Characterization of White Matter Reorganization in Neonatal Hypoxic-Ischemic Cerebral Injury using Diffusion Tensor Imaging

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INTRODUCTION: Hypoxic-ischemic (HI) cerebral injury is an important cause of permanent damage to neuronal cells that may result in neonatal death or be manifested later as cerebral palsy or impaired cognition. Although the neonatal brain undergoes massive cell death and atrophy the first week after injury, it retains the potential to generate new oligodendrocytes up to 4 weeks after injury within and surrounding the infarct (4).Yet, the exact origins of the white matter (WM) reorganization are still unclear. There is emerging evidence that fractional anisotropy (FA), as measured by diffusion tensor imaging (DTI), is a useful marker for early neonatal HI insults (1-3). In this study, we employ *in vivo* DTI to ascertain whether this method can improve the detection of brain injury and reorganization in the late stage of neonatal HI insults.

MATERIALS AND METHODS: Animal Preparation: Sprague-Dawley rats (12-16 g, N=7) underwent unilateral ligation of the left common carotid artery at postnatal day 7 under isoflurane anaesthesia, followed by hypoxia in 8% oxygen and 92% nitrogen at 36-37°C for 2 hours. Two and a half months after HI insults, T1WI, T2WI and DTI were performed to all animals.

MRI Protocol: All MRI measurements were acquired utilizing a 7 T Bruker scanner using a receive-only surface coil. T1WI and T2WI were acquired using 2D RARE pulse sequences. For DTI, multi-shot SE-EPI diffusion weighted images were acquired with FOV = 3.0 x 3.0 cm^2, MTX = 128 x 128, slice thickness = 0.5 mm, number of slices = 33, TR/TE = 5000/28 ms, NEX =10 to 16, b = 0 and 1000 s/mm^2, number of shots = 4 and 30 diffusion directions.

Data Analysis: The volume of the infarct in the ipsilesional hemisphere of the injured rats was measured in T2WIs from Bregma -9.84 mm to 4.68 mm using ImageJ v1.40g, by selecting pixels with signal intensities greater than mean + 2 SD of those in the contralateral hemisphere of each slice. DTI parameters, including FA, $\lambda_{//}$, $\lambda_{\perp}$ and diffusion trace value were obtained using DTIStudio v2.30 after co-registration. Regions of interest were drawn on both sides of the WM components in accordance to the rat brain atlas. Throughout the experiment, the right hemibrain served as the internal control.

RESULTS AND DISCUSSION: In the T2WIs of the injured brains 2.5 months after HI insult, infarct regions contributed to 65.85%±7.73% of the total ipsilateral hemisphere. Significantly less intact tissues were found in the ipsilesional hemisphere compared to those in the contralesional hemisphere ($p<0.001$), whereas no significant difference was found in the total volumes between contralateral hemispheres ($p=0.37$) (Fig. 1). In addition, the ipsilateral external capsule appeared to be split into two branches (Fig. 1, solid arrows). Shrinkage of the ipsilateral superior and inferior colliculi (Fig. 1, open arrow) was also observed. T2W hypointensity was found in the thalamus (Fig. 1, arrowhead) likely due to iron deposition.

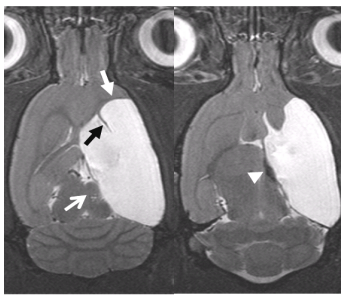

Fig. 1: Dorsal (left) and ventral (right) T2WIs of a representative brain in axial plane 2.5 months after hypoxic-ischemic insult. A large cyst was presented by hyperintensity covering the ipsilateral hemisphere.

