Abnormalities in the Visual System of Streptozotocin-induced Type 1 Diabetic Rats-A Diffusion Tensor Imaging Study

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Introduction Diabetes is a common cause of visual loss [1]. Basic visual function is disturbed in diabetes, e.g., delayed visual evoked potentials [2]. Complex visual disturbances are also a feature of diabetes, since patients are negatively affected in the visuoconstruction, visual-motor skills and visual-spatial skill [3]. In this study, diffusion tensor imaging (DTI) and histology were used to assess microstructural abnormality in the visual system in type 1 diabetic rats induced by STZ injections.

Materials and methods Animal preparation: Eight weeks old male Sprague-Dawley rats (202-278 g) were randomly separated into two groups. In the experimental group, diabetes were induced by a single intraperitoneal injection of streptozotocin (STZ, 62 mg/kg) dissolved in citric acid solution (0.01 M, PH 4.5) (STZ, n=7). Control animals were injected with the same amount of solvent (Con, n=7). After 3 days, the rats in the STZ group with fasting (12-h) blood glucose < 11.3 mM were excluded from this experiment. MRI and histological evaluations were performed at 12 weeks after diabetes induction. MRI scanning: MRI experiments were performed using a Bruker Biospec 7.0 T/20-cm MRI scanner. A 72-cm-diameter volume coil was used for radiofrequency (RF) transmission, and quadrature surface coil for signal detection. DTI was performed with a 4-shot spin-echo echo planar imaging sequence. Diffusion-sensitizing gradients were applied along 30 directions homogenously distributed on the unit sphere. Five non-diffusion-weighted images (b = 0) and 30 diffusion-weighted images (b = 800 s/mm^2) were acquired during each DTI scanning. The other imaging parameters: Repetition time (TR) 5000 ms, echo time (TE) 26 ms, field of view (FOV) 3 cm × 3 cm, slice thickness 0.8 mm, matrix size 128 × 128, time between application of gradient pulses (Δ) 14ms, diffusion gradient duration (δ) 3 ms, and 4 averages. Data processing: For each rat, the diffusion-weighted images were realigned to the non-diffusion-weighted images with an affine transformation used Diffusion Toolbox in FMRIB's software library within FSL (**http://www.fmrib.ox.ac.uk/fsl**) to minimize eddy currents disturbance. Three eigenvalues were derived from the diffusion tensor matrix diagonalization. Fractional anisotropy (FA), axial diffusivity ($ADC_{\parallel}$) and radial diffusivity ($ADC_{\perp}$) maps were calculated used DTIstudio 3.0. The regions of interest (ROIs) were selected on the optic tracts and visual cortex on the FA map (Fig. 1) and then projected onto $ADC_{\parallel}$ and $ADC_{\perp}$ maps. Average FA, $ADC_{\parallel}$ and $ADC_{\perp}$ values in the ROIs were calculated. Independent samples t-test was used for statistical analysis. Histological evaluation: Following the MRI scanning, the rats were executed for histological evaluation. Axonal degeneration and myelin damage were assessed with anti-phosphorylated neurofilaments SMI-31 antibody and and anti-myelin basic protein antibody (MBP) respectively in the cross-sections of the optic tracts (OT). In the cross-sections of the visual cortex (VC), methylene blue staining was used for the assessments of cellular architecture and axonal injury and uranyl acetate-lead citrate staining for the assessments of myelin integrity.

Results Compared to controls, the diabetic rats showed significantly reduced FA in both the OT and VC at 12 weeks after diabetes induction. Correspondingly, significantly decreased $ADC_{\parallel}$ and significantly increased $ADC_{\perp}$ were associated with hyperglycemia. The VC in the diabetic rats had a slight decline in $ADC_{\parallel}$, and a slight increase in $ADC_{\perp}$ relative to those in controls (Fig. 1). In Fig. 2, preliminary results showed that the OT of the STZ-induced rats are characterized by reduced SMI-31 and MBP immunostaining. The VC of the STZ-induced rats is characterized by atrophic soma and axon of pyramidal neuron (methylene blue staining), and rarefied myelin sheath (uranyl acetate-lead citrate staining).

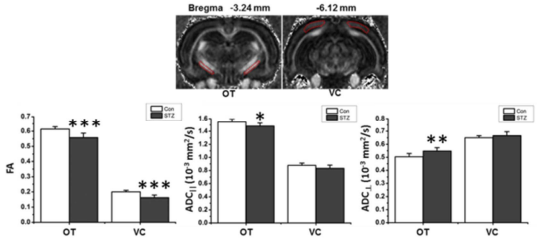

Figure 1. FA, $ADC_{\parallel}$ and $ADC_{\perp}$ values in the optic tracts and visual cortex at 12 weeks after diabetes induction. FA value was significantly lower in the diabetic rats than that in controls both in the optic tracts and visual cortex. Significantly decreased $ADC_{\parallel}$ and significantly increased $ADC_{\perp}$ were seen in the optic tract. No significant difference was found in both $ADC_{\parallel}$ and $ADC_{\perp}$ between the diabetic rats and controls in the visual cortex. * $p < 0.05$, $p < 0.01$, $p < 0.001$, compared to controls.

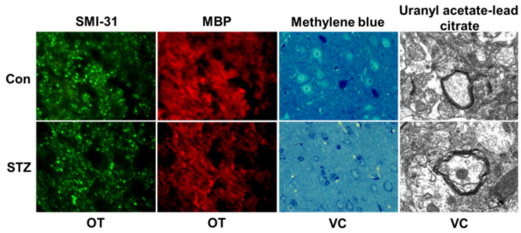

Figure 2. SMI-31 and MBP immunostaining of the optic tracts, and methylene blue and uranyl acetate-lead citrate staining of the visual cortex at 12 weeks after diabetes induction.

Discussion The DTI results suggest the destruction of microstructural integrity (reduced FA, decreased $ADC_{\parallel}$ and increased $ADC_{\perp}$) in the visual pathway (OT and VC) at 12 weeks after diabetes induction. The histology results in our work suggest axonal degeneration and myelin damage in the OT and VC of the diabetic rats at this time point. Reduced $ADC_{\parallel}$ and increased $ADC_{\perp}$ are presumed to mainly reflect the axonal injury and demyelination respectively [4]. Our finding is consistent with this. In conclusion, this preclinical study shows that the DTI technology can detect the potential microstructural abnormalities in the visual system in diabetes.

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