

Longitudinal cerebral metabolic alterations in a novel macaque model of neuro-AIDS

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Introduction: The neurochemical alterations associated with the inflammatory process and neuronal loss have been observed in HIV⁺ patients in the *in vivo* and *ex vivo* studies with magnetic resonance spectroscopy (MRS) [1-3]. Most studies reported significant NAA reduction in the patients with moderate to severe AIDS dementia but not in those who were neurologically asymptomatic [1]. However, these findings in neuroasymptomatic patients remain inconclusive as the results were obtained under variable conditions such as, time of infection, treatment history, sex, age. The non-human primate model of Simian Immunodeficiency Virus (SIV) exhibits neuropathological symptoms similar to those seen in HIV⁺ humans [4]. Therefore it is ideal for studying the CNS dysfunction due to HIV/SIV infection [5-8]. In this study, MRS was employed to investigate the longitudinal cerebral metabolite alterations in a novel SIV model of Neuro-AIDS at 3T.

Methods: Five male pig-tailed macaques (*Macaca nemestrina*, 4 years old) were infected with the SIVsmm9virus, a highly neuropathogenic virus in the pig-tailed macaques [9]. The MRS data were collected in the prefrontal cortex (PFC) and right basal ganglia (BG) with a surface coil on a 3T scanner (Siemens Magnetom/Trio) before SIV inoculation and in the weeks (2, 4, 8, 12, 16, 20 and 24) post inoculation (wpi) until sacrificed at 24-wpi. Blood samples were collected from each animal for monitoring CD4⁺ and CD8⁺ T-cells [9] at each time points. The behavioral tests [10] were carried out to evaluate cognitive functions. Single voxel (6 × 6 × 6 mm³) MRS exam was performed in PFC and BG with a PRESS sequence using TR/TE = 1500/30ms and CHESSE water suppression. During scanning, the animals were anesthetized and maintained with 1-1.5% isoflurane and immobilized with a custom-built head holder, and all vital physiological parameters were monitored continuously. Metabolite levels including, N-acetylaspartate (NAA), creatine (Cr), choline (Cho), myo-inositol (mI) and glutamate/glutamine (Glx) were calculated with LC Model. The total Cr was used as an internal reference for quantification. Analysis of variance (ANOVA) was employed for the statistical analysis of repeated measurements across different time points. Student's t-test was applied to compare the results between pre- and post-inoculation. Correlations between the T cell % and metabolite changes were examined using Spearman correlation coefficients. P-values less than 0.05 were considered statistically significant.

Results: No significant changes were observed in the performance on the behavioral testing battery following viral inoculation. After SIV infection, NAA and Glx (averaged from wpi) were reduced 11.8%, 10.0% in PFC and 8.8%, 9.6% in BG, although no significance was observed in comparison with the baseline (Fig 1). In the acute phase (<1 month), the CD8⁺ T cell % increased significantly while the CD4⁺ T cell % declined (Fig 2). Significant reductions of NAA in PFC and Glx in BG as well as an increase of mI in BG were observed in the 2nd wpi (Fig 3). In the chronic phase, Cho and Glx in PFC significantly decreased in the 4th and 12th wpi (Fig 3a). The NAA in PFC and the NAA and Glx in BG exhibited significantly negative correlations with the CD8⁺ T cell %. Furthermore, the CD4⁺ T cell depletion showed significantly positive correlations with the Cho in PFC (Table 1).

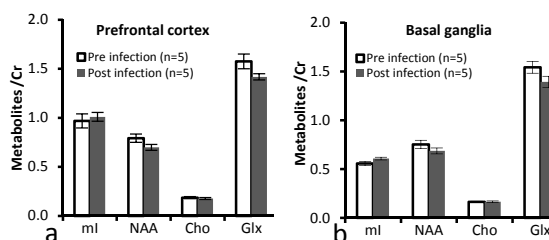


Fig. 1 Cerebral metabolites changes in prefrontal cortex (a) and basal ganglia (b) in macaques after SIV inoculation. Error bars represent standard deviation error. *, P < 0.05 compared with the baseline.

