A longitudinal study of DTI in a nonhuman primate model of Neuro-AIDS

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Introduction: Because of its ability to characterize brain white matter integrity [1-4], Diffusion tensor imaging (DTI) has been proposed as a quantitative marker of the neurological status of HIV$^+$ patients. Longitudinal trials are, however, not feasible for neuro-AIDS patients. The macaque Simian immunodeficiency virus (SIV) model exhibits neuropathological symptoms similar to those seen in HIV$^+$ humans, and has been widely used for studying the cognitive and neuropathological sequelae of AIDS [5]. The aim of this study was to use non-invasive DTI imaging to characterize white matter abnormalities in specific regions of SIV-infected monkeys longitudinally in order to assess the validity of DTI findings as a non-invasive marker of the neurological status of neuro-AIDS patients.

Methods: Three adult male pig-tailed macaques (Macaca nemestrina) were infected with the SIVsmm9virus [6, 7]. DTI scans were obtained at baseline and at intervals until sacrifice 6 months later. During scans, animals were anesthetized and immobilized with a custom-built monkey head holder. Anesthesia was maintained with 1-1.5% isoflurane. Et-CO_2, inhaled CO_2, O_2 saturation, blood pressure, heart rate, respiration rate, and body temperature were monitored continuously. Blood for monitoring of CD4 and CD8 T-cells [4] was collected on 3 occasions prior to inoculation (three times), and at post-inoculation weeks 2, 4, 8, 12, 16, 20 and 24. Cognitive behavioral tests (cued and uncued attention, basic DNMS and mixed delays, DRST spatial, Progressive Ratio) were carried out at corresponding time point as well. Scans were collected in a Siemens 3T Trio scanner, with the Siemens CP extremity coil. A segmented double-spin echo sequence (provided by BITC, Emory University) was used for DTI data. Sequence parameters were: TR= 4800ms/TE = 89 ms, FOV= 96 mm × 96 mm, data matrix = 128 × 128, slice thickness = 1.5 mm, 2 shots, b = 0, 1000 s/cm^2, 30 directions, the scan was repeated three times. The whole brain was covered with 32 slices. The splenium and genu of corpus callosum, and frontal white matter (frontal WM) were selected as Regions of interest (ROIs) for analysis of the fractional anisotropy (FA) and mean diffusivity (MD). SPM software *(www.fil.ion.ucl.ac.uk/spm)* was employed to register DTI scans to each animal's initial baseline scan.CSF was excluded in whole brain analyses of FA and MD. Longitudinal changes in measures of interest were analyzed with repeated measure analysis of variance (ANOVA) and the Student's t-test. P-values less than 0.05 were considered statistically significant.

Results: Whole brain DTI results are shown in Figures 1 and 3. Whole brain FA declined significantly (Fig.1 left). MD showed an increasing tendency following inoculation but this trend did not reach statistical significance (Fig.1 right).The ROI analyses of the FA and MD are shown in Figure 2. FA value in genu of corpus callosum was reduced after the inoculation while there are no changes found in the frontal WM. The longitudinal results for FA and MD for whole brain and ROIs were shown in Figure 3 and 4 respectively. Significant declines in FA were observed in the genu (8th and 12th week after inoculation) and splenium (16th and 24th week after inoculation).There was an increase in MD in genu (20th week, not shown) as compared with baseline; The cross-correlations of the FA and MD in whole brain and ROIs with CD4 counts and CD4:CD8 ratios are shown in Table 1. FA values in whole brain correlated significantly with CD4 cell counts. No significant change was observed in the cognitive performance following viral inoculation. CD4 cell counts declined progressively, with significant differences from baseline at weeks 4, 12, 16, 20 and 24 after inoculation (Fig.5).

