Choline's relationship to pro-inflammatory monocyte chemoattractant protein and glial activation

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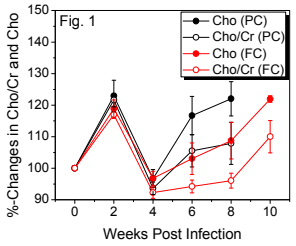
Introduction: *In vivo* ^{1}H magnetic resonance spectroscopy (1H MRS) has emerged as one of the most informative neuroimaging methods for the study of neuroAIDS. Neurochemical changes detectable by MRS include a decline in N-acetylaspartate (NAA) or NAA/Creatine (NAA/Cr), a marker of neuronal health. 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 [1-3]. Choline-containing compounds are primarily related to lipid membrane turnover [4]. Elevations of Cho and Cho/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 [5,6]. However, the neurocellular bases of the changes observed in these resonances remain poorly understood. The objective of this study was to understand these immunodeficiency virus-induced changes in Cho concentrations measured by MRS. Towards this end, we employ the accelerated SIV-infected CD8 T lymphocyte depleted (SIV+/CD8-) macaque model of neuroAIDS. Neuropathological changes due to the virus in the SIV+/CD8 model are extremely comparable to those of HIV, including the accumulation of viral-laden perivascular macrophages and multinucleated giant cells, astrogliosis, microgliosis, and neuronal injury.

Methods: Twenty-seven rhesus macaques were inoculated with SIVmac251 virus, and their CD8+ T-lymphocytes were depleted with antibody targeted against the CD8 (cM-T807) at 6, 8, and 12 days post inoculation (dpi) to ensure rapid disease progression. Sixteen of these animals remained untreated and were sacrificed at predetermined endpoints [4, 6, 8 and 10 weeks post infection (wpi)] or when animals were moribund with AIDS. Four animals were treated with combination anti-retroviral therapy (cART) and seven animals were treated with minocycline, an anti-inflammatory/neuroprotective drug [7], starting at 4 wpi. Single voxel ^{1}H MR spectroscopy was performed in the parietal cortex (N=19) and frontal cortex (N=27) using a point resolved spectroscopy sequence (PRESS) with TE/TR = 30/2500 ms on either a Siemens 3T Trio system or a 1.5T GE Sigma system. Metabolite concentrations were determined with LCModel using the unsuppressed tissue water resonance as the internal standard. *Post mortem*, CNS tissue samples were evaluated and scored in terms of severity of gliosis by a neuropathologist. Furthermore, brain tissue from the parietal cortex (21 samples) and frontal cortex (24 samples) was harvested for quantitative neuropathology. The degree of reactive astrogliosis was assessed with monoclonal anti-glial fibrillary acidic protein (GFAP). Finally, levels of pro-inflammatory monocyte chemoattractant protein 1 (MCP-1) were assayed in CSF by ELISA on a subset of 9 animals (4 untreated and 5 minocycline treated).



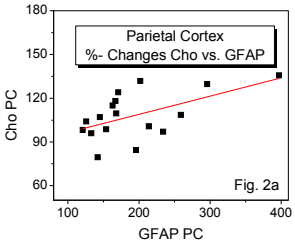
Results and Discussion: Figure 1 shows the percent changes from pre-infection for Cho (filled circles) and Cho/Cr (open circles) levels in parietal cortex (black) and frontal cortex (red) with time post infection. Repeated-measures analysis of variance for all measures were highly significant ($p<0.001$). Subsequent matched-pair t-tests for both Cho and Cho/Cr in all regions indicated large elevations from pre-infection levels at two weeks (t-tests: $p<0.001$) and large reductions between two weeks and four weeks (t-tests: $p<0.001$). Reductions between pre-infection and four weeks were significant ($p=0.007$) for Cho/Cr in the FC. With further disease progression, Cho increases once more at 8 and 10 wpi, (t-tests: $p<0.03$) above baseline levels. Minocycline treated animals did not show the second increase in choline [7].

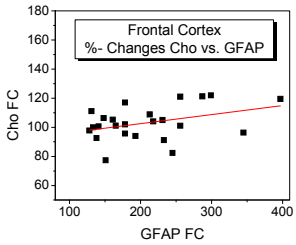
Of these 27 animals seven developed classical hallmarks of SIV encephalitis (SIVE) including the accumulation of viral-laden perivascular macrophages and multinucleated giant cells. Elevated Cho and Cho/Cr at the animal's last scan before sacrifice predicted SIVE (see **Table 1**).

Table 1: Predictions for SIV encephalitis

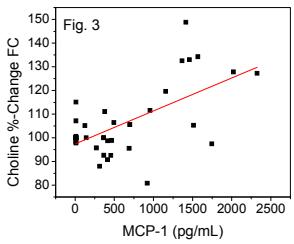
		AUC	Cut-off	Sensitivity	Specificity
Cho	PC	87%	+9%	100%	67%
Cho/Cr		88%	+3%	100%	69%
Cho	FC	80%	+19%	67%	100%
Cho/Cr		66%	+6%	43%	90%

Interestingly, GFAP, a marker for astroglisis that typically becomes elevated with SIV-infection, showed a positive correlation with Cho changes in both the PC ($R=0.56$, $p=0.02$ **Figure 2a**) and FC ($R=0.37$, $p=0.07$, **Figure 2b**). Furthermore, Cho/Cr elevations were associated with severity of gliosis (ANOVA $p=0.048$).





The virus enters the CNS primarily through activated/infected blood monocytes through the BBB. Once in the brain, these immune cells release viral proteins and cytokines that then activate microglia and other macrophages, inducing neuronal damage. MCP-1 seems to have a role in regulating the migration of peripheral blood monocytes across the BBB. The up-regulation of MCP-1 enhances recruitment of virally infected monocytes into the brain. MCP-1 concentration in CSF followed a biphasic pattern with elevations during the first 2 weeks of infection, followed by a decline after two wpi and increases after four wpi. This biphasic pattern is very similar to what is observed for Cho changes. Furthermore, in macaques treated with minocycline, the second peak in MCP-1 levels in CSF was not observed. A least-squares means model was used to identify correlations between the changes in choline and MCP-1. This method allows for the correlation of data points that are not independent of one another, such as repeated measurements of MCP-1 and MRS from the same animal over multiple time points and emphasizes the temporal relationship between these two measures. Both PC and FC Cho ($p=0.03$, $R=0.66$ and $p=0.0001$, $R=0.82$, respectively) and both PC and FC Cho/Cr ($p=0.02$, $R=0.69$ and $p=0.07$, $R=0.66$, respectively) correlate with MCP-1. **Figure 3** shows the relationship between FC Cho and MCP-1.



Conclusion:
Acute/early increases in choline levels correlate with the initial inflammatory response measured by MCP-1 while increases in choline levels during later stages of SIV-infection correlate with astroglial activation measured by GFAP. These dynamic choline changes warrant further study.

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] Hakumaki et al. Trends Biochem Sci. 2000;25,357.[5] Kim et al. AJNR 2005;26:752, [6] Gonzalez et al., AIDS 2000;14:2841, [7] Ratai et al, PLoS One 2010;7(5):e10523.