DTI - Histology Correlation in Spinal Cord

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Introduction: The processes underlying traumatic spinal cord injury (SCI) contribute to numerous complex and significant consequences, many of which are still not well understood. Damage to the fiber tracts is one of the most important consequences of SCI. Diffusion tensor MRI (DTI) may be an effective tool to study the integrity of the spinal cord tracts in vivo. Recent DTI studies of normal spinal cord in rats showed areas of high fractional anisotropy (FA) in the dorsal columns as well as in the lateral columns [1]. However, the morphological correlate for this high anisotropy has not been identified. The purpose of these studies was to identify these regions of high anisotropy within the actual tissue using immunohistochemical techniques.

Methods: Adult male Sprague-Dawley rats weighing approximately 300 g were used in these studies. Each animal underwent surgery under isoflurane anesthesia for implantation of a 11x35 mm RF coil above the spine and was inductively coupled to an external coil for improved signal-to-noise ratio. All MRI scans were performed on a Bruker 7T scanner. The DTI studies were performed using icosa21pn encoding scheme that is balanced and unbiased [1]. Diffusion weighted images were acquired using a multi-shot EPI readout sequence with TR/TE=2000/39 ms. A total of 20 contiguous and interleaved axial slices, each of 2 mm thick, with an FOV of 2.62 cm were acquired. Immediately following the completion of MR studies animals were deeply anesthetized with sodium pentobarbital and were transcardially perfused, first with 150 ml ice-cold phosphate buffered saline (PBS), then 400 ml ice-cold 4% paraformaldehyde (4% PFA) in PBS. The spinal columns were removed and post-fixed overnight in 4% PFA at 4°C; the following day the columns were transferred to a 30% sucrose solution (in buffered saline) and kept overnight at 4°C. The spinal cords were then removed from the bone and marks were made on the dura to indicate the location of the overlying implanted coil. Cords were put back into 30% sucrose to optimize tissue infiltration and cryoprotection. Cords were then blocked into 5mm segments centered at the midpoint of the implanted coil region. Axial sections of the spinal cord tissue were cut at a thickness of 35 µm on a cryostat; sections were collected as free-floating. For immunohistochemical processing, sections were rinsed 3X in tris-buffered saline (TBS), then blocked in 5% pre-immune serum for an hour. Since both myelin and axons contribute to the diffusion anisotropy, a battery of immunohistochemical (IHC) labels was used to elucidate the type of cells involved. First, cytoskeletal properties were evaluated in order to verify neuronal or glial origin. To determine if the areas of elevated anisotropy were neuronal in nature, we assayed a battery of neuron-specific cytoskeletal proteins in the tissue sections, including alpha-internexin (Chemicon), NF-145 kD (Chemicon), NF-200 kD (Chemicon), or type III, beta-tubulin (Sigma). GFAP, the major intermediate protein of astrocytes (Chemicon), was used to determine whether astrocytes contributed to the areas of high anisotropy. Fluorescently-tagged secondary antibodies (Alexafluor dyes, Molecular Probes) were used to visualize target antigens. Sections were mounted onto glass slides and coverslipped with Fluoromount-G (Fisher) which contains anti-fading ingredients. Labeled sections were then imaged using a Bio-Rad Radiance 2000 confocal microscope. Eriochrome staining of additional spinal cord slices was also performed as a histological stain for detection of myelin. The next series of tests were performed in an attempt to classify the type of axons in the region. It was thought that the area of increased FA in the dorsal columns of the spinal cord contained ascending sensory axons; sections of spinal cord were incubated with an antibody against BDNF (R&D Systems), a marker for a subpopulation of ascending sensory populations. To further characterize the potential contribution of ascending sensory axons to the increased FA in the dorsal columns, the tract tracing molecules Cholera toxin B (CTb) and wheat-germ agglutinin (WGA) were injected into the sciatic nerves. Animals were left to recover for ten days to allow for anterograde tracing of the CTb and WGA. Following the period of recovery, animals were sacrificed, the spinal cords were removed for histology, and fluorescent immunohistochemistry was again performed to visualize the location of the fiber tracts.

Results and Discussion:

Fig. 1a shows the FA map of in vivo spinal cord, demonstrating the high FA values in the dorsal and lateral columns. When histology was compared to the DTI images, it was observed that the region of increased anisotropy better corresponded to neuronal cytoskeletal components, rather than that of glial cells. Also, though it was established that the region contained axons, it is interesting to note that only a specific isoform of intermediate filament, NF-200 kD, was found to stain the area that corresponded to increased anisotropy (Figure 1b) in both the dorsal columns and lateral tracts. This is significant in that this population of axons is highly specific to these fiber tracts exhibiting increased FA. Eriochrome staining for myelin also stained regions that corresponded to the high anisotropy values in the DTI images (Figure 1c). Morphologically, the eriochrome labeling detected a region of intense signal that corresponded to a region of high FA, the dorsal columns, located directly above the corticospinal tract. This area is known to contain ascending sensory tracts, so the next steps in the experiment were aimed towards validating this hypothesis. In tests to determine the type of axons in the region, sections of spinal cord were incubated with antibodies against BDNF, a specific marker for a subpopulation of ascending sensory axons, but that antibody did not stain the fiber tracts corresponding to the highlighted regions in DTI images. In the experiments in which rats received sciatic injections containing CTb and WGA, the populations of axons of interest were not labeled by either the CTb or WGA. The selective correlation of only the heavy isoform of neurofilament (NF-200) with areas of high FA suggest that the areas identified in both the dorsal and lateral columns may be due to the presence of large caliber axons. The actual phenotype of the tracts exhibiting a high degree of FA in DTI images is still unknown, requiring further analysis.

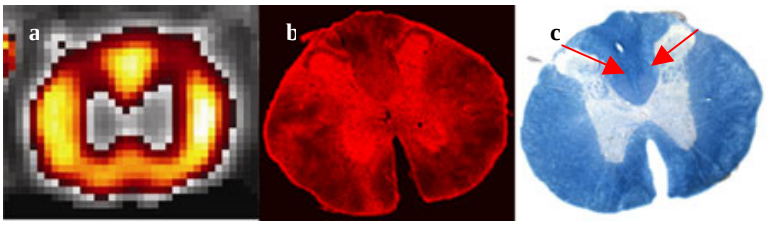

Fig. 1. (a) In vivo FA map of spinal cord, based on DTI, (b) neurofilament (200kD), and (c) eriochrome stained slides. The arrows indicate regions of high FA values.

Conclusions: IHC assays were performed in order to understand the origin of the observed high diffusion anisotropy in the dorsal and lateral columns in normal spinal cords. The observed spatial association between histology and high FA values, observed only with heavy NF-200, suggests that these regions contain large caliber axons. In addition, the correspondence between intense eriochrome staining and the high FA value in the dorsal column suggests a significant myelin contribution to the observed anisotropy in this region.

References: [1] Madi S, Hasan KM, Narayana PA, Magn Reson Med (2004; In Press)

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