# Targeting HIV-infected brain to improve stroke outcome

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## Abstract

In the era of highly active antiretroviral therapy (HAART), the HIV prognostic has changed from a deadly disease to a chronic condition. While the virus is kept repressed, several co-morbidities, including cardiovascular disease are still present in long term survivors. HIV positive individuals are more at risk of having strokes and also suffer from a less favorable recovery prognostic. Our hypothesis is that despite efficient HAART, residual HIV presence can contribute to stroke severity. In addition, we also hypothesize that viral reservoirs in the brain contribute to injury. Previous publications in our laboratory, based on the EcoHIV mouse model, demonstrated that infection affects the integrity of the functions of the blood-brain barrier. In the current study, we observed that brain infection by EcoHIV resulted in a significant increase in infract size both at early (24h) and late (7 days) post-stroke when compared to mock infected animals. A recovery from stroke injury was seen in control animals, this reduction was not visible in EcoHIV infected mice. Upon further examination, we were able to demonstrate that the induction of stroke resulted in an increase in HIV presence in the affected hemisphere, with infected cells situated primarily near or at the border of the infract area. The majority of cells harboring the virus were from the macrophage/microglial lineage. We next employed several immune markers to examine if the immune reaction to the tissue injury and the more prominent viral presence could be responsible for the delay in infract recovery. We observed a trend for an increase in inflammatory markers in EcoHIV infected mice, especially those associated with the monocyte/macrophage/neutrophil response. We are currently investigating the potential therapeutic efficacy of targeting the HIV CNS reservoir using a high CNS penetrating efficacy (CPE) therapy (neuroHAART). The successful implementation of this regiment would be highly beneficial in HIV patients at risk of cerebrovascular disease.

## Introduction

The usage highly active antiretroviral therapy (HAART) enables sero-positive patients to control HIV-1 replication. Despite this quasi absence of virus replication, some pathologies, such as cardiovascular and neurodegenerative diseases, are still present, albeit progressing at a slower pace. We have previously demonstrated that the NNRIT Efavirenz is toxic to brain microvascular endothelial cells (Bertrand and Toborek, 2015). In addition, we also demonstrated that this drug can affect the integrity of the blood-brain barrier, in particular the tight junctions, and lead to an increase in infract size using an experimental stroke model (Bertrand, Dygert and Toborek, 2016). In this study we are investigating the impact of HIV on stroke and potential HAART strategies that could help alleviate the infection's impact on disease outcome. Our hypothesis is that HIV infection will lead to an increase in stroke size and a delay in recovery. This could be the result of several factors related to the infection, such as increased inflammation, compromised blood brain barrier, toxic viral proteins and many more. We are also investigating the increase therapeutic benefit of HAART with a high CNS penetration-effectiveness to target HIV in the brain. For this investigation, we are using the mouse adapted HIV strain EcoHIV and the middle cerebral artery occlusion (MCAO) stroke model.

