LONGITUDINAL MICROSTRUCTURAL WHITE MATTER CHANGES IN A TRANSIENT FOCAL ISCHEMIC STROKE RAT MODEL USING A TRACT-BASED SPATIAL STATISTICS (TBSS) METHOD

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Introduction

Previous studies have shown anatomical changes (i.e. fractional anisotropy (FA) and mean diffusivity (MD)) in rats after experimental stroke using ROI-based analysis[1, 2]. The correspondence of voxels across different rats cannot be assumed for image registration due to the abnormality of white matter (WM) tracks near the infarct areas, for which previous studies limited to ROI-based analysis. Recently, the tract-based spatial statistics (TBSS) method has been shown to provide reliable voxel-by-voxel analysis by the projection of diffusion tensor indices onto an alignment-invariant WM skeleton[3]. Using the TBSS method, in this study, we examined spatial changes of diffusion tensor indices in a voxel-by-voxel manner in rats recovering from experimental stroke.

Methods

Focal ischemic model in rat brain: The transient middle cerebral artery occlusion (t-MCAO) model was used to create a focal cerebral ischemia animal model (n=6, 300-350g). The blood flow of the middle cerebral artery was blocked for 90 minutes. After 90 minutes, the inserted silk thread was removed and reperfused

Acquisition of MR Data: The MRI data were obtained at BL and 4h, 1, 3 and 6 weeks after inducing focal ischemic brain injury in the MCAO rats using a 7.0 Tesla MRI scanner (Bruker Biospin GmbH, Ettlingen, Germany). For MRI experiments, rats were initially anesthetized using an artificial ventilation system, and the body temperature was maintained at 36℃±1. T2 weighted imaging (TR/TE=3000/60 ms, resolution = 120×120 μm^2, slice thickness= 0.75 mm) and DTI (TR/TE=4500/37 ms, resolution= 160×160 μm^2, slice thickness = 0.75 mm, gradient direction = 30, gradient duration (δ) = 5ms, gradient separations (Δ) = 15ms, b-values = 1000 s/mm^2) were acquired for each rat.

MRI data analysis: DTI analysis to evaluate microstructural WM integrity was performed with the FMRIB Software Library (FSL) package[4]. In preprocessing, the diffusion weighted images were linearly aligned to a b0 image to correct for head motion and eddy current effects, and non-brain tissues were masked out. The brain mask was semi-automatically depicted. Next, the diffusion tensor was calculated at each voxel to generate the fractional anisotropy (FA) and mean diffusivity (MD) maps. The b0 images for each animal were linearly registered to the Paxinos & Watson rat brain space[5], and then the derived-transform matrix was applied to the FA and MD maps. In TBSS, the individual baseline FA maps were nonlinearly aligned with each other to identify the best target requiring minimum transformation and others were warped to this target. Following registration, the aligned FA maps were averaged and thinned to construct the mean FA map and skeleton mask used as a template in the subsequent registration. The individual FA maps (baseline and follow up) were nonlinearly registered to mean FA map and projected onto skeleton mask. The registration and projection vectors derived from FA processing were applied to the MD map. Finally, voxel-wise differences were statistically carried out using a paired two-sample t-test with the baseline dataset, setting the number of permutations at 1000 and taking into account significance at $p < 0.05$ with a threshold-free cluster enhancement (TFCE) correction.

Results

At 4 hours post-stroke, FA values remained unchanged in any tracts measured, whereas MD values significantly decreased in perilesional WM. The integrity of these tracts was dramatically changed with a decrease in FA and an increase in MD 1 week later. During the time shift to 3 and 6 weeks after stroke, decreased FA areas showed gradual decline from around the corpus callosum to the external capsule, meanwhile, the areas of evaluated MD values were observed with constant increasing patterns from the external capsule to the corpus callosum.

Discussion and conclusion

In this study, we estimated longitudinal changes in diffusion tensor indices in MCAO rats model at 4 hours (acute stage) and 1 week (sub-acute stage), 3 and 6 weeks (chronic stage). We found the regional changes of MD and FA over time using the TBSS method, which would have been impossible using a traditional voxel-wise method (i.e. voxel-based morphometry (VBM)) due to the spatial abnormality of WM tracks. The decrease in MD at an acute stage may be caused from the cellular swelling of oligodendrocytes which maintains the structural integrity of WM, the increase in MD at a later stage may be involved with the degradation of WM integrity by cell lysis and demyelination. The normalized FA from the corpus callosum to the external capsule over time may be caused from the recovery of ischemic brain damage through reorganization of WM tracks, but further study such as histological evaluation or correlation between neurological and functional recovery are required to verify these findings.

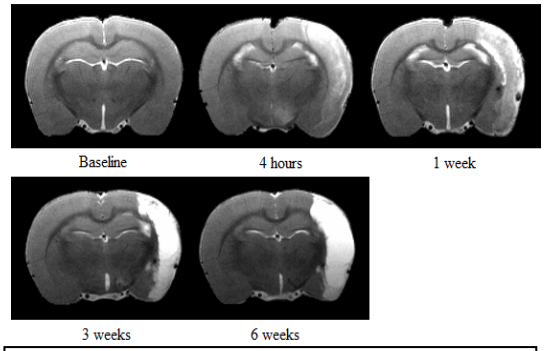

Figure 1. An example of T2-weighted images at multiple time points (baseline; 4 hours; and 1, 3, 6 weeks) after ischemia.

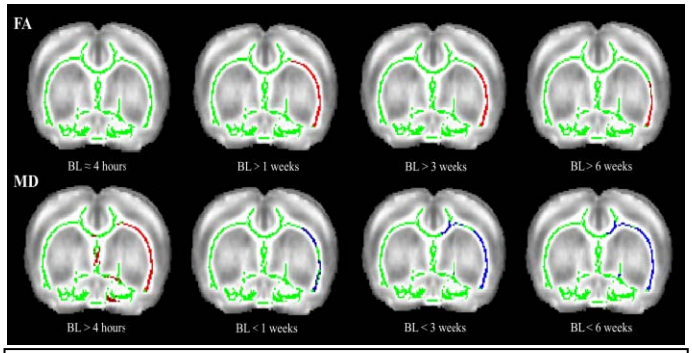

Figure 2. FA and MD changes in right MCAO rat model over time compared with baseline (red: decreased; blue: increased). WM skeleton is shown in green.

References

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