulating water at 37°C while anaesthetized with inhaled isoflurane. T_2-weighted images (T_2WIs) were acquired with TR=4200ms, TEs=38.9 ms, FOV=30×30mm^2, matrix=128×128, slice thickness=1.0mm and NEX=2. Diffusion-weighted images (DWIs) were acquired with a SE 4-shot EPI with 30 diffusion gradient directions and 5 b_0 with TR/TE=3750/32ms, δ/Δ=5/17ms, resolution=273×273×1000μm^3, b=1000s/mm^2 and NEX=3**.** ***Data Analysis:*** FA, axial diffusivity ($\lambda_{//}$), radial diffusivity ($\lambda_\perp$) and trace maps were generated from DWIs using DTIStudio. ROIs in the PY were first established on T_2WIs and corresponding diffusion maps at the last time point, and then copied to the other time points (Fig. 1)[7]. Ratios of T_2W signal intensities between right (ipsilateral) and left (contralateral) side (rT_2SI) were plotted on single animal basis. The signal difference between right and left sides on FA, $\lambda_{//}$, $\lambda_\perp$ and trace maps were compared by two-tailed paired t-test at each time point, after it was confirmed that the left PY of ICH rats showed no significant difference along time as compared with normal control. One-way ANOVA was performed for comparison of the right and left difference across different time points. ***Histology:*** Paraffin sections were prepared from rat brain samples for staining for myelin with Luxol blue staining (LBS), and for intact axons using immunohistochemical staining for phosphorylated neurofilaments (SMI-31)[8].

RESULTS: Typical T_2WIs of one normal rat and one ICH rat were shown in Fig.1. Hypointense T_2W signal were observed in the right PY at D7 and D42 (green arrows), while hyperintense T_2W signal was found at D120 after ICH (red arrow). As shown by the regions of interest (ROI) analysis, rT_2SI scattered around 1.0 at D3 in 12 out of 14 rats, then became lower than 1.0 in 13 rats at D7, followed by gradual normalization until D42. Afterwards, rT_2SI continued to increase till D120, the end of the experiment. As for diffusion metrics as shown in Fig. 2, right PY showed significant decrease of FA, $\lambda_{//}$ and trace at D3, followed by increase of $\lambda_\perp$ at D7, as compared with the left side (two-tailed paired t-test). One-way ANOVA analysis detected a further decrease of FA and increase of $\lambda_\perp$ and trace in ipsilateral PY at D28, as compared with that at D3. Afterwards, FA decrease and $\lambda_\perp$ increase persisted and became more obvious at D120. As shown in Fig. 3, staining intensity of SMI-31 in right PY first decreased at D3 and further decreased at D7, D42 and D120; LBS staining showed increased myelin debris at D7, which cleared away gradually within D90, followed by vacuolization changes by D120.

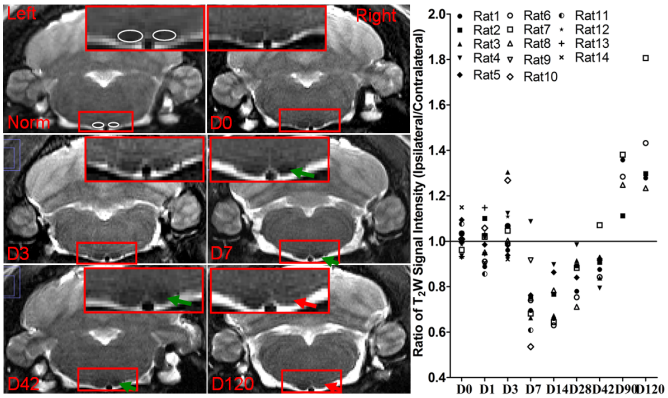

Fig.1 Typical T_2WIs of one normal rat and one ICH rat and longitudinal regions of interest (ROI) analysis of signal intensity ratios between left and right PY (rT_2SI) individually in 14 ICH rats. ROIs in both PYs, as enlarged in the red-line box, were defined as indicated by white circles in the top left image. As compared with the left side, right PY (ipsilateral) showed hypointense signal at D7 and D42 as indicated by green arrows, and hyperintense signal at D120 indicated by red arrow.

