Chapter 2

Aims of the study

HIV-associated neurocognitive disorders (HAND) lead to cognitive, motor and behavioral deficits in approximately 50% of individuals suffering from AIDS. Almost complete eradication of systemic HIV-1 infection is now possible with the advent of HAART. However, its clearance from the central nervous system still remains a huge challenge. In the CNS, macrophage/microglia and astrocytes act as a reservoir for latent HIV infection. This provides the virus with an excellent opportunity to reside and replicate within these cells and spread the infection to uninfected cells. Moreover, as the virus hides in these reservoirs, the antiretroviral drugs become ineffective over time because of incomplete suppression of viral replication, which may develop antiretroviral drug resistance that further compounds the problem of neuroAIDS.

Astrocyte infection by HIV is persistent and non-productive and is frequent in the pediatric and adult brain. Almost 19% of astrocytes in the brain get infected with the virus, though the infection is mostly latent and affect nearby cells by release of viral proteins. HIV-1 Transactivator of transcription, Tat, is one of the viral proteins produced by the proviral DNA in the infected astrocytes that exerts profound damage to other brain cell types concurrently leading to astrocyte activation/dysfunction. For the critical functions, astrocytes play in normal neuronal activity; even minor astrocyte dysfunction can have severe impact on neuronal health and thus brain functions. Therefore, even a small number of infected astrocytes could potentially encompass disastrous consequences for normal brain function. Ample evidence have implicated astrocytes as the major contributors to the glia-mediated indirect neurotoxicity in HIV-E and HAD, however, the exact mechanisms are still unravelled and hence, the study was initiated.

The primary objective of this study has been to determine the cellular and molecular intricacies underlying astrocyte-mediated neuronal injury. To fulfill this lacuna in knowledge, we used HIV protein Tat and focused on the astrocytic purinergic receptors, particularly P2X7R. The research work of the thesis has been divided into following three objectives:

1. To elucidate the role of purinergic receptors (P2X7R) in astrocyte-mediated neuroinflammation (MCP-1/ CCL2 release) upon HIV-1 Tat exposure.

2. To study the involvement of P2X7R in Tat-mediated direct and indirect neuronal damage.

3. To determine the soluble factors released by astrocytes which mediate Tat-induced indirect neuronal toxicity with an emphasis on the role of hemichannels in P2X7R associated mechanisms.

For our work, we utilized a well characterized *in vitro* model system of primary human astrocytes or neurons which closely mimic the *in vivo* conditions. The primary culture of human fetal astrocytes and neurons were differentiated from neural stem/precursor cells (hNPCs) isolated from the aborted fetus. This model system has been well accepted in the field.