R03. HIV Tat protein activates plasma kallikrein-kinin system in the doxycycline-inducible astrocyte specific HIV-1 Tat transgenic mice

Logan Sneed
University of Mississippi, lsneed@go.olemiss.edu

Fakhri Mahdi
University of Mississippi

Salahoddin Mohamed
University of Mississippi

Jason J. Paris
University of Mississippi

Zia Shariat-Madar
University of Mississippi

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HIV Tat protein activates plasma kallikrein-kinin system in the doxycycline-inducible astrocyte specific HIV-1 Tat transgenic mice

Logan Sneed, Fakhri Mahdi, Salahoddin Mohamed, Jason Paris, and Zia Shariat-Madar
The neurovascular unit (NVU) of the blood-brain barrier (BBB) consists of astrocytes, pericytes, neurons and endothelial cells.

Cerebral microvessel, as a necessary component of this unit, are responsible for the selectively of the passage of molecules from capillaries into the brain parenchyma and vice versa.

The NVU as a selective barrier selectivity is compromised when endothelial cells are infected by viruses, including HIV, rabies virus, herpes simplex virus, and West Nile Virus (WNV).

Infection activates endothelial leading to loss of its integrity such as its anti-inflammatory and anti-thrombotic properties.

Upon activation, endothelial barrier functions mainly its permeability and selectivity are altered.

These changes facilitate plasma leakage and the entry of HIV or HIV particles into the brain parenchyma, allowing HIV to spread in the brain.
• We propose that the HIV trans-activator of transcription (Tat) protein alters the structure and function of the endothelium, becoming prothrombotic and proinflammatory state contributing to the activation of the plasma kallikrein kinin system (KKS) and favoring the passage of HIV.
Kallikrein Kinin System

Components of Plasma KKS

• High Molecular Weight Kininogen (HK)
• Prekallikrein (PK)
• Factor XII
High Molecular Weight Kininogen

The contact and intrinsic pathways make up the main amplification loops in the coagulation cascade.
We take advantage of the doxycycline (Dox)-inducible brain-specific HIV-1 Tat (Tat+) transgenic mice to show that an acute very low-level expression of Tat is associated with the activation of the components of the plasma KKS.
**Procedure**

- **Kallikrein quantification**
  - Time course
  - Dose response
  - Enzyme inhibition studies
  - Biotinylated-Tat binding studies

- **Selectivity studies**
  - Measuring kallikrein levels
  - Bradykinin generation
  - Measuring FXII activity
  - Measuring FXI activity

- **Standardizing two in vitro models**
  - A polarized monolayer of human brain endothelial cells (HBECs)
  - Co-culture containing HBECs and astrocytes

- **To explore whether HIV Tat protein induces changes in the structure or function of the endothelial barrier**
  - Measuring PK activation on HIV Tat protein treated endothelial cells
  - The endothelial membrane permeability is quantified in the presence or absence of kallikrein inhibitor
Data/Observations

- Increased Plasma Kallikrein Levels Is Associated With Tat+ Expression in the Doxycycline-Inducible Astrocyte Specific HIV-1 Tat transgenic mice

HIV Tat protein enhances prekallikrein activation
HIV Tat protein enhances plasma kallikrein levels
HIV Tat protein enhances plasma kallikrein levels

Plasma Kallikrein activity is inhibited by Kallistop and Soy been Trypsin Inhibitor (SBTI)

\[ IC_{50} = 1.1 \pm 0.2 \]
Conclusion

• Plasma kallikrein activity was associated with HIV Tat expression ($p < 0.05$).

• Kallikrein was generated in a dose- and incubation time-dependent manner.

• Plasma kallikrein activity was reduced by kallistop and SBTI with IC$_{50}$ =1.1 μM and 1.3 μM.

• Plasma kallikrein activity known to increase the plasma kallikrein level are associated with HIV Tat protein levels in the doxycycline-inducible astrocyte specific HIV-1 Tat transgenic mice, suggesting that plasma kallikrein-dependent signaling might alter the structure and function of the endothelium.