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MILD HYPOXIC-ISCHEMIC INJURY IN THE NEONATAL RAT BRAIN: LONGITUDINAL EVALUATION OF THE WHITE MATTER USING DIFFUSION TENSOR MR IMAGING

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INTRODUCTION: Mild hypoxic-ischemic (HI) neonatal brain injury is known to cause selective damage to the white matter (WM) [1]. Therefore, we apply diffusion tensor MR imaging (DTI) to evaluate longitudinally the changes in the 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, damage to myelination.

MATERIALS AND METHODS: Nineteen 7-day-old SD rats underwent unilateral left common carotid artery ligation followed by exposure to 8% oxygen-balanced nitrogen at 37°C for 50 minutes. Rats were evaluated by DTI at D1(*n=19*), D7(*n=16*), D14(*n=13*), D30(*n=11*) and D90(*n=9*) post HI using a 7T NMR scanner (Bruker, Germany) with a microimaging mouse brain coil (for D1, D7) or a rat brain coil (D14, D30 and D90). MRI sections were performed from 2mm anterior to the corpus callosum to the end of the cerebrum. The following imaging parameters were used: TR/TE =3000ms/32ms, FOV = 32mm^2, thickness= 0.5mm, acquisition matrix = 256 x 256, b value =0 and 1000 s/mm^2. FA, trace, $\lambda_{//}$ and $\lambda_{\perp}$ were created for quantitative analysis using DTIstudio v2.4. Signal intensity of FA, trace, $\lambda_{\perp}$ and $\lambda_{//}$ maps were analyzed by ROI manually drawn over the external capsule (EC) of each hemisphere on 5 consecutive slices using Image J (NIH, U.S) and the values were averaged in each EC for quantitative analysis. Rats were randomly selected for histological evaluation of the bilateral EC at D1(*n=3*), D7(*n=3*), D14(*n=2*), D30(*n=2*) and D90(*n=5*) post-HI using H&E, luxol fast blue (LFB) and pan-axonal neurofilament marker (NF) staining. Image intensity of LFB and NF positive axons were measured in the symmetrical injured and control EC of the histological digital images (200X) by Image J. The ratios of the injured/control EC DTI indices and histological evaluations were computed. Paired t-test was used to detect statistical differences between injured and control EC. Trend of DTI indices in both sides of EC were analyzed by using linear mixed modeling. The longitudinal changes were evaluated by one-way ANOVA post-hoc test. Pearson's correlation analysis was used to determine the relationship between DTI indices and histological evaluation.

RESULTS: Comparison of DTI indices between injury and control EC (Fig 1): Significant decrease of FA was found in injured EC from D1 to D90 post-HI with maximum decrease of 8.4% on D1, and minimum decrease of 3.3% on D90. Apart from significantly increased trace in injured EC on D1 ($p<0.01$), similar trace was found in other time points. Significantly elevated $\lambda_{\perp}$ was found in injured EC at every time point with maximum increase of 8.6% on D1 ($p<0.01$) and minimum increase of 4.3% on D90 ($p<0.01$). No significant difference in $\lambda_{//}$ was found in both sides of EC at all time points. **Longitudinal trend of DTI indices (Fig 1):** Longitudinal trends of DTI indices were similar in both sides of EC with a significant increase in FA ($p<0.01$), decrease in trace ($p<0.01$) and $\lambda_{\perp}$ ($p<0.01$) and stable $\lambda_{//}$ from D1 to D90. We found significant increase in the ratio of injured/control FA ($p=0.016$), decreases in injured/control trace ($p=0.001$) and injured/control $\lambda_{\perp}$ ($p=0.002$) from D1 to D90. **Histological evaluation (Fig 2 and 3):** H&E stain showed mild vacuolation but without necrosis in injured EC on D1 and D7 post-HI. Quantitative analysis of LFB staining intensity showed significant increase in LFB staining from D1 to D90 post-HI in both sides of EC ($p<0.01$ for both). There was significantly decreased LFB staining intensity in injured EC compared to control EC in all time points. Increased axonal count was demonstrated in both sides of EC from D1 to D90 but this did not reach statistical significance. Also, no significant differences were found in axonal count between both EC at all time points. No significant longitudinal trend of injured/control LFB or axonal count was demonstrated. **Correlation between DTI indices and histological evaluation (Table 1):** FA was significantly correlated with both LFB staining intensity ($r=0.68$, $p<0.01$) and axonal count ($r=0.67$, $p<0.01$). $\lambda_{\perp}$ was significantly correlated with LFB ($r=-0.53$, $p<0.01$) only and $\lambda_{//}$ was significantly correlated with axonal count ($r=0.37$, $p=0.04$) only.

