Quantitative Neuropathological Correlates of Change with Primate Brain NAA/Cr

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Introduction Neuropathology and proton magnetic resonance spectroscopy (^{1}H MRS) studies independently demonstrate neuronal injury and loss in both the HIV and simian immunodeficiency virus (SIV) model of neuroAIDS. Previously reported temporal trends between spectroscopic markers of SIV in the acute phase led to speculation of the relationships between spectroscopic and histopathological markers of neuronal health and inflammation [1]. The purpose of this study was to elucidate the pathobiological basis of transient changes in the primate brain NAA/Cr ratio using the SIV-infected macaque model of neuroAIDS. Building upon previously reported transient declines of NAA/Cr levels in the macaque frontal cortex during the first month of SIV infection, frontal lobe tissue was investigated using high resolution ^{1}H MRS of brain extracts and quantitative neuropathological analyses including neuronal counts by stereology and immunohistochemistry of 3 neuronal markers: synaptophysin, microtubule associated protein 2 (MAP2) and calbindin. Historically, these histopathologic markers have been used to characterize neuronal health.

Methods Eleven rhesus macaques underwent MRI/S the day before sacrifice (1.5T GE Signa LX scanner). Four macaques were used as controls while the remaining seven were infected with SIVmac251, and sacrificed at the following time points: 12 days post inoculation (dpi) (3 animals), 14 dpi (3 animals), and 28 dpi (1 animal). Spectral analyses determining the quantities of *N*-acetylaspartate (NAA), myo-inositol (MI), choline (Cho) and creatine (Cr) in the frontal cortex were performed using SAGE-GE software as previously described [2]. Peripheral blood was collected on days 0, 11, 12, 14 and 28 of infection for quantification of viral RNA in plasma. Paraffin tissue samples of frontal cortex were obtained for immunochemical appraisal of neuronal integrity with monoclonal antibodies against calbindin, synaptophysin, and MAP2 (results reported as optical density units/mm^2). The number of cortical neurons was quantified using stereology. A Bioquant Image Analysis System (Nashville, TN) was used to performing stereological neuronal counting, utilizing the optical dissector technique. All assessments were performed blind to clinical and neuropathological diagnoses. Since the entire volume of the STS was not available for study, the results are presented as number of neurons/mm^3 (neuronal density). Neighboring tissue samples were removed for use in ex vivo high resolution NMR analysis (14 T). Metabolites were extracted as previously reported and dissolved in 600 µL D_20 [3]. Ex vivo spectra were analyzed with Peak Research NMR Software (PERCH solutions, Ltd., Kuopio, FI).

Results Analysis of variance (ANOVA) revealed highly significant changes between the SIV RNA viral load in the blood plasma of the 11 necropsied animals ($p<10^{-7}$) with a peak in virema occurring at 11 dpi. The viral load drops significantly by 28 dpi, although it is never eliminated. During acute infection with SIV, the macaque brain exhibited significant changes in NAA/Cr ($p < 0.02$, ANOVA) and synaptophysin ($p < 0.01$, ANOVA) (Figure 1). No significant changes were found in the neuronal counts or the other immunohistochemical neuronal markers (Figure 1). Using the Spearman rank coefficient, a significant direct correlation was detected between synaptophysin and extracted NAA/Cr ($r_s = 0.72$, $p < 0.01$) (Figure 2). Indirect linear correlations were also found to exist between plasma viral load and synaptophysin ($r_s = -0.67$, $p < 0.03$), as well as between extracted NAA/Cr from frontal lobe tissue and plasma viral load ($r_s = -0.72$, $p < 0.02$). There was no correlation between NAA/Cr and neuronal counts, calbindin, or MAP2.

Conclusion In the acute SIV-infected macaque, we observed a transient decline in NAA/Cr levels that followed the peak in viremia and its subsequent control. During this period, quantitative neuropathologic evaluations revealed a decline in synaptophysin, but not in neuronal counts, calbindin or MAP2. NAA/Cr and synaptophysin have been independently used as markers for neuronal health; here, they have been confirmed to show similar patterns of change during the acute phase of SIV. Moreover, we found a statistically significant correlation between NAA/Cr in brain extracts and synaptophysin. We conclude that declines in NAA/Cr observed by *in vivo* and *ex vivo* ^{1}H MRS may be an indicator of early neuronal injury and not necessarily neuronal loss, and that this injury may be related to presynaptic dysfunction.

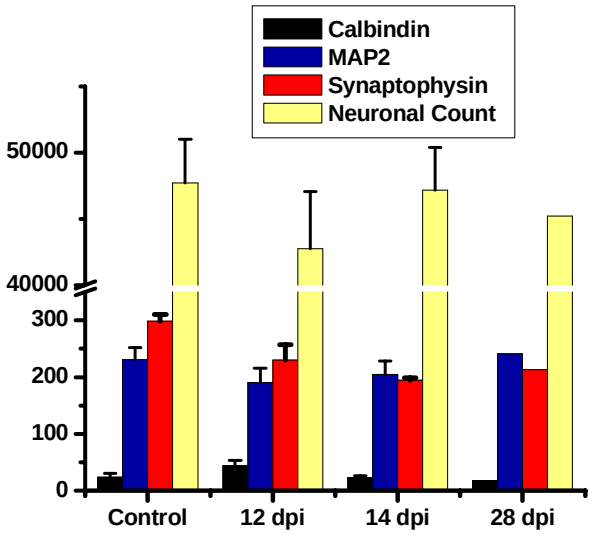

Figure 1. Changes in brain neuronal counts, MAP2 and calbindin in frontal cortical tissue found in macaques sacrificed without SIV, and those sacrificed 12, 14 and 28 dpi. All error bars shown are the standard error of the mean.

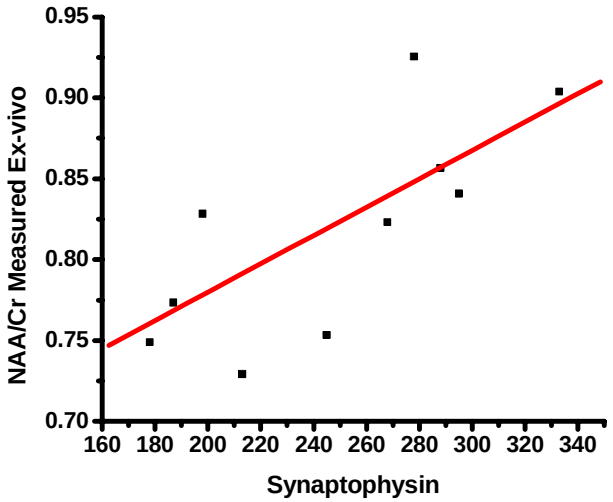

Figure 2. Linear correlations found between NAA/Cr in extracts from frontal lobe tissue and synaptophysin ($r_s = 0.72$, $p < 0.01$).

References

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