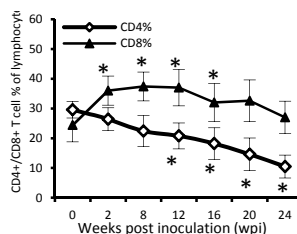


Fig. 2. CD4⁺ and CD8⁺ T-cell % of lymphocyte changes in macaques after SIV inoculation. Error bars represent standard deviation error. *, P < 0.05 compared with the baseline (before SIV inoculation).

Table 1 Correlations of CD4⁺ and CD8⁺ T-cell % of lymphocyte with cerebral metabolites in prefrontal cortex (PFC) and basal ganglia (BG), n=5. *, P<0.05; **, P<0.01

Location	%	mI	NAA	Cho	Glx
PFC	CD4	-0.29	0.03	0.37*	0.08
	CD8	0.23	-0.37*	0.23	-0.23
BG	CD4	0.10	-0.18	0.14	-0.22
	CD8	0.04	-0.41*	0.06	-0.57**

Discussion and Conclusion: The cognitive tests

indicated all the SIV-infected monkeys were asymptomatic in the entire 24 wpi. In comparison with the pre-inoculation MRS results, the ~10% reduction in NAA and Glx in PFC and BG (averaged across time after infection) were observed. The reduction is non-significant but more than that in asymptomatic patients [3]. In the acute phase, the significant reductions of NAA in PFC and BG were observed which is consistent with the reports from HIV⁺ patients and SIV models [1-8, 10]. In addition, elevated mI in BG in the 2nd wpi and reduced Cho in PFC in the 4th wpi consistent with the previous SIV study [5]. In the chronic phase, the progression changes in NAA, mI, and Glx exhibited differently in the region of PFC and BG. The reductions of Glx in PFC and BG are in agreement with the results in HIV⁺ patients [2, 3, 11] but different from previous SIV reports [5], probably due to the virus and model difference. The correlation between the reductions of NAA in PFC and

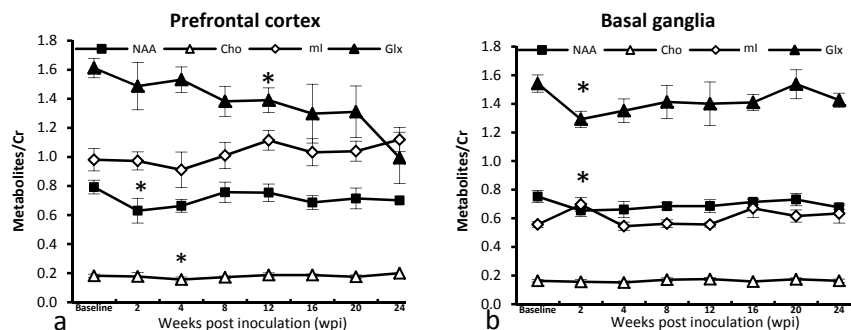


Fig.3 Longitudinal cerebral metabolites changes in prefrontal cortex (a) and basal ganglia (b) after SIV inoculation. Error bars represent standard deviation error. *, P < 0.05 compared with the baseline.

BG and Glx in BG with the CD8⁺ T cell % is consistent with the previous reports from the patients with early HIV⁺ infection [11]. Our results further validated that Glx is the sensitive biomarker in the HIV infection in addition to NAA [1-3, 11].

In conclusion, NAA was reduced significantly in the acute phase and Glx was reduced significantly in both acute and chronic phases in the neuroasymptomatic subjects after the SIV inoculation. As NAA was increased slightly in the chronic phase, non-significant reduction of averaged NAA is resulted accordingly. Also, the progressive alterations in NAA and Glx correlated significantly with the changes of CD8 T cell % in this macaque model of Neuro-AIDS.

Reference: [1] Chang et al, Neuroimage (2004); [2] Sailasuta et al, NMR Biomed (2009); [3] Mohamed et al, MRI (2010); [4] Williams, R., J Neurovirol (2008); [5] Ratai et al, MRM (2009, 2011), [6] Greco et al, MRM (2004); [7] Lentz et al, Radiology (2005); [8] Fuller et al, BMC Neurosci (2004); [9] Novembre et al, J. Virology (1998); [10] Li et al, Neuroimage (2011); [11] Lentz, et al, Neurology (2009)

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