^{1}H MRS and tandem mass spectrometric metabolite signatures herald HIV-1 induced metabolic abnormalities in the brains of humanized mice

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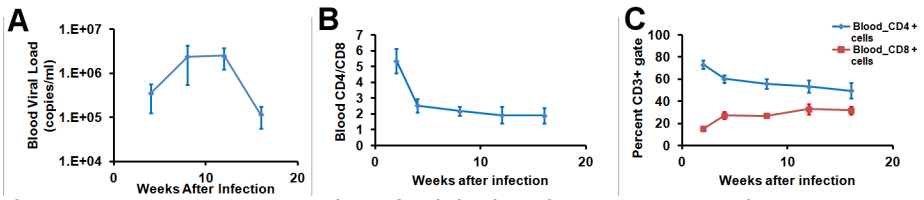

Figure 1. *Mean ± SEM versus time after infection of A: HIV concentration, B: CD4/CD8, C: CD4 and CD8 as a percentage of CD3 positive gates on the flow cytometer.*

Purpose: Chronic human immunodeficiency virus (HIV) infection leads to a spectrum of neurological signs and symptoms termed HIV-associated neurocognitive disorders. Humanized NOD/scid-IL-2Rγ_c^{null} (NSG) mice reconstituted with human hematopoetic stem cells (hu-NSG) then infected with HIV-1 can mirror aspects of human disease through the establishment of persistent viral infection, loss in CD4+ T cell numbers and nervous system (CNS) pathobiology. This includes human monocyte-macrophage ingress from the periphery across the blood brain barrier, meningitis and neuroinflammation[1]. Herein, we investigated the progress of metabolic abnormalities in the cortex and cerebellum using volume localized proton magnetic resonance spectroscopy (^{1}H MRS) validated with quantitative ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS).

Materials and Methods: Newborn NSG mice were irradiated at 1 Gy then injected intrahepatically with 10^5 purified CD34+ human stem cells in 20 µl of PBS. Animals with sustained 20-50% chimerism were infected at 22 weeks of age by intraperitoneal injection of HIV-1_{ADA} using a single dose of 10^5 50% tissue culture infectious doses/ml. Infected hu-NSG mice (n=8) were imaged including ^{1}H MRS (PRESS, TR=4 s, TE = 50 ms, NA = 320) of the cerebral cortex and cerebellum, and had blood FACS analysis for T cell population and viral load over time from preinfection through 4, 8, 12, and 16 weeks post infection. All procedures were done in accordance with the ethical guidelines for care of laboratory animals at the University of Nebraska Medical Center and the National Institutes of Health.

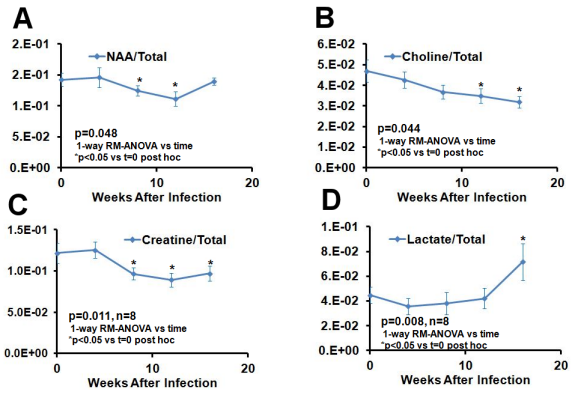

Figure 2. *Time evolution in Cerebral Cortex of HIV infected hu-NGS mice of A: NAA, B: Total Choline, C: Creatine and D: Lactate.*

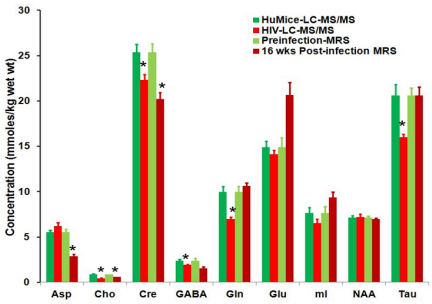

Figure 3. *Comparison of UPLC-MS/MS results (left bars) of metabolite levels in infected (red) and uninfected (green) mice to ^{1}H MRS results (right to bars) preinfection (light green) and 16 weeks after infection (dark red).*

In a parallel study, uninfected and infected hu-NSG mice of the same age, 18 weeks post infection, were sacrificed, brains quickly dissected into cortex, cerebellum, midbrain, hippocampus, caudate, and brainstem, and tissue flash frozen exactly 5 minutes after sacrifice. Frozen tissue was then processed and submitted for quantitative UPLC-MS/MS analysis of most ^{1}H MRS visible metabolites[2].

Results: Figure 1 shows the time course of viral load and CD4/CD8 ratio over time. While a stable viremia is seen over the first 12 weeks, between weeks 12 and 16 some reduction in viral load is apparent. Figure 2 shows results with significant changes over time ($p<0.05$, one way RM-ANOVA vs time) with individual points marked which show significant differences versus preinfection ($p<0.05$ LSD post-hoc analysis). No significant changes over time were seen in cerebellum of infected mice or in both regions in uninfected mice studied up to 8 weeks (n=7, later times in progress). Comparison of UPLC-MS/MS results from cerebral cortex in uninfected and infected mice at 18 weeks after infection (or same aged uninfected controls) are displayed in Figure 3. In agreement with ^{1}H MRS at 16 weeks, UPLC-MS/MS shows no change in NAA, as well as reductions in creatine and choline. Lactate quantification method is under development.

Discussion: Results from quantitative UPLC-MS/MS analyses of brain samples demonstrate general agreement with ^{1}H MRS and provide absolute quantitation of metabolites. NAA is found to reduce and recover with blood levels of viral load. Other changes are progressive with time of infection. These changes are in general agreement with long term changes in primate models.

Conclusions: UPLC-MS/MS analysis will be useful for validation of new spectroscopic acquisition, analytical methods, and tissue preservation methods. Additionally, high resolution ex-vivo UPLC-MS/MS analysis will allow further metabolomic characterization of the biochemical mechanisms underlying ^{1}H MRS detected metabolic alterations. ***References:*** *1. Dash, P.K., et al., The Journal of neuroscience, 2011.* ***31****(9): p. 3148-57. 2. Bathena, S.P., et al., J Chromatogr B Analyt Technol Biomed Life Sci, 2012.* ***893-894****: p. 15-20.*