Differentiating the early stage ischemic stroke severity in mice using diffusion tensor imaging

T-H. Wu[1,2], H-F. Liang[2], C-I. Chen[2,3], C-Y. Hsu[4], and S-K. Song[2]

[1]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, [2]Department of Radiology, Washington University School of Medicine, St. Louis, MO, United States, [3]Department of Neurology, Taipei Medical University - Wanfang Municipal Hospital, Taipei, Taiwan, [4]Graduate Institute of Neuroscience, Taipei Medical University, Taipei, Taiwan

Introduction

Stroke is the third leading cause of death and disability in western countries. Each year 500,000 – 700,000 patients are affected, of which roughly 30% die and another 20% – 30% become permanently disabled in US. Currently, there is still no quantitative method to determine the severity of ischemic stroke in the early stage. Recent reports on diffusion tensor imaging (DTI) derived biomarkers of white matter injury may potentially be used as the needed acute diagnostic method[1, 2]. The goal of the present study is to verify whether DTI may be used to determine the severity of early stage ischemic stroke in mice. In this study, the areas of infarct in cortex and external capsule (EC) were examined for evaluating the severity of ischemic stroke mice.

Materials and Methods

Stroke model

Male C57BL/6 mice of 8 – 12 weeks old, weighing 22 – 28 g, were used for this study. A craniectomy was performed followed by electrocoagulation of Middle Cerebral Artery (MCA). Different energy depositions at 1.4, 1.8, and 4.5 mJ were employed to generate mild, moderate, and severe ischemic stroke respectively. Each group consists of 6 mice.

Diffusion Tensor Imaging

Data were acquired using a standard spin-echo diffusion weighted imaging sequence. Acquisition parameters are: TR = 1.7 sec, TE = 50 msec, Δ = 25 msec, δ = 8 msec, NEX = 4, slice thickness = 0.5 mm, field-of-view = 3 cm, and data matrix = 256 × 256 (zero filled to 512 × 512). Diffusion sensitizing gradients were applied along six directions: [Gx,Gy,Gz] = [1,1,0], [1,0,1], [0,1,1], [-1,1,0], [0,-1,1], and [1,0,-1]. Two b-values (0 and 0.768 ms/μm^2) were used. Axial ($\lambda_{\parallel}$) and radial diffusivities ($\lambda_{\perp}$) were measured in the area of infarcted EC. Apparent diffusion coefficient (ADC) was also measured in the area of infarcted cortex. All in vivo DTI measurements were performed at 3 hours after the MCA occlusion.

Immunohistochemistry Evaluation

Cross sectional examinations were performed to histologically validate the *in vivo* DTI findings. Mice (N = 18) were perfusion fixed with phosphate buffered saline (PBS) followed by 4% paraformaldehyde. 5-μm-thick slices matching the DTI images were cut for immuno-histochemical examinations. The integrity of axons was evaluated using a primary antibody against phosphorylated neurofilament (pNF) and myelin integrity was assessed with a primary antibody against myelin basic protein (MBP). Histological sections were examined with a Nikon Eclipse 80i microscope, and images were captured with a Photometrics CCD digital camera.

Results

The volume of infarcted cortex increases with the increased power deposition, i.e., increased severity of the stroke (Fig. 1). The relationship of infarcted cortex volume and energy deposition from 1.4 to 4.5 mJ in this stroke model is not linear (Fig. 2). The value of axial diffusivity ($\lambda_{\parallel}$) (Fig. 3) and radial diffusivity ($\lambda_{\perp}$) (Fig. 4) in the area of infarcted EC are statistically different ($p < 0.05$) among these three groups. The ADC value in the infarcted cortex showed significant difference between the mild and severe groups but not between the mild and moderate, or moderate and severe groups.

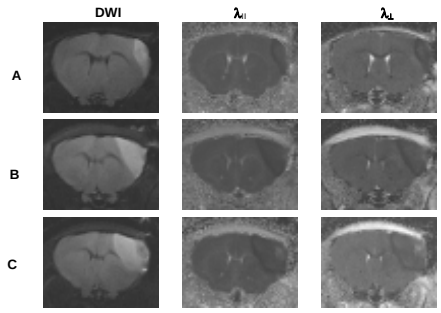

Fig. 1 DTI maps of the mild (A), moderate (B), and severe (C) ischemic stroke mice.

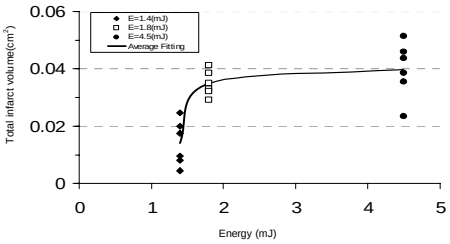

Fig. 2 The total infarct volume vs. energy deposition

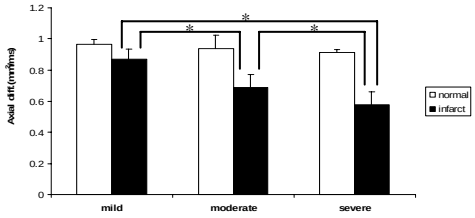

Fig. 3 Axial diffusivity of external capsule from stroke mice of different severities. *$P<0.05$

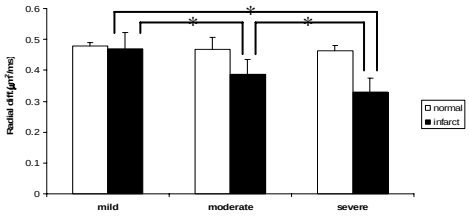

Fig. 4 Radial diffusivity of external capsule from stroke mice of different severities. *$P<0.05$

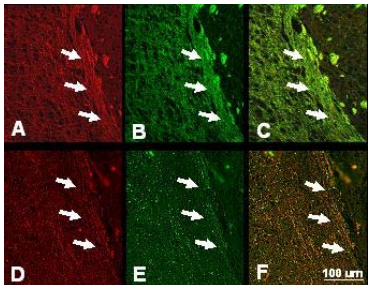

Fig. 5 Immunohistochemistry of external capsule from the control (A – C) and ischemic stroke (D – F) mice. Intact EC from the control mouse is evident as the normal appearance in myeline basie protein (MBP, A) and phosphorylated neurofilament (pNF, B) staining. The loss of pNF staining suggestive of axonal injury is apparent on the merged images (C and F), where the loss of green pNF staining is clearly seen in the stroke mouse (F).

Discussions and conclusions

We generated a stroke mouse model with various degrees of injury to determine if DTI can differentiate the severities of ischemic stoke in the early stage. The degrees of severity were produced by varying the deposition of coagulating energy in the MCA. In the early stage of ischemic stroke in mice, ADC measurement in the area of infarcted cortex differentiates the severities of stroke although it is not as sensitive as the changes in axial and radial diffusivities measured from EC. The pattern of changes in axial and radial diffusivities, i.e., both decreased acutely after ischemic stroke, suggests that axonal injury is significant in the early stage of stroke in the infarcted EC. The corresponding immunohistochemistry findings also suggest that the significant axonal injury without demyelination was present in the infracted EC (Fig. 5). Our results support the notion that DTI derived axial and radial diffusivities may be used as the biomarker of white matter injury in the early stage of ischemic stroke in mice.

References

1.Song et al., Neuroimage 2005; 26: 132-40.

2.Sun et al., Magn Reson Med 2006; 55: 302-8.