Increases in Creatine detected by MRS utilizing a macaque model of neuroAIDS suggests glial activation and inflammation

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Introduction:

^{1}H magnetic resonance spectroscopy (MRS) has emerged as one of the most informative neuroimaging methods for the study of neuroAIDS. Neurochemical changes detectable by MRS include a decline in N-acetylaspartate (NAA) or NAA/Creatine (NAA/Cr), a marker of neuronal health. Prior to declines in NAA, increases in choline (Cho) and *myo*-inositol (MI) have been observed in HIV-infected individuals, as well as in the SIV-infected macaque model [1,2,3]. Elevations of Cho, MI, Cho/Cr and MI/Cr have also been documented in a variety of neuroinflammatory and neurodegenerative diseases and are thus considered markers of ongoing central nervous system (CNS) inflammation or gliosis [4,5]. However, the neurocellular bases of the changes observed in these resonances remain poorly understood.

Increases in Cr levels have been observed in antiretroviral-naïve HIV patients [6,7] and during the acute phase of SIV infection [8]. We propose that in neuroAIDS creatine may be an excellent maker to monitor inflammatory processes in the brain. Towards this end, we employ the SIV-infected CD8 T lymphocyte depleted (accelerated) macaque model of neuroAIDS to understand the biological and chemical foundation of changes in Cr caused by the virus. The SIV-infected, CD8+ T lymphocyte depleted macaque model (SIV+/CD8 model) shares similar biological properties with HIV including the accumulation of viral-laden perivascular macrophages and multinucleated giant cells, astrogliosis, microgliosis, and neuronal injury.

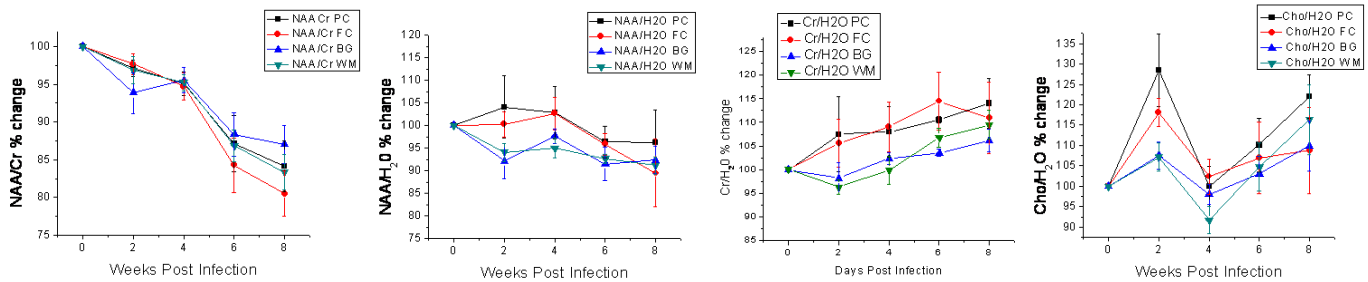
Methods:

Eight rhesus macaques (*Macaca mulatta*) were inoculated with SIVmac251 virus (20 ng SIVp27, i.v.) and their CD8+ T-lymphocytes were depleted with antibody targeted against the CD8 (cM-T807) at 6, 8, and 12 days post inoculation (dpi). Animals were scanned two times pre-infection, and biweekly until sacrifice at 4 weeks post infection (wpi) (4 animals), or 8 wpi (4 animals), on a Siemens 3T Trio system. Single voxel ^{1}H MR spectroscopy was performed in the parietal cortex (PC), frontal cortex (FC), basal ganglia (BG) and white matter semiovale (WM) using a point resolved spectroscopy sequence (PRESS) with TE/TR = 30/2500 ms. Metabolite concentrations (NAA, Cho, MI, and Cr) were determined offline using LCModel. CD8+ T lymphocyte depletion was monitored by flow cytometry. Plasma and CSF viral loads were quantified using a commercially available enzyme immunoassay (EIA) for SIVmac p27. Repeated measures analysis of variance (RM ANOVA) and univariate ANOVA in conjunction with Holm's t-tests were performed using Primer of Biostatistics.