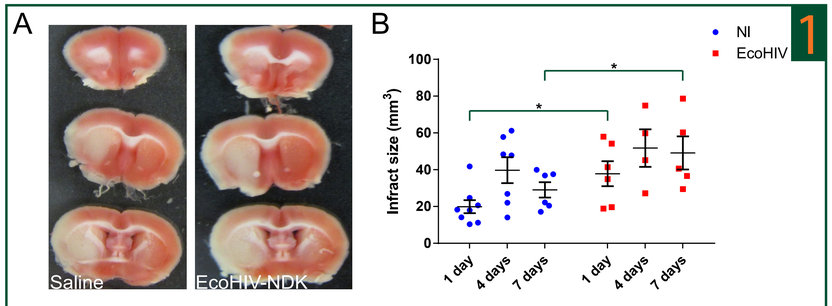


**Figure 1: EcoHIV infection leads to increased stroke size and delays injury recovery.** Mice were infected with 1ug of p24 of EcoHIV-NDK through the carotid artery. Three weeks post-infection, stroke was induced using the middle cerebral artery occlusion model (MCAO) extensively reviewed in (Bertrand, Dygert and Toborek, 2017). The suture is left in place for 60 minutes and then removed to allow reperfusion. (A) Brans were stained at 24 hours post stroke using Triphenyl tetrazolium chloride (TTC) to evaluate infract area. (B) When comparing 24hr infract size between Mock or EcoHIV-NDK infected mice, a significant increase was observed in the latter group. In addition, when evaluating injury size at 1, 4 and 7 days post stroke, we were able to detect a recovery in infract size in mock infected animals, however no such reduction was visible in EcoHIV-NDK infected animals. (*: p<0.05)

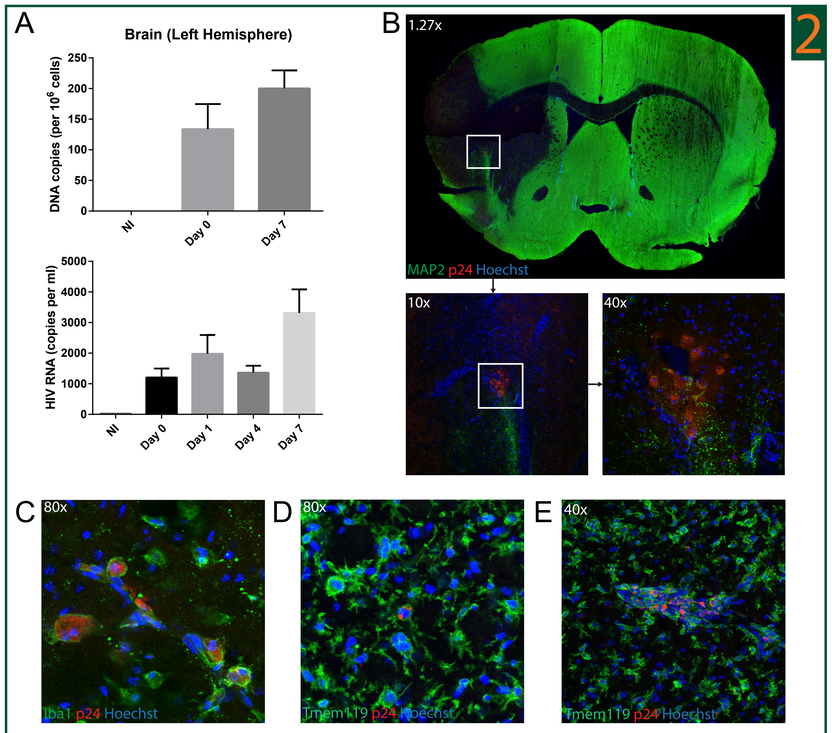


**Figure 2: Stroke leads to an increase in EcoHIV levels.** Animals were infected as described before and then stroke was induced at 3 weeks post infection. (A) Plasma was collected at several time point and EcoHIV was quantified using rt-qPCR. We detected an increase in RNA copy levels at 7 days post-stroke. (B) EcoHIV integrated DNA copies was also quantified using quantitative qPCR on isolated DNA from left brain hemisphere. We detected an increase at all time points following stroke, however statistical significance was only at 7 days post stroke. (C) Using floating section immunofluorescence microscopy, we were able to detect EcoHIV p24 staining at the edge of the infract area (Loss of Map2 staining). We used several markers to try and identify the p24 positive cells. We did not detect any GFAP/p24 double positive cells (astroglial), but a majority of p24 positive cells also co-stained for Iba1 (Microglia/Monocyte/Macrophage). (*: p<0.05)

