Utilizing 1H MRS to Examining the Relationship between SIV Encephalitis and Neuronal Injury.

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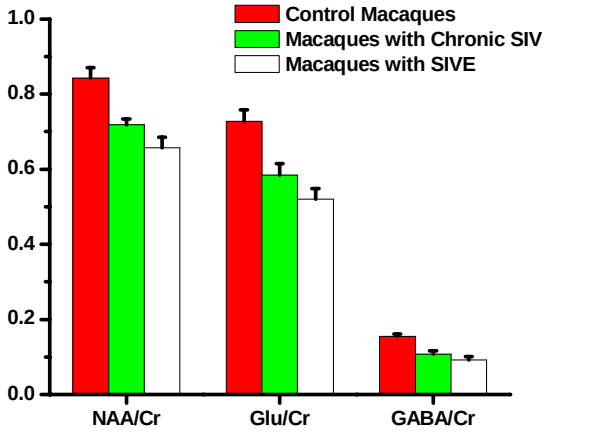
Introduction Human immunodeficiency virus (HIV) invades the central nervous system (CNS), causing neurological disease that may range from a mild cognitive disorder to frank dementia [1]. The largest recently published autopsy series of data reveals that the CNS is second only to the lungs as the most commonly diseased organ in patients dying with AIDS.[2] With the advent of anti-retroviral therapy (particularly HAART), the incidence of neurological disease has declined. However, the prevalence is increasing as patients live longer [3]. Encephalitis is known to occur in both HIV as well as simian immunodeficiency virus (SIV). It has not been established whether encephalitis caused by SIV, classically defined by the presence of perivascular macrophages/microglia and multinuclear giant cells (MNGC), is essential for neuronal injury in this model of neuroAIDS. Previous work [4,5] found that neuronal injury was present in the absence of SIV encephalitis (SIVE). To conclusively answer this question we used high resolution proton magnetic resonance spectroscopy (1H MRS) to analyze brain extracts from three cohorts of macaques; those with SIVE (n=16), those chronically infected with SIV, but lacking encephalitis (n=8), and uninfected controls (n=6). Furthermore, we subdivided the SIVE category into mild (n=5), moderate (n=4) and severe encephalitis (n=7), making a total of 5 cohorts.

Methods Twenty-four SIV infected rhesus macaques (SIVmac251 or SIVmac239) were sacrificed by intravenous overdose injection of sodium pentobarbital once they were found to be moribund with AIDS. Frontal cortex tissue samples from each SIV infected macaque and 6 uninfected control macaques were collected, snap frozen immediately upon necropsy, and stored at –70°C. For each tissue sample, a portion (50-90 mg) were separated and immersed in a fast prep tube filled with methanol (1.2 mL) and pulverized. Two successive rounds of grinding/centrifugation followed this step with the supernatant being decanted and placed in a 15 mL tube followed by grinding/centrifugation with 0.8 mL H_2O and then 1 mL $CHCl_3$. After the grinding procedure, the entire contents of the fast prep tube were transferred to the 15 mL tube containing the supernatant. The fast prep tube was then cleaned with 0.8 mL methanol, 1 mL $CHCl_3$, and 0.2 mL H_2O, and the contents transferred to the 15 mL centrifuge tube. The aqueous layer was removed and dried using a Savant dryer. The samples were rinsed and dried twice with D_2O. Once drying is complete the sample is ready for NMR analysis. High resolution 1H MRS (14.1 T) experiments were performed on a Bruker AVANCE™ spectrometer (Bruker Instruments, Inc., Billerica, MA). The spectral processing software Peak Research NMR (PERCH solutions, Ltd., Kuopio, FI) was used to determine the quantities of N-acetylaspartate (NAA), myo-inositol (MI), choline (Cho), creatine (Cr), γ-aminobutyric acid (GABA), glutamate (Glu), taurine (Tau), lactate (Lac) and glycine (Gly) in these frontal cortex samples. Chronically infected macaques consisted of macaques that died from any opportunistic infection other than those which affect the brain. A primate pathologist at New England Regional Primate Center determined SIVE categories.