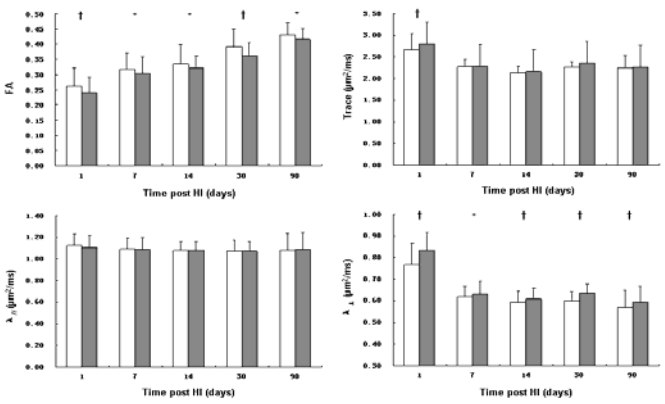

Fig 1: Longitudinal changes of DTI indices FA, trace, $\lambda_{//}$ and $\lambda_{\perp}$ in control EC (white bar) and injured EC (black bar) from D1 to D90 post mild HI. Paired t-test was used to evaluate statistical significance, * $p<0.05$; †, $p<0.01$.

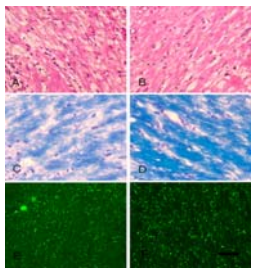

Fig 2: H&E: shows mild vacuolation changes in the injured EC (A) compared to control EC (B) on D1 post-HI. LFB staining: injured EC (C) shows decreased staining intensity compared to control EC (D) on D30 post-HI. NF staining: distribution and number of NF positive axons were similar in both sides of EC on D90 post-HI (E,F). (Scale bar =25μm, applies to A-H).

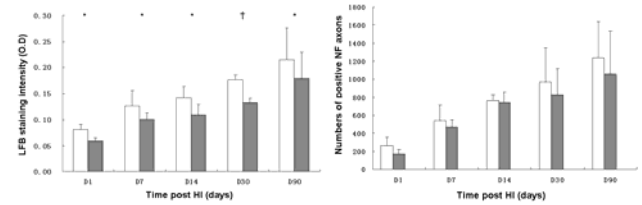

Fig 3: shows longitudinal quantitative histological analysis of LFB staining intensity and numbers of NF positive axons between control EC (white bar) and injured EC (black bar) from D1 to D90 post-HI. Paired t-test was used to evaluate significant differences between both sides of EC. *, $p<0.05$; †, $p<0.01$

	FA		Trace		$\lambda_{//}$		$\lambda_{\perp}$	
	r	p	r	p	r	p	r	P
Axonal count	**0.67**	**<0.01**	-0.21	0.27	**0.37**	**0.04**	-0.35	0.06
LFB intensity	**0.68**	**<0.01**	-0.35	0.06	0.35	0.06	**-0.53**	**0.003**

Table 1: Pearson's correlation analysis comparing DTI indices and histological evaluations of LFB staining intensity and axonal count in the EC of neonatal rats after mild HI injury. $p<0.05$ are in bold.

CONCLUSION: We found reduced myelination in the WM after mild HI injury reflected by reduced FA and increased $\lambda_{\perp}$ in the injured EC compared to the control EC. The longitudinal changes of increase in FA, decrease in $\lambda_{\perp}$ and trace with stable $\lambda_{//}$ in both the injured and control EC are in keeping with the changes of normal development and continual maturation of WM in the injured EC. Furthermore, the trends of decreasing differences in FA and $\lambda_{\perp}$ between the injured compared to control EC from D1 to D90 suggest partial recovery in the injured EC. Our results demonstrated that mild HI induced WM damage has the potential to continue the maturation process with partial recovery post-HI, and this could be reflected by DTI in *vivo*. Our results support the use of DTI as a biomarker to non-invasively monitor the longitudinal changes of mild HI induced WM damage. Moreover, this model may be used to test the effectiveness of potential neuroprotective therapies.

REFERENCES: 1. Qiao M, et al. Neurosci Lett. 2004; 368:332-6. 2. Song SK, et al. Neuroimage.2002;17:1429-36.