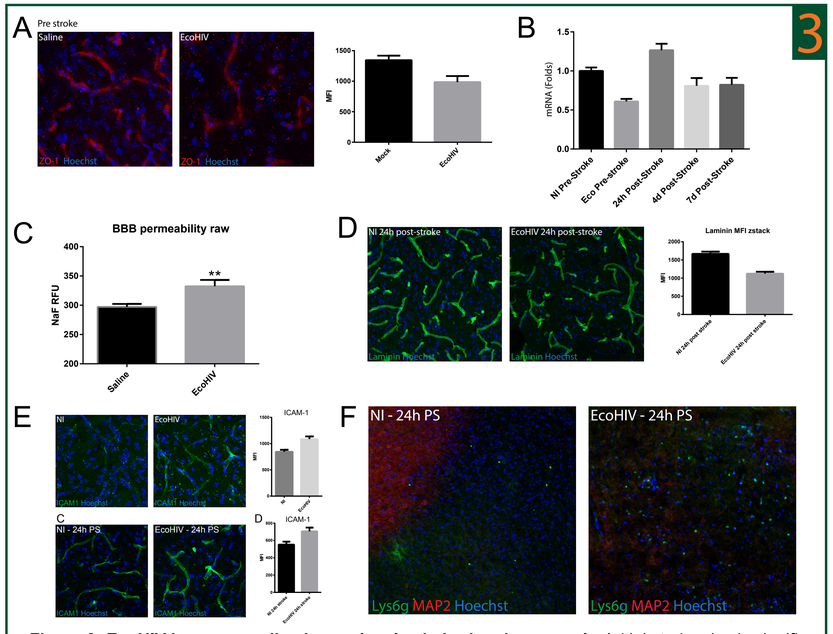


**Figure 3: EcoHIV increases adhesion molecules in brain microvessels.** Initial stroke size is significantly bigger in mice infected with EcoHIV. HIV can cause persistent inflammation and in addition exposure of endothelial cells to HIV protein tat can result in increased expression of attachment molecules. Using our model as described before, we analyzed the levels of three attachment molecules (CD31, ICAM-1 and P-Selectin). Using floating section immunofluorescence and MFI quantification, we were able to detect an increase in attachment molecules either in the infract area (ICAM-1 (A,C)) or at the edge of it (P-Selectin (B,D)) in animals infected with EcoHIV. For CD31 (A,C), we observed an increase in the contralateral side.

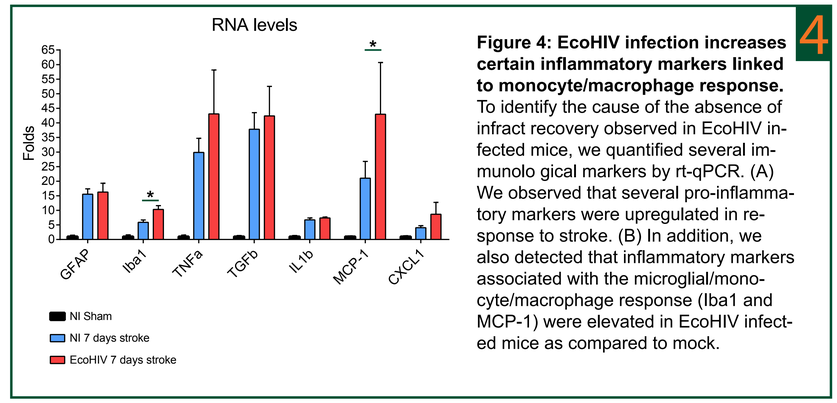


**Figure 4: EcoHIV infection increases certain inflammatory markers linked to monocyte/macrophage response.** To identify the cause of the absence of infract recovery observed in EcoHIV infected mice, we quantified several immunolo gical markers by rt-qPCR. (A) We observed that several pro-inflammatory markers were upregulated in response to stroke. (B) In addition, we also detected that inflammatory markers associated with the microglial/monocyte/macrophage response (Iba1 and MCP-1) were elevated in EcoHIV infected mice as compared to mock.