Results:

SIV infection and CD8 depletion resulted in a rapid decline in NAA/Cr levels in all four brain regions measured (PC – 15% $p<0.0001$, FC -20% $p<0.0001$, BG -13% $p=0.004$, WM -17% $p<0.0001$). Figure 1 displays the changes of all 8 animals studied. The decrease is significant at 6 and 8 wpi in the 4 animals studied to 8 wpi. However, when analyzing all 8 animals at 4 wpi, decreases are already significant in PC ($p=0.02$), FC ($p=0.024$) and WM ($p=0.024$) but not BG yet ($p=0.081$). When measuring NAA and Cr changes with respect to tissue water as the internal standard, much more variability was observed. NAA/H_2O decreases in every brain region in 4 animals at 8 wpi, however, only WM changes are statistically significant ($p=0.03$); the FC and BG only show trends (Figure 2). When analyzing all 8 animals that were studied at 4 wpi NAA changes are already significant in WM at 2 wpi. ($p = 0.022$). In addition, we found an increase in Cr in every brain region in the SIV+ CD8- animals (statistically significant in the PC [$p=0.01$] and WM [0.01], a trend in the BG [0.07], and not significant in the FC [Figure 3].

Increases in Cho and MI are considered to reflect increases in glial activity. We determined the absolute Cho and MI concentrations using the unsuppressed tissue water resonance as the internal standard. During the first four weeks of SIV infection, Cho/H_20 significantly changes in all brain regions (PC $p<0.0001$, FC $p=0.001$, BG $p=0.025$ and WM $p=0.001$) (Figure 4). All brain regions show an initial increase in Cho at 2 wpi, significant in the cortices, then a decrease to baseline values or below, as observed in the WM. With further disease progression, Cho increases once more at 8 wpi, (significantly only in the PC). MI shows complex regional variations over time with increases at 2 and 4 weeks and then normalization to baseline values (data not shown).



Discussion:

Changes in total Cr (phosphocreatine and creatine) are associated with altered energy metabolism. As stated previously, elevated Cr levels have been reported in the frontal white matter and basal ganglia of chronic HIV patients [6,7] and during the acute phase of SIV infection in the white matter[8]. It is believed that the virus enters the brain through infected monocytes that later differentiate into macrophages [9]. During this process of monocytic cell infiltration, astrocyte and microglial activation and proliferation, a high metabolic demand may explain an increase in Cr. At the same time there is a concomitant decline in the energy demand of neurons: NAA decreases as a result of neuronal cell injury and thus a decline in Cr would be expected in these cells. However, in the MR spectrum, this change is overwhelmed by Cr elevations occurring within the more metabolically highly active immune and glial cells. The postulation of creatine increase as a surrogate marker for inflammation/active gliosis is supported by the facts that elevated Cr decreases during treatment with anti-inflammatory drugs such as minocycline [10] and treatment with antiretroviral therapy in macaques and patients [7]. These dual changes of decreased NAA and increased Cr make the NAA/Cr ratio a sensitive marker for brain disease status.

References: [1] Barker et al. Radiology 1995;195:58, [2] Tracey et al. Neurology 1996;46:783, [3] Greco et al. Magn Reson Med. 2004;51:1108, [4] Kim et al. AJNR 2005;26:752, [5] Gonzalez et al., AIDS 2000;14:2841, [6] Chang et al. Neuroimage. 2002;17:1638, [7] Chang et al. Antivir Ther. 2003;8:17, [8] Ratai et al. Manuscript in Preparation, [9] Gartner et al. *Science* 2000;287:602, [10] Ratai et al. ISMRM abstract 2009 submitted