For *in vivo* DTI data, color-encoded FA maps of the principal eigenvector showed that the perilesional areas with high FA values had similar fiber orientations as the contralateral external capsule in the anterior section of the brain (Fig. 2, blue arrows), and as the internal capsule and the fimbria of hippocampus in the posterior section of the brain (Fig. 2, white and yellow arrows). These high FA fiber bundles appeared to colocalize with our H&E stains (Fig. 3) and with previous histological studies on WM reorganization in late severe HI insults (5). Our previous study showed that severe HI insults led to cystic WM injury characterized by reduction in all DTI indices, i.e., FA, $\lambda_{//}$, $\lambda_{\perp}$, and diffusion trace value, at 1 day post-injury (3). In the current study, compared to the contralateral components, quantitative evaluation of the late stage of neonatal HI insult showed significantly lower FA in the ipsilateral corpus callosum and the anterior commissure, accompanied by higher $\lambda_{//}$, $\lambda_{\perp}$ and diffusion trace value. Significantly lower FA, and higher $\lambda_{//}$, $\lambda_{\perp}$ and diffusion trace value were also found in the proximal end of the fibers extended from the ipsilateral corpus callosum compared to the contralateral external capsule; whereas in the distal end of the extension, significantly higher FA, $\lambda_{//}$, and diffusion trace value were observed (Fig. 2, blue arrows). Lower FA and $\lambda_{//}$ were also noticed in the ipsilateral optic nerve in 6 out of 7 animals ($p=0.053$ and 0.061 respectively) (Fig. 2, red arrows). Studies in ischemia models in adults and hypoxia-ischemia models in immature animals have suggested that the mechanisms contributing to injury and the modes of neuronal death are age-dependent, and that the immature brain is more plastic than the adult brain (6). While increasing evidence also suggested the axonal remodeling indicated by FA increase in the perilesional areas after ischemic stroke in adult rodents (7,8), further experiments will be performed to differentiate the plastic changes upon injuries between mature and developing brains.

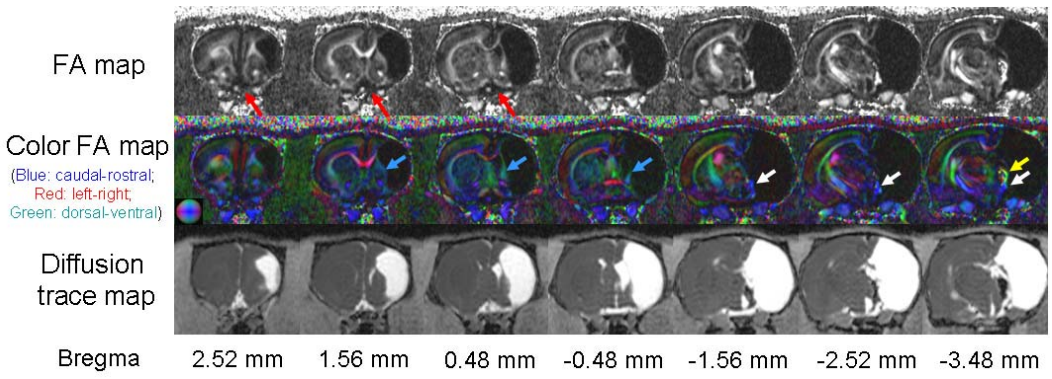

Fig. 2: Serial FA, color-encoded FA and diffusion trace maps of a representative injured brain in the coronal plane. In the color-encoded FA maps, the high FA fiber bundle along the lesion appeared to extend from the corpus callosum (blue arrows) in the anterior section of the brain, and was connected to the caudal-rostral segment of the internal capsule (white arrows). In the posterior section, the bundle extended from the internal capsule along the cyst apparently connected to structures with similar directionality to the dorsal-ventral segment of the internal capsule and the fimbria of hippocampus on the contralateral side (yellow arrow). Note also the lower FA in the ipsilateral optic nerve (red arrows).

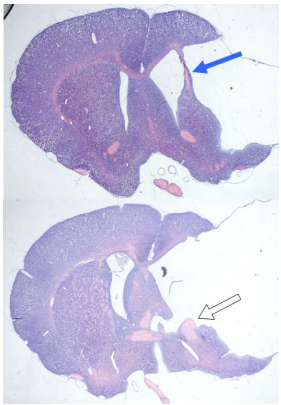

Fig. 3: H&E staining in a late stage severe HIE brain at about Bregma = 0.48 mm (left) and -0.48 mm (right). Note the apparent colocalization of white matter structures (arrows) with DTI data.

CONCLUSION: To our knowledge, the results presented here constitute the first report in detecting long-term microstructural changes in the neonatal HI brains using *in vivo* DTI in company with conventional MRI. This may possess direct clinical applications for humans given its non-invasive nature, and can be useful to test longitudinally in the same subject the effectiveness of trophic factors and neuroprotective drugs that may enhance maturation and survival of immature neurons in different microstructures for improving recovery after neonatal brain injury.

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