Correlation between water apparent diffusion values and histopathological cerebral white matter changes in fixed tissue from a primate model of premature birth.

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Synopsis: To understand changes in diffusion measures in developing brain in relation to white matter injury, diffusion tensor images were acquired from fixed baboon brains. Water apparent diffusion coefficient (ADC) values were obtained from six regions of interest located in the centrum semiovale. Brains were also analyzed histologically, and a white matter abnormality score (WMAS) was obtained. A statistically significant relationship was found between ADC and WMAS for all regions analyzed, with higher ADC values found for more severely injured tissue. These findings provide evidence that diffusion measures reflect changes in white matter microstructure, even after fixation.

Background: Premature birth is associated with adverse neurodevelopmental outcome, with up to 10% of survivors born at weight less than 1500 g developing cerebral palsy, and a higher proportion having developmental and behavior abnormalities by school age. Cerebral white matter injury is the most common neuropathology in the preterm infant and may be responsible for these adverse outcomes. Diffusion tensor magnetic resonance imaging (DTI) has been used to assess white matter microstructural maturation and injury in the developing human brain. In this study, we used DTI, alongside histopathological analysis, to evaluate the white matter fixed tissue from a baboon model of premature birth and brain injury.

Methods:

Subjects: The premature baboon (sp. Papio) is investigated at the Southwest Foundation in San Antonio, Texas to study the impact of postnatal therapies on respiratory outcomes. For this study, 19 baboons were delivered at 125 d gestational age (GA, equivalent to 28 weeks GA in the human) and were sacrificed between 139 and 160 d post-conceptional age (PCA). Support included exogenous surfactant administration, ventilator respiratory support, inotropic support, total parenteral nutrition, central venous access, and routine monitoring of heart rate, oxygen saturation, and blood gas status. Following sacrifice, the brains were immersion fixed in 10% formalin. Control data were obtained from 6 animals who were sacrificed immediately after delivery at GAs ranging from 125 to 160 d.

Image Acquisition: Data were obtained using a 4.7-T MR system controlled by a Varian INOVA console. The sample was placed in a 7-cm i.d. linear Litz rf coil (Doty Scientific). Diffusion images were generated using a standard, two-dimensional spin echo pulse sequence. TR was 2.0 s, TE was 80 ms, gradient pulse duration was 15 ms, gradient spacing was 50 ms, and spatial resolution was 0.6 mm isotropic. Two averages were obtained. Seven images were obtained, with b = 0 for one. For the other six, b = 2.0 ms/μm^2 and was directed along six non-collinear axes. A monoexponential signal attenuation function was used to calculate ADC values.

Histology: Forebrain sections were stained with H&E and glial fibrillary acidic protein (GFAP) to assess cerebral injury and gliosis. To determine the degree of white matter abnormality, an index was formulated using quantitatively assessed parameters, including: the presence of ventriculomegaly, areas of axonal damage, white matter gliosis, and the presence of GFAP immunoreactivity in radial glial fibers. Damage was scored throughout the rostral-caudal extent of the brain. For each brain, the presence and severity of white matter abnormality was ranked on a scale from 0 (no abnormality) to 22 (severe abnormality).

Results: There was a higher white matter injury score (5 ± 2, mean ± SD) for the 19 experimental brains as compared with controls (score 0). ADC values were analyzed as pairs in the frontal, central, and occipital areas (Figure 1). Regression analyses showed a statistically significant relationship between ADC and WMAS for all three areas (Figure 2), which persisted when adjusted for PCA ($p < 0.01$ for all ROI's).

Figure 1. Axial, T2-weighted image (left) and ADC map (right) showing ROI's.

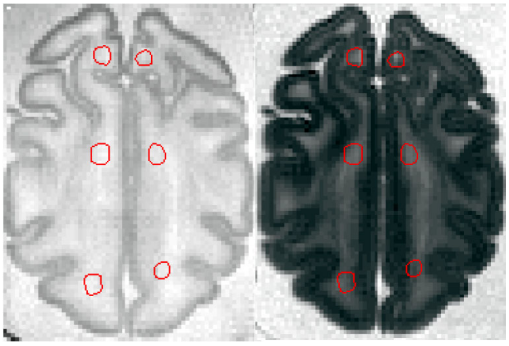
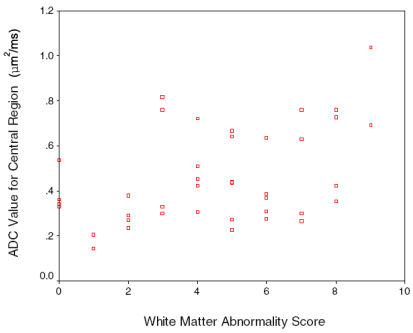


Figure 2. Scatter plot of WMAS *vs.* ADC for the central ROI.



Discussion: Previous studies of fixed tissue have demonstrated that water ADC values decrease approximately three-fold following fixation, while measures of diffusion anisotropy remain unchanged (1). Further, preliminary data from our lab indicate that, following acute stroke in rat, the relatively reduced ADC values found in the area of injury are no longer present following fixation (i.e., ADC values are the same in injured and uninjured tissue). In the study presented here, differences in ADC due to injury are preserved after fixation and correlate with histologic findings of white matter abnormality. Our findings may differ from the rat stroke model data due to the timing of MR imaging after the injury, which occurred several weeks following birth - the highest risk time for acute injury. The delay in image acquisition may allow time for changes in underlying tissue structure to develop, and these changes are preserved following tissue fixation. The findings of white matter gliosis and axonal damage being associated with elevated ADC supports the hypothesis that elevated ADC values, as described in premature infants, reflect white matter injury with resulting alterations in white matter microstructure.

Reference: 1. Sun SW, Neil JJ, Song SK. *Magn Reson Med* 2003;50(4):743-8.