

# Reduction of Brain Virus by minocycline and combination anti-retroviral therapy produces neuronal protection in a primate model of AIDS

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## Introduction:

Infection with the AIDS virus can produce neurocognitive disorders including dementia. In developed countries over 50% of HIV-infected patients manifest HIV-Associated Neurocognitive Disorders (HAND) despite receiving antiretroviral therapy. Development of effective treatments for HIV related brain injury is hindered by an incomplete understanding of its neuropathogenic pathways. Utilizing a well-established rhesus macaque simian immunodeficiency virus (SIV) model allows investigation of the full chain of causality from viral infection to neuronal injury in the same animal. MR imaging and spectroscopy (MRS) are noninvasive tools able to monitor the the brain during disease progression.

Previously, our group has reported that neuronal injury caused by the simian immunodeficiency virus can be ameliorated by administration of combination antiretroviral therapy consisting of PMPA and RCV (William et al. JCI 2005) as well as minocycline (Ratai et al PLoS 2010). The similarity of the response suggests that there is a final common pathway involved in neuronal injury that explains the salubrious effects produced by these 2 very different therapeutic approaches. The aim of this study was to understand the neuroprotective mechanism of cART and minocycline using *in vivo* MRS to measure N-acetylaspartate/Creatine (NAA/Cr) as a sensitive, reliable marker for neuronal integrity, flow cytometry to measure monocyte trafficking into the CNS and polymerase chain reaction (PCR) to measure the amount of SIV RNA in plasma, cerebrospinal fluid (CSF) and brains of SIV-infected/CD8 depleted rhesus macaques (SIV+/CD8-).

## Methods:

Twenty three SIV+/CD8- macaques were included in this study. Four animals were studied until 4 weeks post infection (wpi), four until 6 wpi, and four until 8 wpi. Of the 23 animals, seven were treated with minocycline (MN) for four weeks starting at 4 wpi and four animals were treated with combination antiretroviral therapy (cART) consisting of PMPA, FTC and Stavudine for 6 weeks starting at 6 wpi. Brain metabolites were monitored *in vivo* by MRS on a 3 Tesla whole-body imager using a point resolved spectroscopy sequence (PRESS) with water suppression enhanced through T<sub>1</sub> effects using TE=30 ms, TR=2500 ms, n<sub>acq</sub>=192. All spectra were processed offline using LCModel to determine metabolite concentrations. Peripheral CD14 CD16 monocytes were monitored by flow cytometry. Viral RNA was quantified by PCR in plasma and CSF longitudinally and in brain tissue after sacrifice.

## Results:

*In vivo* neuronal marker NAA/Cr steadily declined following SIV infection reaching decreases as low as 20% below baseline by 8 wpi in untreated animals. The decline in NAA/Cr was arrested with this regimen of cART as well as minocycline treatment resulting in higher NAA/Cr levels when compared to untreated animals sacrificed at 8 wpi, (cART P=0.09; MN P=0.029, **Figure 1**).

In SIV-infected (SIV+) untreated animals, plasma viral RNA levels reached a plateau at  $4.9 \times 10^8$  copies eq./mL (data not shown). Viral levels in both plasma and CSF persisted following the administration of cART and minocycline but treated animals revealed  $\sim 1$  log lower plasma viral RNA levels at endpoints when compared to untreated animals at 8 wpi (cART P=0.019; MN P=0.0063). SIV progression in the accelerated model is characterized by the expansion of activated CD14+CD16+ monocytes in the periphery which play a major role in trafficking virus across the BBB into the brain (Campbell et al. PLoS 2011). During minocycline as well as cART treatment a significant reduction of circulating activated CD14+CD16+ monocytes was observed (cART P=0.014; MN P=0.004, **Figure 2**). Comparing NAA/Cr levels with CD14+CD16+ monocytes reveals a strong negative correlation (P=0.0008, R<sup>2</sup>=0.65). NAA/Cr levels also correlated inversely with plasma viral RNA. P=0.008, R<sup>2</sup>=0.41).

Most importantly, we were able to show that virus in the brain in treated animals were lower than those in untreated animals (cART P = 0.007; MN P = 0.006) indicating clearance of brain viral RNA with both treatments (**Figure 3**). Interestingly, severity of neuronal injury correlated with productive viral infection in the CNS. SIV-induced alterations in neuronal metabolism in the frontal cortex, measured by NAA/Cr at last MRS scan, correlated with virus in the frontal cortex (P=0.02, R<sub>p</sub>=-0.48, **Figure 4**).

## Discussion:

The current findings identify a pivotal link in the

neuropathogenesis of HAND. Treatment with both cART and minocycline resulted in partial clearance of virus in the brain. Severity of neuronal damage was shown to be dependent on CNS viral load, and the processes that lead to neuronal injury can be sufficiently ameliorated once the virus in the brain is reduced below a threshold level. The degree of CNS viral infection is directly influenced by plasma viral load as well as activated/infected CD14+ CD16+ monocytes in systemic blood. The data indicates that injury to neurons can be reversed if the influx of monocytes is less than their turnover rate. We hypothesize that the major mechanisms of action by which cART and minocycline treatment resulted in neuroprotection is by reducing the levels of activated monocytes in the periphery, and thus their trafficking into the CNS. These findings suggest a new direction for HAND treatment. Investigation into monocyte-directed therapies is warranted, as circulating activated monocytes mediate CNS infection and injury.

