Neurodengeneration in Optic Tracts of Rats Subjected to Bilateral Common Carotid Artery Occlusion-A Longitudinal DTI Study

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Introduction Permanent bilateral common carotid arteries occlusion (BCCAO) induces chronic cerebral hypoperfusion. It has been widely used as a model to mimic cerebrovascular impairment observed during human aging and Alzheimer's disease[1,2]. Many previous studies have shown that animals subjected to BCCAO develop white matter lesions as a result of chronic hypoperfusion[3,4]. In this study, diffusion tensor imaging (DTI) and immunohistochemistry were used to investigate the development of white matter lesion longitudinally in the brain of rats subjected to BCCAO.

Materials and methods Male Wistar rats (180-220 g) were anesthetized by i.p. injection of 5% chloral hydrate (6 ml/kg). For the animals in the BCCAO group (n=10), both common carotid arteries were exposed and double-ligated with silk sutures. The rats in the control group (n=10) received sham surgeries, but without having the common carotid arteries ligated. On day 2 (d2), day 7 (1w), day 14 (2w) and day 28 (4w) after the surgeries, all animals were imaged on a 7 T/20 cm Bruker Biospec scanner. A volume coil was used for RF transmission, and a quadrature surface coil for signal detection. DTI was performed with a 4-shot spin-echo EPI sequence, an encoding scheme of 30 gradient directions homogenously distributed on the unit sphere and following parameters: TR 5000 ms, TE 26 ms, FOV 3 cm×3 cm, slice thickness 0.8 mm, matrix size 128×128, Δ 14ms, δ 3 ms, b=0 and 800 s/mm^2 and 4 averages. The rats were transcardially perfused after the last MRI examination. The brains were removed and stored in the fixative overnight. Coronal sections of 30 μm thickness were used for SMI-31 (hyperphosphorylated neurofilament) and GFAP (glial fibrillary acidic protein) immunostaining. Coronal sections of 10 μm thickness were used for LFB staining (Klüver-Barrera staining). For each rat, raw DTI data were used to obtain the fractional anisotropy (FA), mean diffusion coefficient (MD), axial diffusivity ($ADC_{//}$) and radial diffusivity ($ADC_{\perp}$) maps. Regions of interest (ROI), such as the optic tracts (OT), were drawn manually on these maps (Fig. 1A). Average FA, MD, $ADC_{//}$ and $ADC_{\perp}$ values in the ROIs were calculated. Inter-group comparisons were performed with independent-samples *t*-test. Repeated measures ANOVA was used to assess statistical significance of time-related changes and group× time interactions.

Results Compared to the control animals, BCCAO rats showed significantly reduced FA in the OT at all time points (Fig. 1B). $ADC_{//}$ reduction in the OT of the BCCAO rats was statistically significant at 7d, 2w and 4w, but not at 2d (Fig. 1D). In contrast, $ADC_{\perp}$ in the OT of BCCAO rats was found significantly increased at 1w and 2w (Fig. 1E). Within 4w after BCCAO, no statistically significant changes were observed for MD of the OT between the groups (Fig. 1C). FA changes in the OT showed significant group× time interaction (Fig. 1B), as revealed by repeated measures ANOVA. Immunohistology data in Fig. 2 showed that the OT of the BCCAO rats are characterized by presencet of vacuoles and increased crookedness of myelin fibers (LFB staining), significantly reduced SMI-31 immunostaining and gliosis.

Discussion Previous studies[4] have found that the number of fibers immunolabeled for encephalitogenic peptide (EP) increased in the OT of BCCAO rats over time from day 1 to 30 after surgery, and the amyloid β/A4 precursor protein (APP) immunoreactivity in the OT increased from day 1, peaked at day 7-14 and then diminished at day 30. As indicators of WM degeneration, the EP and APP immumopositivity are thought to represent demyelination and some degree of axonal injury respectively. Our immunohistology results (Fig. 2) also suggest that myelin degeneration and axonal damage were present in the OT of BCCAO rats at 4w.

$ADC_{\perp}$ represents water diffusivity perpendicular to the myelin sheath. $ADC_{\perp}$ in the OT of BCCAO rats was significantly higher than that in the control rats at 1w and 2w, likely as a result of myelin degeneration. $ADC_{//}$ represents water diffusion parallel to the axon fibers. $ADC_{//}$ in the OT of BCCAO rats became significantly lower than that in the control rats already at 1w, and this trend lasted up to 4w. This result was consistent with SMI-31 staining and the evolution of APP immunoreactivity in the OT of BCCAO rats mentioned above. In addition, astrogliosis may also have played a role in causing changes in the water diffusivity in the OT of the BCCAO rats.

References [1] Kuo T, etc, Stroke, 21:1205-1209, 1990. [2] Farkas E, etc, Brain Research Reviews, 54:162-180, 2007. [3] Schmidt-Kastner R, etc, Brain Research, 1052:28-39, 2005. [4] Wakita H, etc, Brain Research, 924:63-70, 2002.

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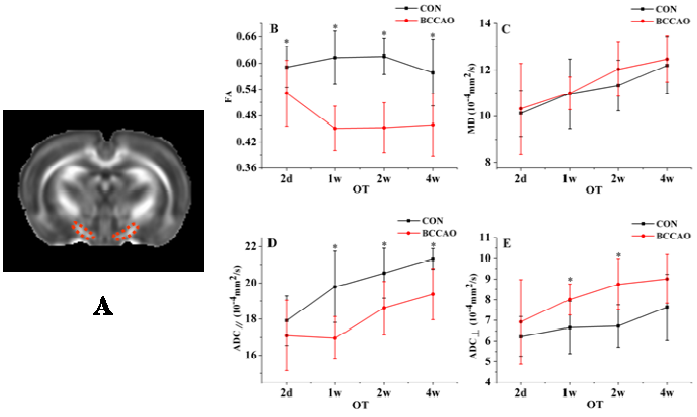

Figure 1. FA, MD, $ADC_{//}$, $ADC_{\perp}$ values in the optic tract as a function of time after BCCAO. A significant group× time interaction was observed for FA, but not for MD, $ADC_{//}$ and $ADC_{\perp}$. * p< 0.05, statistical inter-group significance at each time point.

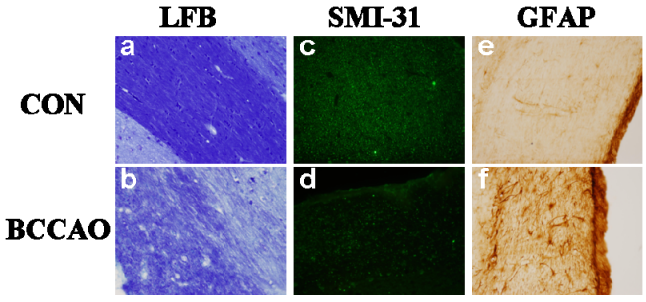

Figure 2. LFB staining, SMI-31 and GFAP immunostaining of the optic tract at 4 weeks after BCCAO.