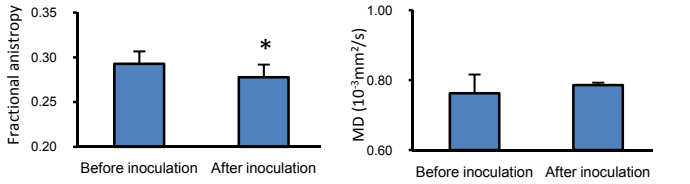

*Fig.1 Means and SDs of FA (left) and MD (right) of the whole brain before and inoculation, *P<0.05*

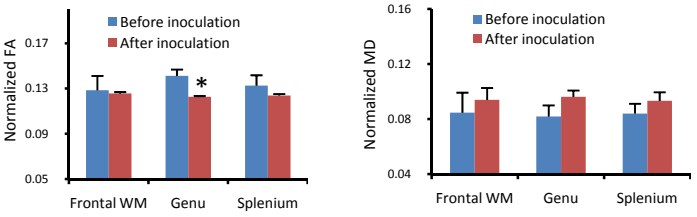

*Fig.2 Means and SDs of FA (left) and MD (right) of the ROI, before and inoculation, *P<0.05*

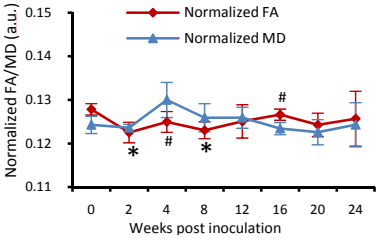

*Fig.3: Longitudinal normalized FA and MD changes in whole brain, Error bars denote standard deviation. *, #, respectively showed that P<0 .05 compared with baseline and 2nd week.*

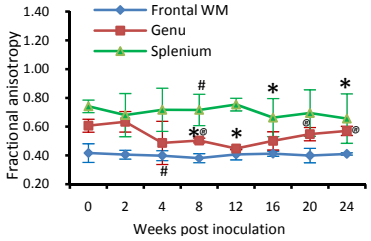

*Fig.4: Longitudinal FA changes in selected ROI, Error bars denote standard deviation. *, #, and®, respectively showed that P < 0.05 compared with baseline, 2nd week and 12th week.*

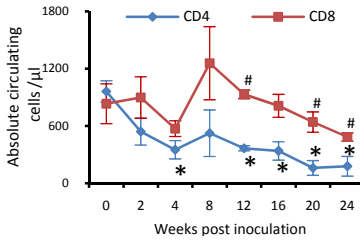

*Fig.5 Longitudinal analysis of circulating CD4 and CD8 cells in SIV-infected macaques. Error bars denote standard deviation error. *P<0 .05 compared with baseline; # P< 0 .05 compared with baseline 16th week.*

Region	CD4	CD4/CD8
Whole brain	0.53*	0.22
Frontal WM	0.37	0.35
Genu	0.16	0.14
Splenium	0.18	0.33

*, P<0.05

Table 1 Correlation of CD4 counts and CD4:CD8 ratio with FA.

Discussion and Conclusion: The neurological examinations result showed SIV-infected monkeys were not any significantly abnormal behavior, which indicated SIV-infected monkeys were not obvious cognitive abnormities. The whole brain reduction in FA and MD increase after viral inoculation are consistent with the results in HIV$^+$ patients [1], and the FA reduction in the genu and splenium of the corpus callosum are as same as reported in humans [1-4]. The results indicate the SIV-infected monkey is an excellent model for resembling the brain tissue changes after viral inoculation. MD after inoculation tends to increase in all the ROIs, but no significant changes were observed (Fig 2), which is consist with Thurnher et al's report [3], but different from Wu et al's result [2], perhaps because of the small study cohort and involved in different disease stage. In the longitudinal data, the FA value of the whole brain correlated significantly with CD4 cell counts, which further suggest that DTI may identify changes in brain tissue status during asymptomatic period. However, the correlation between the CD4 counts (or CD4:CD8 ratio) and ROI results of FA and MD are not significant. This study suggests that further investigation with large sample size is needed to validate the effectiveness of DTI measurement to access and evaluate the progress and severity of the disease.

In conclusion, a reduction in FA reduction and an increase in MD were observed evidently after viral inoculation and whole-brain FA changes correlated significantly with CD4 depletion. Findings from this investigation support the use of DTI for measurement of HIV associated neuropathologic changes. Further longitudinal study is needed to investigate the validation of DTI measures as a marker for disease progression.

References: [1] Ragin, J Neuroradiol (2004); [2] Wu et al, AJNR (2006); [3] Thurnher et al, AJNR (2005); [4] Tucker et al, Journal of Neuroimmunology (2004); [5] Williams et al, J Neuroviral (2008); [6] Francis J. Novembre et al. Journal of Virology (1998); [7] O'Neil et al, Am J Pathol (2004)