Manganese Enhanced Magnetic Resonance Imaging (MEMRI) Reflects Human Neuropathology in a Murine Model of HIV-1 Associated Neurocognitive Disorders (HAND)

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Introduction: HIV-associated neurocognitive disorders (HAND) is a common disorder of cognitive and motor dysfunction in virus-infected people. HAND reflects the early disease course or asymptomatic neurophysiological impairment (ANI) but can evolve into HIV-associated mild neurocognitive disorder (MND) and in the most severe form, HIV-associated dementia (HAD). Although antiretroviral therapy has decreased the prevalence of HAD, the overall prevalence of HAND has remained unchanged affecting from 39 to 52% of infected patients. The means for better diagnosis and monitoring of HAND from its earliest stages to more severe disease could improve disease outcomes by separating HAND from other neurologic disorders as well as better defining when to initiate antiretroviral or adjunctive therapies. Importantly, the diagnosis of HAND is one of exclusion of other CNS disorders and as such could be improved if disease biomarkers were available and acquired either from cerebrospinal fluid tests or by neuroimaging. Here, we used Mn^{2+} enhanced MRI (MEMRI) to evaluate changes in the brain of humanized mice due to HIV-1 Clade-C infection, providing imaging biomarkers of brain dysfunction. These methods will be useful for testing experimental therapies for HIV-1 infection and HAND.

Materials and Methods: *Animal Model:* NOD/scid-IL-2Rγ_c^{null} mice (n = 4) were bred, reconstituted with CD34+ human stem cells isolated from human cord blood then infected with HIV-1 Clade-C to generate murine HAND. On the day of birth, newborn mice were irradiated at 1 Gy using a C9 cobalt 60 source. Mice were then injected intrahepatically with 10^5 purified CD34+ hematopoietic stem cells in 20 µl of PBS. Starting from 18 weeks after reconstitution, mice with stable grafts were infected with HIV-1 by i.p. at 10^2 $TCID_{50}$/mouse. Three to four weeks after infection, the levels of viral RNA copies/ml were analyzed to confirm HIV-1 infection. Humanized mice without HIV-1 injection were used as controls. *MEMRI:* MEMRI was performed 16 weeks after HIV-1 injection. A baseline scan was performed before $MnCl_2$ injection. $MnCl_2$ was then injected consecutively at 24 hours interval 4 times before MRI. In each injection, 50 mM $MnCl_2$ was administrated intraperitoneal (i.p.) with a dose of 60 mg/kg. MRI was performed 24 hours after the last $MnCl_2$ injection on a 7T/21 cm horizontal bore scanner (Bruker, Billerica, MA) with a volume coil for RF transmission and a 4-channel phased-array coil for signal reception. Gradient recalled echo sequence was used to acquire T1-wt MRI with TR = 20 ms, flip angle = 20^o, 3D isotropic resolution = 0.1 mm^3.

Data Analysis: Brains were first extracted from MR images[1]. Image inhomogeneity caused by the phase-array coil was corrected using the N3 algorithm[2]. Inter-subject differences were minimized by normalizing the whole brain signal intensity (SI) to the mean SI. Brains were then registered to a MEMRI based standard mouse brain of the same strain created by our group. The SI difference (SI_d) between MEMRI and baseline scan was calculated by subtracting baseline images from MEMRI. Region-of-interest (ROI) analysis was performed on cortex, hippocampus, thalamus, hypothalamus, caudoputamen and amygdala to compare the SI_d changes between HIV-1 infected mice and the control group.

Results: SI_d changes on a HIV-1 infected (left) and a control mouse (right) are demonstrated in Figure 1. It can be seen that SI_d of hippocampus (especially on the pyramidal layer of CA3) was higher in the infected mouse compared to in the control mouse. SI_d was suppressed on amygdala in the infected mouse. Statistical analysis showed that SI_d of hippocampus and amygdala were significantly different ($p < 0.05$) between control and infected mice. No significant difference was found on other regions including cortex, thalamus, hypothalamus and caudoputamen.

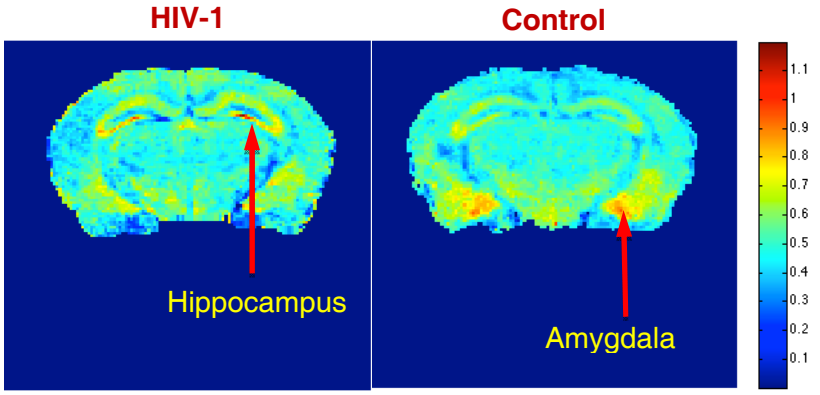

Fig. 1. T1-wt MRI images. Red arrow indicates change in signal intensity.

Discussion:

The changes of MEMRI signal intensity indicated that HIV-1 infection caused the deviation of Mn^{2+} accumulation in hippocampus and amygdala from uninfected state, suggesting neuronal pathology in these regions. The elevated SI_d on hippocampus in infected mice suggests excitatory neurotxicity causing increased Mn^{2+} uptake, whereas the decreased SI_d on amygdala may result from neuronal dropout[3]. The function of both hippocampus and amygdala include memory. The abnormal signal intensity in the infected mice may suggest memory deficit in these animals. Histological analysis is being performed for glial activation and neuronal pathology. In future studies, behavior analysis will also be included.

References:

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