Characterization of Cortex and White Matter Injury in a Mild Hypoxic-Ischemic Neonatal Rat Model by Diffusion Tensor MR Imaging

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Introduction: Mild hypoxic-ischemic (HI) neonatal brain injury is known to cause astrogliosis and radial glial disruption to the cortex1 and dysmyelination to the WM2. We apply diffusion tensor MR imaging (DTI) to evaluate the changes in the cortex and WM of a mild HI neonatal rat brain injury model. We hypothesize that the quantitative indices of DTI are able to reflect the histological changes of HI injury, namely, astrogliosis in the cortex and dysmyelination in the WM.

Materials and Methods: Seven-day-old rats underwent unilateral left common carotid artery ligation followed by exposure to 8% oxygen-balanced nitrogen for 50 minutes (n=9) in order to create mild HI induced brain damage. DTI was performed using a 7T NMR scanner (Bruker, Germany) and microimaging mouse brain coil on 24h post HI. Images were obtained in the coronal plane using the following parameters: FOV=32mm; TR/TE=3000ms/32ms; matrix size 128 x 128; slice thickness=0.5mm. FA, trace, λf and λc maps were created for quantitative analysis by using DTIstudio (Johns Hopkins University, U.S.). ROIs were manually drawn over the primary somatosensory cortex of each hemisphere on the FA, trace, λf and λc maps on two consecutive slices (Bregma -1.08mm and Bregma -2.4mm) (Fig 1). ROIs were also manually drawn over the external capsule (EC) of each hemisphere on five consecutive slices (Fig 1). Paired t test was used to detect statistical differences in the DTI indices between the injury/control cortex and EC. Rats were randomly sacrificed (n=3) for histological analysis of the morphological characteristics of cortex and EC (H&E stain), myelin in the EC by using Luxol fast blue (LFB) stain and astrocytes in the cortex by using immunohistochemistry analysis of gli fibrillary acidic protein (GFAP) (SMI-22, 1;1000, USA).

Results: Comparison of DTI indices between injury and control cortex (Table 1): FA was significantly decreased in the injured cortex compared to the control cortex with a reduction of 11.7%, (p<0.01). A significantly decreased λf was found in the injured cortex compared to control cortex with a reduction of 3.8%, (p<0.05). However, there were no significant differences in λc and trace. Comparison of DTI indices between injury and control EC (Table 1): FA was significantly decreased in the injured EC compared to control EC with a reduction of 10.1%, (p<0.01). Significantly increased λf (13.5%, p<0.01) and trace (11.0%, p<0.01) were demonstrated in the injured EC compared to control EC. However, λc was similar in both sides of EC. Histological evaluations of cortex (Fig 2): H&E stain showed scattered pyknotic cells in the injured cortex (c) compared to control cortex (d). (Scale bar =25µm).

Fig 1. (a) shows ROI manually drawn over the primary somatosensory cortex (red area) on the FA map which is corresponding to rat brain at Bregma -1.08mm (c). (b) shows ROI drawn over the EC on the FA map (red area) which is corresponding to rat brain at Bregma -2.4mm (d).

Fig 2. Histology findings in the cortex: H&E stain (a-b). Injured cortex (a) shows scattered pyknotic cells (arrow) compared to normal appearance in control cortex (b). GFAP stain: (c-d) shows much increased GFAP positive cells in the injured cortex (c) compared to control cortex (d). (Scale bar =25µm).

Fig 3. Histology findings in the EC: H&E stain (a-b). Injured EC (a) shows vacuolation changes compared to normal appearance in control EC (b). LFB stain (c-d): injured EC (c) shows decreased LFB staining intensity compared to control EC (d). (Scale bar =25µm).

Conclusion: Our findings suggest that DTI indices can characterize mild HI induced injury in the cortex and WM: significantly decreased FA and λf with no change in λc appears to characterize astrogliosis in the cortex whilst significant reduction of FA with increased λf and trace characterize dysmyelination in the WM. It has been proposed that reactive astrogliosis imposes diffusion barriers due to hypertrophy of astrocytic processes, although how this affects λc is not well understood. Dysmyelination causes increased water diffusion perpendicular to the axon, therefore increased λf but decreased FA. Combined analysis of DTI indices may be useful potential markers for the non-invasive monitoring of mild HI induced brain injury.