Minocycline reveals neuroprotection in an accelerated rhesus macaque model of neuroAIDS by 1H MR Spectroscopy

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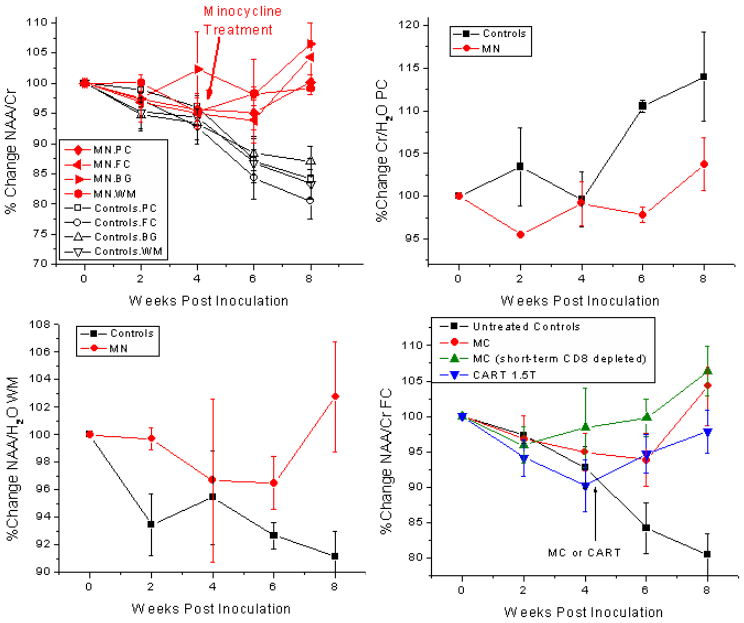
Introduction: A significant number of HIV-infected patients develop neurological symptoms ranging from minor cognitive impairment to severe dementia (neuroAIDS), which are thought to be a result of injury to neurons in the CNS. There is a consensus that HIV enters the CNS during the early stages of infection primarily through virally infected/activated monocytes from the blood. Macrophages and microglia are considered to play a key role in the pathogenesis of neuroAIDS, as they are the primary targets of productive infection in the brain. Once in the brain, infected macrophages or microglia release neurotoxic substances that induce neuronal injury and apoptosis. The goal of this study was to investigate minocycline (MN) as a neuroprotective agent for neuroAIDS. Minocycline has been tested in a variety of neuronal diseases including stroke, multiple sclerosis, Parkinson's disease and neuroAIDS [1]. The benefits of using minocycline are multifaceted since it has been found to have advantageous effects against inflammation, microglial activation, apoptotic cell death, and viral production [1]. However, some studies have found inconclusive data regarding MN's neuroprotective effects and some have even reported a harmful effect on patients with ALS [2]. The focus of our study was to serially assess neuronal health in an accelerated macaque model of neuroAIDS by measurement of N-acetylaspartate/creatine (NAA/Cr), a reliable marker of neuronal injury [3] using *in vivo* proton MR spectroscopy. The SIV-infected, CD8+ T lymphocyte depleted macaque model (SIV+/CD8-) results in a reliable accelerated model of neuroAIDS; 95% of persistently depleted animals [>21 days post inoculation (dpi)] demonstrate histopathological signs of SIV encephalitis. The goal of the present study was to test whether minocycline could reverse neuronal injury induced by this model. The results were compared to untreated SIV+/CD8- depleted animals, SIV infected short-term CD8 depleted animals (<21 dpi) treated with minocycline, and data from a previous study in which SIV+/CD8- animals were treated with combination antiretroviral therapy (CART) as previously reported in [4].

