

Neuroprotective mechanism of minocycline in an accelerated macaque model of neuroAIDS

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Introduction: HIV-associated neurocognitive disorders continue to be a significant problem despite the use of highly active anti-retroviral drugs (HAART), and this has motivated a search for adjunctive therapies. The accelerated macaque model of neuroAIDS in combination with in vivo 1H MR spectroscopy (MRS) provides an exceptional opportunity to efficiently explore drug therapies that can control neuronal injury (Williams et al 2005 JCI). One of those adjunctive therapies includes minocycline a well-tolerated, inexpensive anti-inflammatory tetracycline-type antibiotic. We previously reported that minocycline prevented further decline of NAA/Cr, a marker for neuronal damage using MRS (ISMRM 2008). The benefits of using minocycline are multifaceted since it has been found to have advantageous effects against apoptotic cell death, inflammation, microglial activation, and viral production. However, the mechanism of MN is still unknown. There is a consensus that HIV enters the CNS during the early stages of infection primarily through virally infected/activated monocytes from the blood. The increase in the pro-inflammatory CD14+CD16+ monocyte subset is associated with HIV dementia and predictive of SIV encephalitis. 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.

Methods: Eleven 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. Seven 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. In addition, monocytes were fractionated based on CD14 and CD16 expression. 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) twice before and biweekly following SIV infection until 8 wpi. Single voxel 1H MR spectra were acquired from the parietal cortex (PC) 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 using the unsuppressed water peak as reference. Post mortem, CNS tissue samples were evaluated by a neuropathologist and microglial activation was assessed by quantifying calcium binding adaptor protein 1 (IBA-1).

Results and Discussion:

Flow cytometry showed that 3 animals that received MN treatment were not persistently CD8 T lymphocyte depleted. Thus, all analyses were performed on 3 groups of animals: 1) untreated, persistent CD8-depleted animals, 2) MN-treated, persistent CD8 depleted animals, and 3) MN-treated, short-term CD8-depleted animals. While the plasma viral load in untreated animals continued to increase no further increase in plasma viral loads was observed in the MN-treated animals. Significant declines from peak levels in CSF viral loads were only observed in the MN-treated animals.

Minocycline treatment beginning at 4 wpi was found to arrest further decrease in NAA/Cr in SIV-infected, persistently CD8 T cell depleted animals. In addition, a complete recovery of NAA/Cr was observed in MN-treated animals that had partial immune reconstitution of the CD8 T cell population (Figure 1). The recovery of this ratio was found to be due to increases in NAA, suggesting neuronal recovery, and decreases in Cr, possibly reflecting downregulation of glial and inflammatory cell activation. These salutary effects observed by brain imaging were accompanied by clinical improvement including weight gain.

Brain sections were stained with rabbit anti-Iba-1 to determine the level of microglial activation. There was substantially less Iba-1 expression in the FC of MN-treated cohorts (p=0.004), indicating reduced microglial activation (Figure 2). NAA/Cr and Iba-1 levels show significant correlations in the FC and PC (RS=-0.83, p=0.011 and RS=-0.70, p=0.05, respectively) (Figure 3). Though mitigated, microgliosis was still observed in MN-treated animals.

MN-treated animals showed reduced CD14/CD16 activation in the plasma (figure 4). CD14/CD16 monocytes are considered to be responsible for viral infection of the CNS by cell trafficking mechanisms. CD14+/CD16+ and neuronal marker NAA/Cr show an inverse correlation in all regions (PC p=0.002, FC p=0.0003, BG p=0.002, WM p=0.0001) (Figure 5).

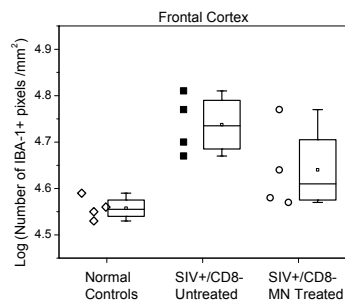
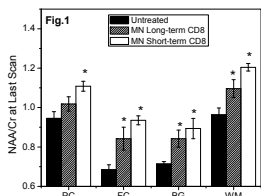


Figure 2

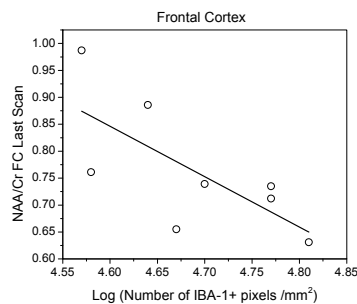


Figure 3

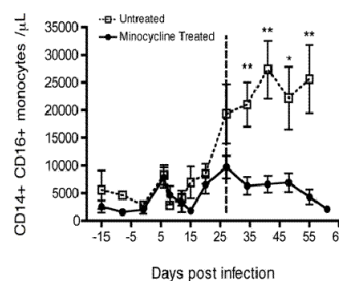


Figure 4

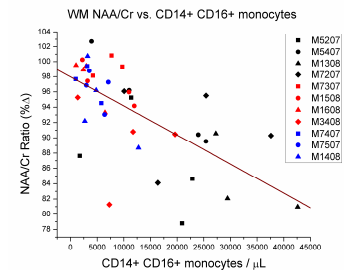


Figure 5

Figure 2: Calcium binding adaptor protein 1 (IBA-1), a marker for microglial activation. Figure 3: Neuronal marker NAA/Cr inversely correlated with IBA-1. Figure 4: MN-treated animals exhibited reduced levels of a proinflammatory CD14+/CD16+ monocyte subset associated with infected cell trafficking into the CNS. Figure 5: Neuronal marker NAA/Cr inversely correlated with the proinflammatory monocyte subset.

Conclusions:

Our results suggest that while MN halts neuronal injury, longer treatments of MN may be necessary to fully deactivate microglia. In addition, MN-treated animals showed a reduction in viral loads in plasma and CSF. MN-treated animals exhibited reduced levels of a proinflammatory CD14+/CD16+ monocyte subset associated with cell trafficking into the CNS. Thus possible mechanisms towards MN's neuroprotection include 1) reduction of inflammatory response by downregulation of glial cell activation in the brain, 2) reductions of CSF and plasma viral loads during treatment and 3) a reduction in a subset of circulating monocytes considered to be responsible for viral infection of the CNS by cell trafficking mechanisms.