Noninvasive detection of increased optic nerve cellularity in the DBA/2J mouse model of glaucoma

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Purpose:

Diffusion tensor imaging (DTI) measures the random motion of water molecules in tissues and has demonstrated utility as a sensitive noninvasive biomarker of optic nerve injury in rodent models and patients with optic neuritis [1]. However, increased cellularity and vasogenic edema associated with inflammation has escaped detection by current MRI approaches, including DTI. We recently developed a novel approach, diffusion basis spectrum imaging (DBSI), to noninvasively detect increased cellularity and vasogenic edema associated with inflammation as well as axonal injury and myelin damage with improved accuracy [2]. The ability of this technique to noninvasively quantify optic nerve glial cellularity in a mouse model of glaucoma was assessed.

Methods:

DBA/2J mice (n = 4) at 9 months of age were compared with age-matched C57BL/6J mice (n = 5). Mouse heads were imaged ex-vivo following tissue fixation. The MRI protocol involved the acquisition of diffusion-weighted images taken in 99 directions using a multi-echo spin-echo sequence (TR = 1000ms, TE = 31.87ms, inter-echo delay = 21.18ms, b-value = 3000s/mm^2). Image datasets were processed using a DBSI algorithm designed to convert diffusion measurements into isotropic and anisotropic tensors so as to allow selective analysis of individual tissue components based on diffusion properties [2]. DBSI-derived cellularity, axial diffusivity, and radial diffusivity were calculated in manually selected image regions of interest corresponding to cross-sections of the optic nerves (Fig. 1). Standard DTI analysis was also performed to assess DTI-derived axial and radial diffusivity and compare results with DBSI. Histology was performed to compare the number of DAPI-stained cell nuclei within optic nerve tissue sections to the cellularity calculated with DBSI.

Results:

DAPI cell counts within optic nerve tissue sections increased by 24% in DBA/2J mice ($p < 0.001$) as compared to controls, suggesting possible glial cell proliferation or inflammatory cell infiltration. A corresponding rise in DBSI-calculated cellularity was seen in DBA/2J mice as compared to controls ($p < 0.001$). Notably, DAPI counts were highly correlated with DBSI cellularity ($r = 0.86$, $p < 0.001$, Fig. 2). DBSI axial diffusivity decreased by 18% in DBA/2J mice ($P < 0.001$), suggesting axonal injury. DBSI radial diffusivity increased by 20% in DBA/2J mice ($p = 0.005$), indicating possible myelin injury. Standard DTI axial diffusivity decreased by 27% ($p < 0.001$) and radial increased by 21% ($p = 0.02$) in DBA/2J mice. Comparison of axial diffusivity derived from DBSI and DTI showed that DTI detected a larger decrease in axial diffusivity in DBA/2J mice (Fig. 3).

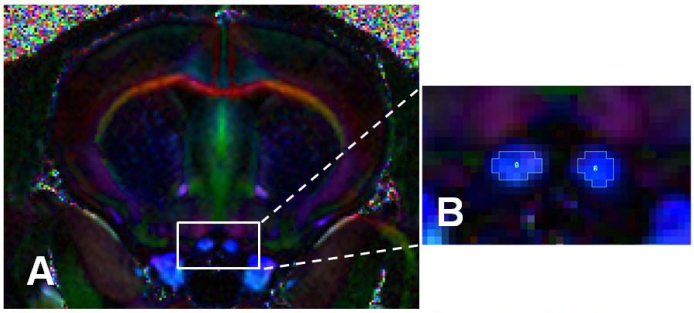

Fig. 1. Color-coded relative anisotropy map of a coronal image through the mouse brain: red, left-right; blue, anterior-posterior; green, superior-inferior. Panel (A) shows the full slice with the optic nerves highlighted. Panel (B) shows the magnified regions of interest selected over the optic nerves for DTI and DBSI calculations.

Discussion and Conclusions:

The detection of histological features such as cellular proliferation and infiltration has eluded modern MRI techniques. The present study demonstrates that DBSI can achieve accurate noninvasive quantification of optic nerve cellularity in a mouse model of glaucoma in addition to axon and myelin integrity. The exaggerated decrease in axial diffusivity in DBA/2J mice seen with DTI as compared to DBSI may be attributed to the restricted diffusion resulting from the increased cellularity.

The capability of DBSI to quantify optic nerve cellularity is encouraging. While most studies concerning reactive gliosis in glaucoma have focused on the optic nerve head, recent work has demonstrated that astrocyte reactivity and proliferation within the myelinated portion of the optic nerve occurs very early in the progression of glaucoma in DBA/2J mice and is a reliable marker of injury [3]. As the role of these important gliotic changes in glaucoma is further elucidated, the ability to noninvasively detect optic nerve cellularity using DBSI could have clinical utility both as a diagnostic tool and as a means of assessing or predicting therapeutic response in patients. Additionally, the ease of applying MRI-based methods to human studies gives this approach promising translational potential.

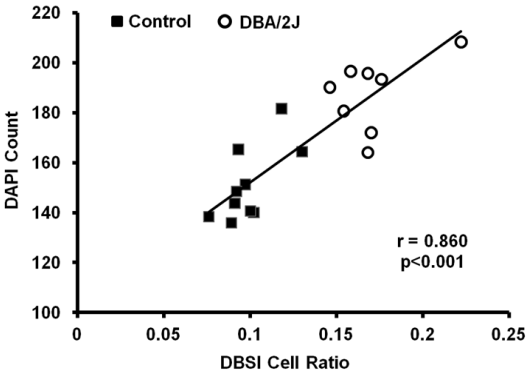

Fig. 2. Correlation between DAPI counts and DBSI-derived cell ratio calculations across 10 control optic nerves (closed squares) and 8 DBA/2J optic nerves (open circles) is significant ($r = 0.860$, $p<0.001$).

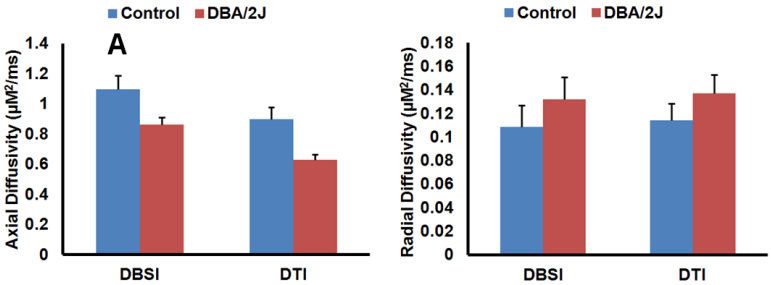

Fig. 3. DBSI and DTI results for axial and radial diffusivity. Panel (A) illustrates the decrease in axial diffusivity seen in DBA/2J mice using both DBSI ($p < 0.001$) and DTI ($p < 0.001$). A larger decrease is seen from control to DBA/2J with DTI than with DBSI (27% versus 18% decrease, respectively). Panel (B) illustrates the increase in radial diffusivity seen in DBA/2J mice using DBSI ($p=0.005$) and DTI ($p=0.02$). Increases detected by DBSI and DTI are comparable (20% versus 21%, respectively).

References:

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