Methods: Ten rhesus macaques were infected with SIVmac251 and treated with the anti-CD8 antibody cM-T807 to deplete CD8 T lymphocytes at 6, 8 and 12 dpi. Six of these animals received daily treatments of minocycline (4 mg/kg/day) starting 4 weeks post inoculation (wpi) for four weeks. Flow cytometry was used to monitor CD8+ T lymphocyte depletion. Plasma and CSF viral loads were quantified using a commercially available enzyme immunoassay (EIA) for SIVmac p27. Animals were examined with MRI and MRS (3.0 T TIM Trio Siemens) 2-3 times before and biweekly after SIV infection until 8 weeks post infection. Single voxel 1H MR spectra were acquired from the parietal cortex (FC) and frontal cortex at the midline (FC), white matter of the centrum semiovale (WM), and the basal ganglia (BG) using a point resolved spectroscopy (PRESS) sequence with TE/TR = 30/2500ms. Metabolite concentrations N-Acetyl-aspartate (NAA), choline (Cho), myo-Inositol (MI), creatine (Cr) and glutamine/glutamate (Glx) were quantified using the LCModel software package as ratios over Cr and using the unsuppressed water peak as reference. T-tests between the cohorts' metabolite changes were performed to assess for statistical significance.



Results and Discussion: 1) The four SIV infected/persistently CD8 depleted animals showed a rapid decline in NAA/Cr levels (13–20%) at 6 and 8 weeks post inoculation (wpi) in all four brain regions measured (PC $p<0.0001$, FC $p<0.0001$, BG $p=0.004$ and WM $p<0.0001$) indicating neuronal injury. Three SIV-infected macaques were persistently CD8 depleted and were treated with minocycline beginning 4 weeks after infection. T–tests between the NAA/Cr changes at time points before MN treatment were not significantly different in these cohorts. Minocycline treatment resulted in a significant increase in NAA/Cr in all 4 regions of the brain compared to untreated control animals (PC at 8 weeks $p=0.021$, FC at 8 weeks $p=0.044$, BG at 8 weeks $p=0.014$ and WM at 6 weeks $p=0.008$ and 8 weeks $p=0.003$) Figure 1 (top left) shows NAA/Cr changes in all 4 regions of control and MN treated animals.

2) To separate changes between NAA and Cr, their levels were calculated with respect to tissue water as the internal standard. Despite increased variability, we detected increases in [Cr] in every brain region in the SIV+/CD8- untreated control animals (PC $p=0.01$, FC not significant, BG trend with $p=0.07$ and WM $p=0.01$). We speculate that the data suggests that Cr is increased due to increased need for energy demands by the upregulated activity of astrocytes and microglia [5]. [NAA] decreases in every brain region, however, due to more variability in these measurements [NAA] was only significantly decreased in the WM ($p=0.03$).

Treatment with MN results in a decrease in [Cr] in every brain region (significantly in PC $p=0.0001$ at 8 wpi and WM $p=0.024$ at 6 wpi) and an increase in NAA in every brain region (significantly in WM $p=0.03$ at 8 wpi) (Figure 2 and 3, top right and bottom left, respectively). Other proposed inflammatory markers such as [Cho] were also decreased in all brain regions due to MN treatment (significant in PC $p=0.007$ at 8 wpi).

3) In addition, we analyzed NAA/Cr changes in a) SIV+/CD8 untreated controls, b) MN treated SIV+/CD8- c) MN treated that were short-term CD8 depleted and d) combination antiretroviral therapy (CART) animals that had been studied previously using a 1.5T GE scanner [4]. Figure 4 (bottom right) shows representative results from the FC. Data reveals that CART and MN have the ability of reversing neuronal injury. The mechanisms of action are different though. CART reduces viral infection of monocytes/macrophages and thus the trafficking of more virus into the CNS [4]. However, minocycline acts via different mechanisms, and instead most likely reverses neuronal injury via its anti-inflammatory and apoptotic properties. Further *post-mortem* studies will verify these mechanisms.

Conclusions: These preliminary data indicate that minocycline has a beneficial effect on the brain in an accelerated model of neuroAIDS in nonhuman primates. A clearly detected reversal of NAA/Cr decline to normal levels after treatment is due to both a reversal of elevation of Cr and reversal of NAA decline suggesting that minocycline produces its result by control of glial activation with subsequent neuroprotection The data also suggest that best strategy to treat neuroAIDS may be the use of antiretroviral therapy in combination with minocycline.

References: [1] Zink et al. JAMA 2005;293:2003, [2] Gordon et al. Lancet Neurol. 2007;6:1045 [3] Lentz et al. Radiology. 2005;235:461, [4] Williams et al. J Clin Invest. 2005;115:2534, [5] Ratai et al. submitted for ISMRM 2009.

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