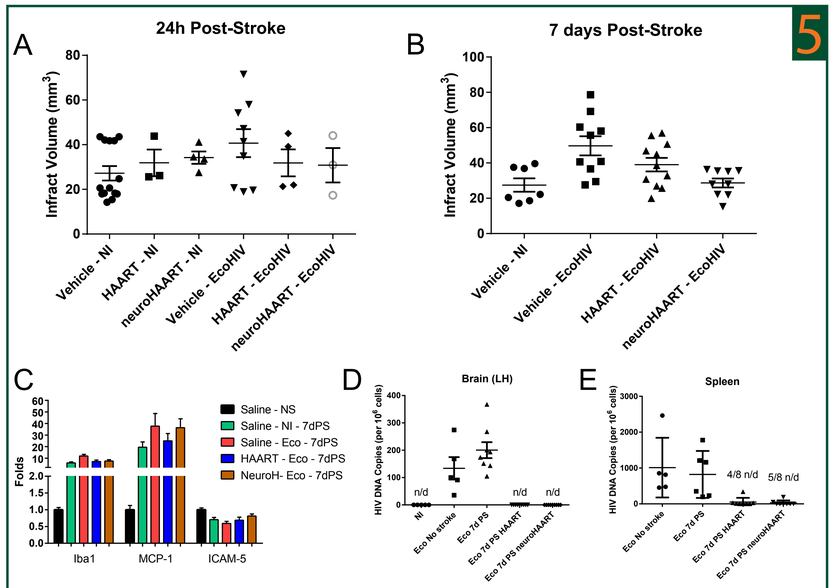


**Figure 5: HAART and neuroHAART reduces HIV induced stroke severity.** Mice were infected with EcoHIV as previously described. One week post infection, animals were gavaged using the following treatments: **HAART** (CPE: 7; Raltegravir 10mg/kg, Emtricitabine 3.33 mg/kg, Tenofovir 5 mg/kg), **neuroHAART** (CPE: 11; Zidovudine 6 mg/kg, Emtricitabine 3.33 mg/kg, Nevirapine 3.33 mg/kg) or vehicle control for 2 weeks. (A) We first demonstrated that treatments did not affect infract size. (B) At 24h post stroke, both treatments reduced infract size to a level similar to vehicle. (C) At 7 days, both treatments were again effective at reducing infract size on a level similar to vehicle, with neuroHAART exhibiting a slight trend to be lower than HAART.

## Conclusion

These results demonstrate that in our experimental model, HIV can have serious repercussion on stroke severity and recovery. The presence of the virus resulted in an increase in infract size. This is possibly due to the increased basal levels of attachment molecules in brain vascular endothelial cells. This occurrence can increase tissue damage in two ways. First, increased attachment of blood mononuclear cells can impede circulation, increasing infract damage. Secondly, increased tissue infiltration by neutrophils can lead to further tissue damage. In addition to initial injury, we also did not observe a contraction of the injury area in EcoHIV infected animals. This is possible due repercussion of the increased initial pro-inflammatory state, but also a repercussion of an increased in HIV presence in the tissue, which could by itself lead to tissue damage but also increase inflammation. This is evidenced by our results that show an increase in EcoHIV genome copies and an increase of certain pro-inflammatory molecules. Finally, using HAART, we were able to reduce HIV mediated stroke severity. While preliminary results does not enable us to conclude that neuroHAART is better than HAART, we can observe a slight trend to lower infract size at 7 days post stroke.

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## References:

1. Bertand, Dygert and Toborek (2016) Antiretroviral Treatment with Efavirenz Disrupts the Blood-Brain Barrier Integrity and Increases Stroke Severity. Sci Rep. 23;6:39738
2. Bertrand and Toborek (2015) Dysregulation of Endoplasmic Reticulum Stress and Autophagic Responses by the Antiretroviral Drug Efavirenz. Mol Pharmacol. Aug;88(2):304-15
3. Bertrand, Dygert and Toborek (2017) Induction of Ischemic Stroke and Ischemia-reperfusion in Mice Using the Middle Artery Occlusion Technique and Visualization of Infarct Area. J. Vis Exp. Feb 2;(120)
3. Leda, Dygert, Bertrand and Toborek (2017) Mouse Microsurgery Infusion Technique for Targeted Substance Delivery into the CNS via the Internal Carotid Artery. J Vis Exp. Jan 31;(119)