A Comparative Study of Diffusion MRI and Histology After Ischemic Stroke in Rats

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Introduction

Diffusion-weighted (DW) MR imaging can be used to follow the progression of tissue injury caused by cerebral ischemia and can detect damaged tissue earlier than other imaging methods.[1] DW imaging is sensitive to the random translational motion of water molecules in tissue and relates image intensity to their relative mobility,[2] and diffusion images can be used to map the apparent diffusion coefficient (ADC) of water. The tissue damaged by cerebral ischemia shows a decrease in ADC believed to be due to cytotoxic edema as water moves from a less restricted extracellular space to a more restricted intracellular space.[3] Although early changes in diffusion images have been documented, early histological correlates have not been clearly established. This study describes early histological changes in ischemic brain tissue in regions showing alterations in DW imaging.

Methods

Animal Model: All animal procedures were approved by the Institutional Animal Care Committees at the University of Manitoba. Cerebral ischemia was induced in 19 male Sprague-Dawley rats by the injection of the vasoconstrictive peptide endothelin-1 (ET-1) adjacent to the middle cerebral artery (MCA), while sham rats (n = 2) were injected with saline.

Imaging: DW images were obtained following surgery using a Bruker Biospec 7T/21cm spectrometer with a volume coil 30mm in diameter using a magnetization-prepared fast low angle shot (FLASH) sequence[4] with repetition time (TR)=3.7ms, echo time (TE)=2.3ms, flip angle=12°, field of view (FOV)=5x5cm^2, matrix size=128x128, Gaussian pulse=1ms, 64 averages. One image served as a control with low diffusion gradient strength with b value of 21s/mm^2, while DW images were obtained with high gradient strength with b value of 1030s/mm^2 applied in each of the x, y, and z directions. Serial DW images were obtained immediately after MCA occlusion, and animals were subsequently killed at times ranging from 1-6 hours after MCA occlusion. Histological features were then compared to features in the MR images obtained immediately before each animal was killed.

Histology: Brain sections were stained for IgG (Immunoglobulin G), MAP-2 (microtubule-associated protein-2), injured neurons (Fluoro Jade), and general features of tissue damage (Hematoxylin and Eosin, H&E).

Data Processing: From the DW images mean ADC maps were generated**.** Regions of the cortex and striatum that showed degenerating neurons stained with Fluoro Jade were superimposed onto the diffusion images to demonstrate the correspondence between early changes in DW images and histological evidence of tissue injury. The percent decrease in ADC in the regions of striatum and cortex that showed Fluoro Jade staining was determined, and the threshold ADC level giving the best correlation of lesion area read from the MR images with the area showing neuronal injury in Fluoro Jade stained sections was determined separately for cortical and striatal tissue.

Results and Discussion

Sham rats showed no changes in ADC maps or histology. In rats that were injected with ET-1 the region of increased intensity on DW images, or decreased ADC, increased in area and intensity over time (Figure 1). Histological signs of tissue damage in brain regions that showed a decrease in ADC on the DW images were evident from as early as one hour after MCA occlusion. The H&E stained sections revealed the presence of patchy areas of spongy edematous tissue containing shrunken neurons with pyknotic nuclei in regions of induced ischemia. Loss of dendritic MAP-2 staining was also observed in these same areas, suggesting a breakdown of the dendritic cytoskeleton. However, lack of IgG staining in the brain tissue indicated that there was no damage to the blood-brain barrier. Fluoro Jade stained sections showed clear evidence of neuronal injury in the brain regions with altered ADC. In regions of Fluoro Jade staining that were superimposed onto the ADC maps (Figure 2), the average decrease in ADC was observed to be 19.6% and 10.9% in the cortex and striatum respectively. Lesion areas determined from the ADC maps corresponded to regions of neuronal injury with threshold decreases of 15% and 10% in ADC for the cortex and striatum respectively (Figure 3). In conclusion, alterations in DW MR imaging parameters that occur in the hyperacute phase (1-6 hours) after cerebral ischemia occur in brain regions with histologically evident neuronal damage.

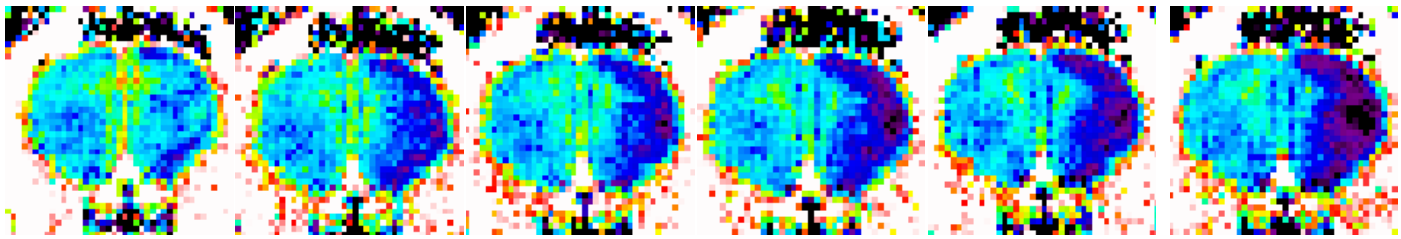

Figure 1. ADC maps obtained at the indicated times after MCA occlusion in a representative rat.

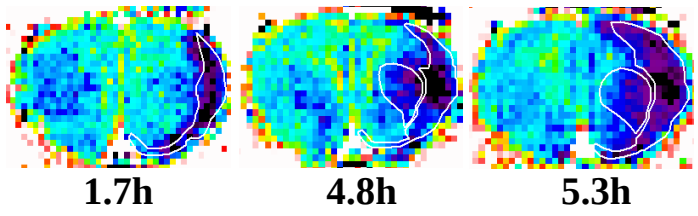

Figure 2. An outline of Fluoro Jade stained regions superimposed onto ADC maps for rats killed at the indicated times after MCA occlusion.

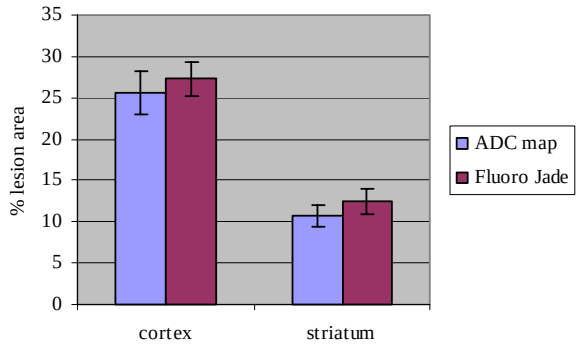

Figure 3. A comparison of lesion areas (as % of the ischemic hemisphere) determined from Fluoro Jade staining with ADC map lesion areas using thresholds of 15% and 10% for the cortex and striatum respectively.

References

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