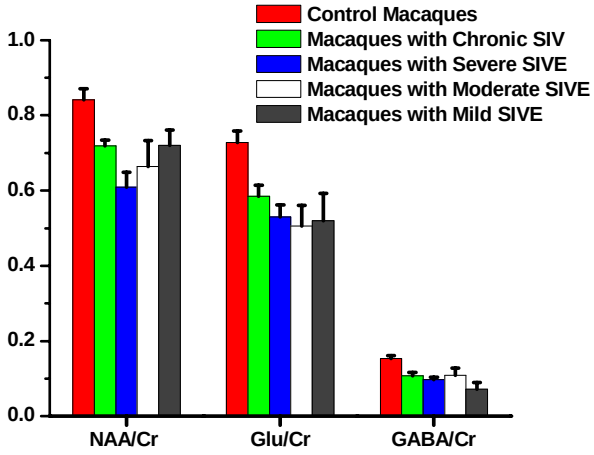


Figure 1 (top): Spectroscopic markers NAA/Cr, Glu/Cr, and GABA/Cr using ex vivo 1H NMR as measured in 3 cohorts: Controls (n=6), Chronic SIV (n=8), and those with SIVE (n=16).
Figure 2 (bottom): Spectroscopic markers NAA/Cr, Glu/Cr, and GABA/Cr using ex vivo 1H NMR as measured in 5 cohorts: Controls (n=6), Chronic SIV (n=8), and those with mild SIVE (n=5), moderate SIVE (n=4). & severe SIVE (n=7).

Results *3 Cohorts*: ANOVA revealed significant differences in levels of NAA/Cr ($p<0.001$), Glu/Cr ($p<0.001$), and GABA/Cr ($p<0.001$) between controls, SIV chronically infected macaques and those with encephalitis (Figure 1). Significant differences in NAA/Cr levels by t-tests were demonstrated between controls and macaques with SIVE ($p<0.0003$), and between controls and those with chronic SIV ($p<0.02$). No significant difference was found between the chronic SIV and SIVE groups themselves. Additionally, significantly lower levels of Glu/Cr and GABA/Cr were found between controls and both SIV and SIVE brains, but no difference between macaques with chronic SIV and those with SIVE could be observed. No significant differences were found in the levels of taurine, glycine, lactate, total and specific cholines, and myo-inositol.
5 Cohorts: ANOVA revealed significant differences in levels of NAA/Cr ($p<0.002$), Glu/Cr ($p<0.01$), and GABA/Cr ($p<0.001$) between controls, SIV chronically infected macaques and those with various levels of encephalitis (Figure 2). Glu/Cr and GABA/Cr levels decreased between control and both SIV and SIV encephalitic brains, but no differences between SIV and any SIVE group was observed. However, significant differences between control levels of NAA/Cr and all other groups (those with chronic SIV, mild SIVE, moderate SIVE, or severe SIVE) were found ($p<0.03$). Differences in NAA/Cr levels amongst those with signs of inflammation but lacking MNGC (mild SIVE) and those with large MNGC (severe SIVE) were significant ($p<0.04$). No difference in NAA/Cr levels at death were observed between those macaques who were chronically infected with SIV and those that had signs of inflammation (mild SIVE).

Conclusion This study confirms our prior observations of neuronal injury in SIV infected animals even in the absence of classic encephalitis. Our findings that glutamate and GABA are also diminished, support injury to both excitatory and inhibitory neurons. While there is neuronal injury in both macaques with SIVE and those without, there are also differences in NAA/Cr levels with respect to the severity of the encephalitis. The most severe encephalitis is accompanied by the greatest decrement in NAA/Cr and neurotransmitters. In considering therapy for neuroAIDS, the findings here support prevention or minimizing the encephalitic process, but suppression of the formation multinucleated giant cells alone would be insufficient to fully prevent neuronal injury.

References

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