Monitoring Ischemic Cerebral Injury in Spontaneously Hypertensive Rats by Diffusion Tensor Imaging

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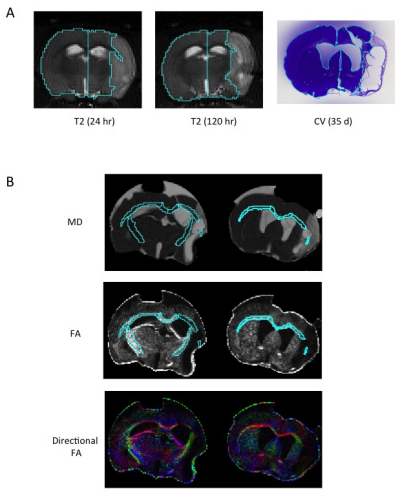
Introduction

Diffusion tensor imaging (**DTI**) is increasingly being used to interrogate microstructural changes in white matter integrity during developmental and pathological processes. We have applied DTI to the widely used middle cerebral artery occlusion (**MCAO**) model of cerebral ischemia.

Methods

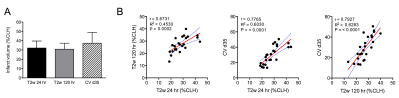
We performed ex vivo DTI 35 days after 60 min transient focal ischemia in male spontaneously hypertensive rats and generated fractional anisotropy (**FA**), mean diffusivity (**MD**), axial diffusivity (**AD**) and radial diffusivity (**RD**) maps. Regions of interest (**ROI**) corresponding to external capsule (**EC**), corpus callosum (**CC**) and internal capsule (**IC**) were compared among sham and stroked rats. We also compared tractographic projections of white matter fiber patterns and examined white matter integrity by Luxol fast blue histological analysis. We determined infarct lesion volumes at 24 hr and 120 hr post-ischemia by T2-weighted magnetic resonance imaging (**T2w**-MRI) and at 35 days by histological staining with cresyl violet (**CV**) and examined correlations between infarct volume and relative FA.

Fig. 1: Regions of interest for infarct volume and DTI measurements



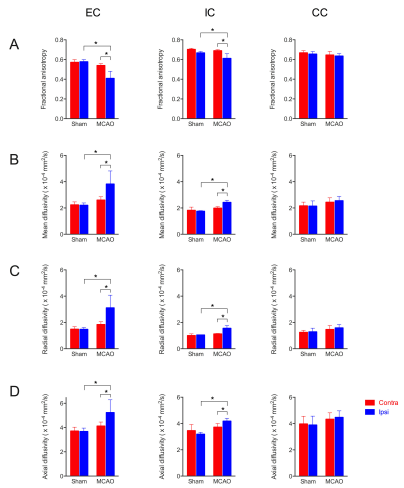
(A) Representative ROI of healthy ipsilateral or contralateral tissue were used to calculate the infarct volume across rat brain slices. (B) White matter ROI for EC, IC or CC were used to measure FA, MD, AD, and RD. Right DTI images are from the same rat as in Fig. 1A.

Fig. 2: Infarct volumes & correlations



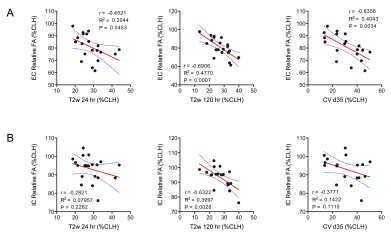
Infarct volume measured by the indirect method (healthy ipsi minus healthy contra, summed across slices) off of T2w-MRI or CV-stained rat brain slices 24 hr, 120 hr or 35 d after MCAO. Mean±SD. %CLH, percent contralateral hemisphere.

Fig. 3: Anisotropy and diffusivity measurements



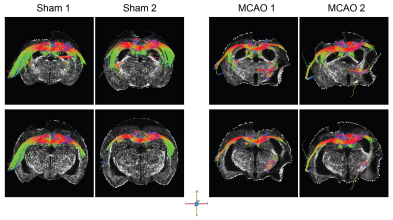
(A) Mean FA, (B) mean MD, (C) mean RD, and (D) mean AD in ipsi and contra EC, IC, and CC of sham or MCAO rats 35 d post-surgery. Mean±SD. *, P<0.05

Fig. 4: Correlation of infarct volume to relative FA



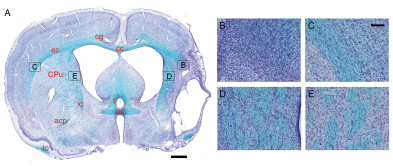
Correlation among T2w-MRI-derived or CV-derived infarct volumes and relative FA for EC (A) or IC (B). Relative FA represents the ratio of mean ipsi FA to mean contra FA. %CLH, percent contralateral hemisphere. P values are uncorrected for multiple comparisons.

Fig. 5: Fiber tractography of sham and MCAO rat brains



Fiber tracking by a deterministic algorithm was seeded with the CC ROI. Results for two rats per condition are shown against an axial FA image for orientation. The tract directions are color-coded: green, dorsal-ventral; red, left-right; blue, anterior-posterior.

Fig. 6: Histological assessment of white matter integrity



(A) Axial section of an MCAO rat brain stained with Luxol fast blue (myelin) and CV, stitched from multiple 4X images. Boxed regions shown in B–E. Scale bar=1 mm. 20X fields from (B) ipsi EC, (C) contra EC, (D) perilesional caudate/putamen, (E) contra caudate/putamen. Scale bar in C=100 m and applies to panels B–E.

Results

We found evidence of alterations in external capsule and internal capsule, but not corpus callosum, as represented by decreased fractional anisotropy. We also observed increased mean, axial and radial diffusivities in the EC and IC. The size of the ischemic lesion detected subacutely by T2w-MRI or at 35 days by CV staining correlated with the extent of decline in FA in the affected structures in multiple cases. Tractography revealed disruption of fiber trajectories through the EC and reorientation of fibers within the caudate/putamen of rats subjected to MCAO. Similarly, loss of white matter integrity in the EC and increased white matter density in the caudate/putamen along the infarct border zone was also evidenced by Luxol fast blue staining of myelin.

Conclusions

Diffusion tensor imaging allows for monitoring of white matter injury and reorganization in hypertensive rats.

Learning Objectives

Diffusion tensor imaging succesfully detects white matter injury and reorganization in hypertensive rats.