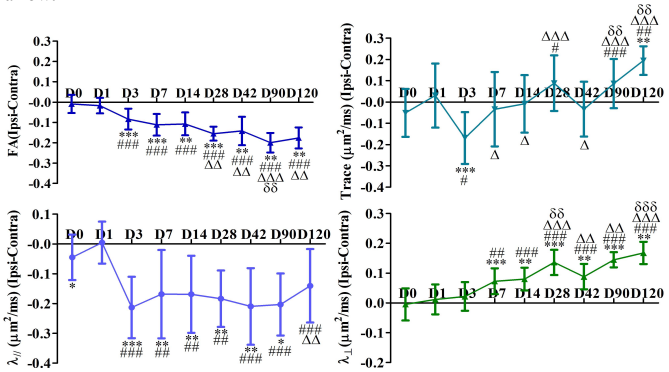

Fig. 2 Longitudinal changes of the difference of diffusion metrics in pyramidal tracts (Ipsilateral minus contralateral, Mean±SD). Two-tailed paired t-test was performed at each time point (***P < 0.001, **P < 0.01), while one-way ANOVA was used for comparison between different time points ($^{\#\#\#}P$ < 0.001, $^{\#\#}P$ < 0.01, $^{\#}P$ < 0.05 vs. D0, $^{\Delta\Delta\Delta}P$ < 0.001, $^{\Delta\Delta}P$ < 0.01, $^{\Delta\Delta}P$ < 0.05 vs. D3, $^{\delta\delta}P$ < 0.01, $^{\delta}P$ < 0.05 vs. D7).

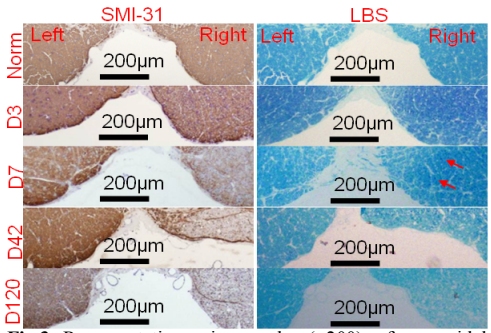

Fig.3 Representative micrographs (×200) of pyramidal tracts of SMI-31 staining for intact axons (brown color) and Luxol blue staining for myelin (blue color). Decreased signal intensity of SMI-31 staining was first found at D3 and further decrease at D7, D42 and D120, indicating progressive axonal injury after ICH. Increased myelin debris (dark blue dots indicated by red arrows) was first found at D7, followed by obvious signal intensity decrease at D42 and D120, suggesting myelin loss.

DISCUSSIONS AND CONCLUSION: Our results demonstrated that WD of PY started within D3 after experimental ICH, which was first detected with DTI with characteristic decrease of FA and $\lambda_{//}$, followed by $\lambda_\perp$ increase within D7. As shown in the previous DTI studies[4, 8], the decrease of FA and $\lambda_{//}$ could be related with disintegration of axonal structure identified by SMI-31 staining at D3, while the increase of $\lambda_\perp$ was related with myelin loss identified by increased myelin debris at D7. While T_2W signals of injured PY failed to show signal abnormality at D3, and underwent a pseudo-normalization between D42 and D90, DTI could detect such injury within D3 and provide further proof of injury deterioration along time. To conclude, DTI can serve as a reliable tool for detecting WD in early phase and for longitudinal monitoring of WD with much better accuracy than T_2-weighted imaging. In line with previous study[3] but with a different temporal profile, WD of PY after experimental ICH could be staged as the following: stage 1 (D0 to D3), with decreased FA and $\lambda_{//}$, and normal T_2W signals; stage 2 (D3 to D7), with decreased FA and $\lambda_{//}$, increased $\lambda_\perp$ and normal T_2W signals; stage 3 (D3 to D28), with decreased FA and $\lambda_{//}$, increased $\lambda_\perp$ and T_2W hypointense signals; stage 4 (D28 to D120), with decreased FA and $\lambda_{//}$, increased $\lambda_\perp$ and T_2W iso- or hyper-intense signals.

REFERENCES : [1] Qureshi AI, et al. Lancet. 2009; 373: 1632-1644. [2] Yoshioka H, et al. Neurosurgery. 2008; 62: 97-103. [3] Kuhn MJ, et al. Radiology. 1989; 172: 179-182. [4] Thomalla G, et al. NeuroImage.2004; 22:1767-1774. [5] Xi, G, et al. Lancet Neurol. 2006; 5: 53-63. [6] Imamura N, et al. Neurosci Res. 2003; 46: 289-298. 1767-1774. [7] Yu C, et al. NeuroImage. 2009; 47: 451-458. [8] Budde MD, et al. Magn Reson Med. 2007; 57: 688-695.