Axial and radial diffusivities as potential markers for characterization of white matter lesions and predicting lesion outcome in a neonatal rat hypoxia-ischemia model

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Introduction: The severity of white matter damage after hypoxic-ischemic (HI) injury can be variable resulting in cystic necrosis to mild more diffuse non-cystic injury with reduced myelination, gliosis/atrophy (1). We evaluate white matter injury following hypoxic-ischemic insult in a neonatal rat model using diffusion tensor MR imaging (DTI) to determine if directional diffusivities can be used to characterize white matter lesions and predict severity and lesion outcome. We hypothesize that the histopathologic processes of hypoxic-ischemic injury in the white matter may be reflected by radial ($\lambda_{\perp}$) and axial ($\lambda_{//}$) diffusivities.

Materials and Methods: Seven-day-old rats underwent unilateral left common carotid artery ligation followed by exposure to 8% oxygen-balanced nitrogen for 1 hours (n=9) and 2 hours (n=9), in order to create HI injuries of different severity. DTI and T2W MRI was performed using a 7T animal scanner (Bruker BioSpin MRI PharmaScan, Germany) and microimaging mouse brain coil on D1 post injury and D7 post injury (T2W only). Images were obtained in the coronal plane using the following parameters: DTI: FOV 32mm x 32mm, TR 3000ms, TE 32ms, matrix size 128 x 128, slice thickness 0.5mm. T2W: TR 11189ms, TE 20ms, FOV 256mmx256mm, slice thickness 0.5mm. Apparent diffusion coefficient (ADC), fractional anisotropy (FA), $\lambda_{//}$, and $\lambda_{\perp}$ maps were created for quantitative analysis using DTIstudio software (Johns Hopkins University, Baltimore, U.S) Region-of-interest (ROI) were manually drawn over the corpus callosum (CC) of each hemisphere on the ADC, FA, $\lambda_{//}$, and $\lambda_{\perp}$ maps on D1 post injury, on the same 5 consecutive slices. ROI was defined first on the FA map, and then placed on identical sites on the ADC, $\lambda_{//}$ and $\lambda_{\perp}$ maps. Percentage value change of ADC, FA, $\lambda_{//}$, and $\lambda_{\perp}$ on the ipsilateral side compared to the 'normal' contralateral side was calculated. Rats were divided into two groups, according to the presence or absence of cystic lesions on the D7 T2W image (Fig. 1); rats without cystic lesions were included in Group A (n=11, comprising 9 rats with 1 hour hypoxia and 2 rats with 2 hours hypoxia) whilst rats with cystic lesions were included in Group B (n=7, comprising rats with 2 hours hypoxia). Cystic lesions were defined as lesions of signal intensity similar to cerebro-spinal fluid on T2W. Percentage volume of cystic lesions on the D7 T2W image was measured. Student's t test was used to detect statistical differences in the DTI quantitative indices between the ipsilateral and contralateral hemispheres ($p<0.05$ considered statistically significant). Three separate rats were sacrificed at D7 for histological analysis of the CC and stained with myelin-basic protein (MBP) and Haematoxylin and Eosin (H&E).

Results: In group A, T2W showed a small area of high signal in 8 rats and no signal change in 3 rats on the ipsilateral hemisphere on D1. Subsequently at D7, T2W showed persistent high signal in only 1 rat whilst the other ten rats had no signal change. Atrophy in the ipsilateral hemisphere was detected in 8 rats. In group B, T2W showed an area of high signal occupying a large portion of the ipsilateral hemisphere on D1 these subsequently developed into cystic lesions on D7 in all rats. Values of FA, ADC, $\lambda_{//}$, and $\lambda_{\perp}$ in group A and B are shown on table 1. Mean FA was significantly lower in the ipsilateral CC compared to the contralateral CC in both group A and B with FA of ipsilateral CC significantly lower in Group B compared to Group A (0.21 vs 0.31, $p<0.0001$). For Group A, ADC was significantly higher, $\lambda_{\perp}$ was significantly lower and no significant change was found in $\lambda_{//}$ in the ipsilateral CC compared to the contralateral CC. For Group B, ADC, and both $\lambda_{//}$, and $\lambda_{\perp}$ were significantly lower in the ipsilateral CC compared to the contralateral CC. In group B, mean±SD % cystic necrosis volume was 24%±5%. The injured side showed much reduced myelination (stained green) in the CC compared to the normal side (Fig. 2). H&E stain showed varied amounts of necrosis, tissue loss and atrophy on the ipsilateral side.

Fig.1 showing T2W image of a Group A and B rat on D7

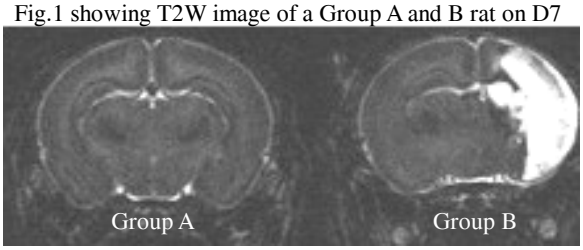

Fig.2 showing MBP stained corpus callosum (green) on D7 post-HI

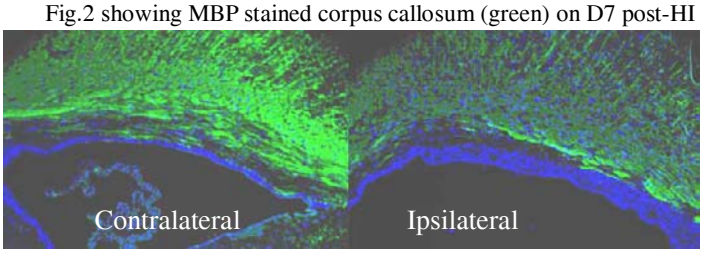

Table 1 showing DTI quantitative indices of the corpus callosum of neonatal HI rats on D1 after injury

	Group A (n=11)				**Group B (n=7)**			
DTI indices	contralateral	ipsilateral	Δ %	P value	contralateral	ipsilateral	Δ %	P value
FA	0.329±0.039	0.310±0.039	-5.5	0.01	0.238±0.041	0.210±0.030	-11.2	0.001
$\lambda_{//}$ x 10^{-3} (mm^2/s)	0.998±0.010	0.994±0.044	-0.4	0.54	1.000±0.019	0.900±0.076	-9.0	<0.001
$\lambda_{\perp}$ x 10^{-3} (mm^2/s)	0.693±0.065	0.748±0.096	8.0	0.001	0.778±0.059	0.682±0.090	-12.1	<0.001
ADC x 10^{-3} = (mm^2/s)	2.509±0.505	2.818±0.389	15.8	<0.001	2.800±0.412	2.314±0.473	-15.7	<0.001

Conclusion: Our findings suggest that axial and radial diffusivities can characterize hypoxic-ischemic injury in white matter; increased radial diffusivity with no significant change in axial diffusivity appears to characterize milder white matter injury with reduced myelination (2) and atrophy whilst reduction in both radial and axial diffusivities characterize severe damage with loss of structural integrity and subsequent necrosis. Therefore, these indices of diffusivity may be potential markers of severity of hypoxic-ischemic injury, able to characterize white matter lesions and predict lesion outcome. This is useful in prognostication and for selecting animals for neuro-protective therapy.

References:

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