White matter injury in germinal matrix hemorrhage in rabbit pup model detected by DTI

H. Chahboune[1], C. Chua[2], P. Ballabh[2,3], and F. Hyder[1,4]

[1]Diagnostic Radiology, Yale University School of Medicine, New HAven, CT, United States, [2]Pediatrics, New York Medical College - Westchester Medical Center, Valhalla, NY, United States, [3]Cell Biology, New York Medical College - Westchester Medical Center, Valhalla, NY, United States, [4]Biomedical Engineering, Yale University School of Medicine, New HAven, CT, United States

INTRODUCTION

Diffusion tensor imaging (DTI) has increasingly been used as a noninvasive diagnostic marker to characterize perinatal brain injury in animal models [1, 2]. The propose of this study was to employ *ex vivo* DTI for the evaluation of the long-term consequences of Germinal matrix hemorrhage-intraventricular hemorrhage (GMH-IVH), found in low birth weight preterm infant, in a rabbit pup model**.** This animal model exhibits neurological sequeale including post-hemorrhagic hydrocephalus (44%) and hypertonic cerebral palsy (25%) associated with histological evidence of reduced myelination of the white matter [3]. Accordingly, our MRI data revealed evidence of white matter damage.

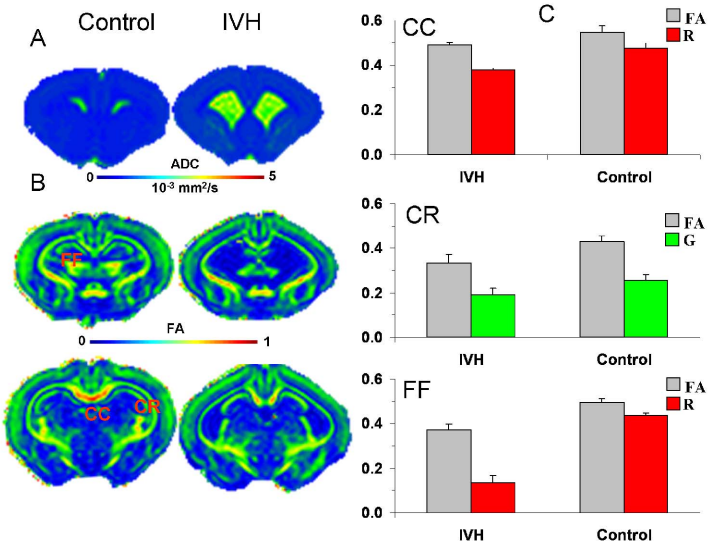
MATERIALS and METHODS

Animal preparation*:** Premature rabbit pups, delivered by C-section at 29 day gestational age (term=32d), were treated with 50% glycerol (6.5g/kg) intraperitoneally to induce IVH. Head ultrasound was performed at 24h age to determine for the occurrence of IVH. At post natal day14, the IVH and non IVH pups were anesthetized with ketamine (50mg/kg), acepromazine (0.8 mg/kg) and xylazine (5 mg/kg), then transcardially perfused with saline followed by 4% paraformaldehyde. The brains were harvested and immediately placed in 4% formalin. Before imaging the brains were placed in PBS for 24 h to wash out the fixation solution and transferred them to into home built MRI compatible tube. The tubes were then filled with fluorinert an MRI susceptibility matching fluid. ***DTI All imaging was completed on a 9.4-T spectrometer (Bruker, Billerica, MA, U.S.A.) with custom-made cosine coil. A set of contiguous coronal slices were acquired to cover the whole brain DTI experiments were performed using the Stejskal-Tanner spin-echo diffusion-weighted sequence using the following parameters, δ = 5 ms; Δ= 8 ms (where δ and Δ are the durations of diffusion gradient and time elapsed between the two diffusion gradients, respectively), repetition time (TR)/echo time (TE) 1000/18 ms; the matrix size was 128 x 64, zero-filled to 256 x 128. The slice thickness = 0.5 mm and the in-plane resolution is 230 µm x 230 µm, NEX = 8. Fifteen images with noncollinear diffusion weighting with b = 1000 s/mm2 and one reference image with no diffusion weighting. The Eigenvalues $\lambda 1$ $\lambda 2$ $\lambda 3$ were driven from the diffusion tensor matrix. Quantitative maps of fractional anisotropy (FA) were calculated and the primary eigenvectors were used to calculate directionally encoded color (DEC) maps to highlight the orientation of anisotropic tissues using medial-lateral (R for red), dorsal-ventral (G for green), and anterior-posterior (B for blue) color maps [6]. Five regions were examined: corpus callosum (CC), internal capsule (IC), Corona Radiata (CR), Fimbria Fornicis (FF) and fasciculus Subcallosus (FS).

RESULTS and DISCUSSION

The IVH pups exhibited ventriculomegaly shown in the ADC maps Fig. 1-A, the ventricles were scarcely visible in control pups. There were no difference in tissue anisotropy in IC and FS between the IVH and the control pups. However, the FA values were significantly decreased in CC ($P<0.05$), FF ($P<0.005$) and CR ($P<0.03$) in the IVH pups compared to control Fig. 1-C. Evidences of reduced FA in these regions in IVH pups are presented in Fig. 1-B, the reduction is illustrated with a decrease in pixel intensity of the FA maps. With respect to directional anisotropy, the anisotropy differences in CC and FF were predominant in the medial-lateral (R) direction and in the dorsal ventral direction (G) in CR. Reduced FA and directional anisotropy of the white matter regions on DTI correlated with reduced myelination in pups with IVH as evaluated by myelin immunohistochemistry and Western blot analysis. While reduced myelination contributes to DTI changes, other factors might also be contributing to the DTI values. The factors impacting the water diffusion could be ionic imbalances in cellular function, alteration in sodium channel activity and changes in the axonal propriety. In conclusion, the premature rabbit pups with GMH-IVH displays white matter injury and ventriculomegaly on DTI, which is consistent with the histological findings in the model. The study highlights a novel animal model of the long-term consequences of IVH, which can be used to evaluate strategies in the prevention and treatment of post-hemorrhagic complications.

Figure 1



REFERENCES

[1] A. Drobsyshevsky et al (2005) J Neurosci 25:5988–97. [2] S. Wang et al (2008) Stroke 39:2348-53. [3] P. Georgiadis et al (2008) Stroke (in press)

ACKNOWLEDGEMENT

Supported P30 NS-052519 (FH), Supported by Pfizer (PB), NIH/NINDS NS050586 grant (PB)