In vivo MR- Spectroscopy demonstrates reversal of neuronal injury with antiretroviral therapy in an SIV model of neuoAIDS

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Introduction:

HIV invades the central nervous system (CNS) causing neurological disease that may range from a mild cognitive disorder to dementia [1]. With the advent of anti-retroviral therapy (particularly HAART), the incidence of neurological disease has declined. However, its prevalence is increasing as patients live longer [2]. Development of effective treatments for HIV related CNS disease is hindered by a lack of understanding of the responsible mechanisms. There is a consensus that neuronal injury is the basis for the cognitive disorder, and that this injury is most likely due to indirect mechanisms since HIV does not infect neurons. Neuroimaging has helped in understanding HIV related brain injury, and in recent years *in vivo* 1H MR spectroscopy has emerged amongst the most informative methods. The best animal model, the simian immunodeficiency virus (SIV) infected macaque, has been highly informative, but it is also beset by the facts that SIV encephalitis occurs in less than a third of infected animals and requires a lengthy time course of two or more years. To elucidate the pathogenesis of HIV-associated CNS injury, we utilized noninvasive proton magnetic resonance spectroscopy (1H MRS) in an accelerated animal model of neuroAIDS in which SIV-infected macaques are depleted of CD8+ T lymphocytes.

Methods:

Eight rhesus macaques (*macaca mulatta*) were included in the study. To date the study of six animals has been completed, and two animals are still under investigation. After inoculation with SIVmac251, CD8+ T cells were depleted *in vivo* at day 6, 8 and 12 post infection (pi) [3]. A subset of two animals underwent combination antiretroviral therapy of PMPA and FTC. All of the animals underwent magnetic resonance imaging (MRI) and spectroscopy (MRS) before infection, and approximately every 2 weeks thereafter until the endpoint of the study. Both MRI and ^{1}H-MRS were performed on a clinical 1.5 Tesla General Electric (Milwaukee, Wisconsin) Signa Scanner with a Horizon 8.3 operating system using a standard GE linear extremity coil. An axial dual echo pulse sequence was utilized, from which the ^{1}H-MRS voxels were defined. In each animal, MRS is carried out in frontal cortical gray matter, white matter semiovale and basal ganglia with a voxel size of 15mm × 15mm × 15mm. Data was acquired using a PRESS sequence (TE=35 ms, TR=3000 ms) with CHESS water suppression. Subsequently, all spectra were processed off-line using the SAGE-GE spectral analysis program to determine the quantities of the brain metabolites N-acetylaspartate (NAA), choline-containing compounds (Cho), *myo*-inositol (MI), and creatine-containing compounds (Cr) as previously described [4]. Metabolite levels were calculated as ratios to creatine as internal intensity reference. After MRI examination blood samples were drawn from each animal for the determination of lymphocytes as well as viral loads. Statistical analysis was performed using ANOVA and Repeated Measures Analysis of Variance (ANOVA). Paired t-tests as well as Dunnett's tests were performed to isolate differences between time points. Linear regression analyses were used to determine the relationship between metabolite values, viral loads, and flow cytometry.

Results:

The four SIV-infected, CD8+ T lymphocyte depleted animals were found to develop AIDS within 10 weeks of infection and had neuropathological evidence of severe encephalitis, defined by infiltrating perivascular macrophages, multinucleated giant cells (MNGC), and virus in the CNS. Using MRS we have observed a substantial, consistent decrease in the neuronal marker NAA/Cr in the frontal cortex (ANOVA, p=0.03) (Figure 1). Dunnett's test was used to isolated differences from pre-inoculation values, and the NAA/Cr was decreased at 8 weeks (p=0.02) and the combined 9/10 week time-point (p=0.016). Linear regression analysis revealed an inverse relationship between time of infection and NAA/Cr in the frontal cortex (r=-0.71, p<0.001), basal ganglia (r=-0.57, p<0.005) and the white matter (r=-0.68, p<0.001). A statistically significant 14% decreases in NAA/Cr in both the frontal gray (p<0.05) and the white matter (p<0.05) between the pre-inoculation scan and the final scan were observed. Regression analysis revealed a statistically significant inverse correlation between NAA/Cr changes in the frontal cortex and plasma viral load (p = 0.01, r = -0.64). Cho/Cr and MI/Cr showed no significant changes over time.

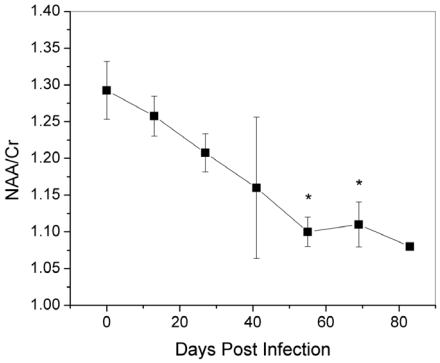

Figure 1: Average NAA/Cr levels in the frontal cortex of four SIV infected, CD8+ T cell depleted animals. Error bars represent Standard Error of the Mean (SEM).

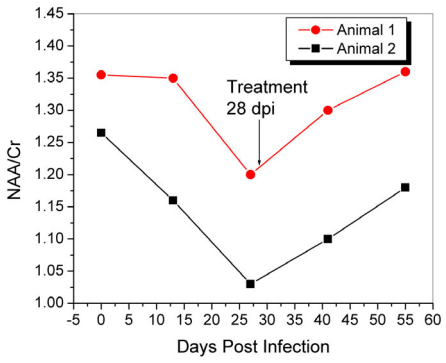

Figure 2: NAA/Cr levels in the frontal cortex in the two SIV infected, CD8+ T cell-depleted macaques that were treated at 28 dpi.

To directly address the question of reversibility of neuronal injury observed in the accelerated model of neuroAIDS, we performed a study in which macaques are SIV-infected and CD8+ T lymphocyte-depleted, then treated with antiretrovirals four weeks pi. Postmortem examination revealed that both animals had no evidence of encephalitis. Repeated Measures ANOVA showed significant changes in the NAA/Cr level in just two animals. (p=0.03). Initially, a substantial decrease was observed in the NAA/Cr ratio by 16% at 27 dpi (p<0.05). After therapy initiation, the levels of NAA/Cr improved dramatically; after two weeks of treatment, the direction of NAA loss is not only stopped, but appears to be reversed. After four weeks we observed an increase of 14% in NAA/Cr (p=0.02).

Furthermore, MI/Cr levels showed significant changes by Repeated Measures ANOVA (p=0.02). We observed a statistically significant decrease between pre-infection values and two weeks after treatment in just two animals (p<0.05). This experiment will be repeated in two additional animals.

Conclusion:

Utilizing proton MRS we have shown a substantial, cumulative decrease in the neuronal marker NAA over a relatively short time period in rhesus macaques infected with SIV and subsequently depleted of CD8 T lymphocytes. Remarkably, all of these animals developed AIDS within 10 weeks pi. This novel SIV-infected, CD8-depleted macaque model overcomes the limitations of those previously described with rapid development of brain MRS abnormalities, which are highly reproducible. Combination of anti-retroviral therapy during primary infection resulted in a significant reversal of NAA/Cr 14 and 28 days after treatment suggesting recovery of neuronal injury.

References:

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