HIV-1 Tat Protein Promotes Amyloid β Generation and Tau Phosphorylation in Rat Hippocampal Slices

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HIV-1 Tat protein has been implicated as a causative agent in the pathogenesis of HIV-1-associated neurocognitive disorder (HAND) and Alzheimer's disease (AD)-like pathology in HIV-1 infected patients. Here, we provide insights into the potential roles of extracellular HIV-1 Tat protein in amyloid β (Aβ) generation and Tau phosphorylation, two major neuropathological features of AD. Exposure of the rat hippocampal slices to the full-length HIV-1 Tat protein (Tat1-86) for 3 days led to the increased levels of Aβ precursor protein (APP) accumulation, which accompanied by Aβ generation in the hippocampus, the brain region most commonly damaged in HIV-1-associated dementia (HAD). Moreover, extracellular HIV-1 Tat significantly stimulated the level of phosphrylated Tau (pTau) identified using immunoblotting with AT8 antibody, which recognizes abnormally hyperphosphorylated Tau. Collectively, our data suggest that HIV-1 Tat plays important roles in increasing the levels of APP accumulation, Aβ generation and Tau phosphorylation in the hippocampus, and thereby might contribute to the development of AD-like pathology in HIV-1-infected patients.
Key Words: HIV-1-associated neurocognitive disorder, HIV-1 Tat, pTau, Aβ, Hippocampus, Alzheimer's disease

INTRODUCTION

Human immunodeficiency virus-1 (HIV-1) infection can cause HIV-associated neurocognitive disorders (HAND), a set of conditions ranging from subtle neuropsychological impairments to HIV-associated dementia (HAD), the most severe form of HAND (1, 2). The introduction of highly active antiretroviral therapies (ART/HAART) has effectively increased the life span of people living with HIV-1/AIDS. However, recent epidemiological studies indicate that the prevalence of HAND with some form of neurocognitive impairment appears to be increasing up to 50% of HIV-infected individuals despite access to ART (3, 4). Furthermore, the incidence of Alzheimer's disease (AD)-like neurocognitive problems and neuropathological features of AD in older HIV-1-infected patients is increasing as HIV infection has become a chronic and manageable condition under ART (5, 6).

AD, primarily affecting the population over the age of 60, is the most common and still incurable form of dementia (7). AD is primarily an auto "proteopathic" neurodegenerative

disease initiated in neurons by misfolding and aggregation of two specific proteins, Aβ and Tau, which in later stages of disease form the characteristic lesions of Aβ-containing senile plaques, usually surrounded by reactive astrocytes, activated microglia and dystrophic neuritis, and intracellular neurofibrillary tangles (8). Inflammation plays a prominent role in AD but it appears to be secondary to Aβ and Tau accumulation (9). These major pathogenetic mechanisms of AD, in concert, lead to neocortical and hippocampal atrophy, memory dysfunction and decline of cognition in AD.

HIV-1 transactivator of transcription protein (Tat), a viral nonstructural protein of 86∼104 amino acids in length, is the product of two exons and contributes to the induction of viral and cellular genes (10). Tat can be secreted by infected macrophages and microglia, and transported across the blood-brain barrier (11) has been detected in brain of patients with HAD (12), implicating a possible role in the progression of HAND. Furthermore, some HIV-positive patients exhibiting the HAND phenotype with an increase in Aβ deposition (13, 14) or increased levels of Tau and phosphorylated Tau (pTau) protein (15, 16) suggest that Tat might contribute to the development of AD-like pathology in HIV-1 infected patients.

In this context, we present data supporting that extracellular HIV-1 Tat can contribute to the increased levels of Aβ precursor protein (APP) accumulation, Aβ generation and Tau phosphorylation in the hippocampus, which is primarily linked to cognitive function. These findings might suggest one common pathway in HAND and AD pathogenesis.

MATERIALS AND METHODS

Materials

Anti-Tau (mouse monoclonal antibody clone Tau-5), which can identify total Tau, was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phosphorylated Tau (Ser$^{199/202}$/Thr^{205}, mouse monoclonal antibody clone AT8), which binds to highly phosphorylated Tau (pTau), was purchased from Innogenetics (Gent, Belgium). Aβ1-40 ELISA kit was acquired from Immuno-Biological Labora-tories (Minneapolis, MN, USA). Anti-APP (mouse monoclonal antibody clone 22C11) corresponding to residues 66-81 of APP was purchased from Boehringer Mannheim Biochemica (Mannheim, Germany). Horseradish-peroxidase-conjugated antimouse IgG was obtained from Jackson Immuno Research (West Grove, PA, USA). Anti β-actin antibody and other chemicals were acquired from Sigma (St. Louis, MO, USA).

Preparation of Tat

Biologically active recombinant HIV-1 Tat1-86 of (HIV-1$_{IIIB}$) was obtained from Advanced Bioscience Laboratories, Inc. (Kensington, MD, USA). A stock concentration of 50 μM Tat peptides in dH$_2$O was made up fresh each time and added to slice culture medium. Specificity of Tat1-86-mediated effect was controlled by hippocampal treatment with heat-inactivated Tat1-86$_{hi}$, prepared by incubation at 85℃ for 1 h.

Hippocampal slice cultures

All experimental procedures were performed using protocols approved by the Animal Care Committee of the Ewha Medical Research Institute. Hippocampal slice cultures were prepared from 10-day-old Sprague-Dawley rat pups (Harlan, Indianapolis, IN, USA) as previously described (17, 18). In hippocampal slices, cells, synapses, and neuronal connectivity mature in parallel with their counterparts *in vivo* (19). Thus, these preparations are deemed appropriate for studies of prolonged pharmacological treatment and recovery without blood flow, which would be difficult to perform in an intact animal system.

Experimental treatments

Treatments were initiated at 10 days post-dissection. All reagents were added to serum-free medium (no horse serum) equilibrated at 37℃ in 5% CO$_2$ prior to addition to the slices. Slices were stimulated with Tat1-86 (1 μM) for 3 days as previously described (18). Vehicles were prepared identically to experimental treatments but did not contain Tat. At the indicated time after treatment initiation, the slices were rinsed twice in 1x PBS, then harvested via the removal of

Millicell membrane inserts after sample freezing on dry ice. They were then processed for immunoblotting as described below. The culture media were also collected to analyze the level of Aβ release.

Enzyme-Linked Immunosorbent Assay (ELISA)

The levels of Aβ1-40 in the culture media were determined using commercially available ELISA kit in accordance with the manufacturer's protocol. In short, 100 μl of sample was added into the precoated plate and was incubated overnight at 4℃. After washing each well of the precoated plate with washing buffer, 100 μl of labeled antibody solution was added and the mixture was incubated for 1 hr at 4℃ in the dark. After washing, chromogen was added and the mixture was incubated for 30 mins at room temperature in the dark. After the addition of stop solution, the resulting color was assayed at 450 nm using a microplate absorbance reader.

Polyacrylamide gel electrophoresis and western blotting

Western blotting was conducted as recently described (17, 18). Whole cell extracts obtained and equal quantities of sample proteins were separated by molecular weight on 10% SDS polyacrylamide gels and transferred onto PVDF membranes (Millipore, Bedford, MA, USA). The membranes were then blocked for 0.5 h with 3% milk in TBST and probed with primary antibody diluted in 1% milk and incubated overnight at 4℃. Signal was acquired with enhanced chemiluminescence after incubation with horseradish-peroxidase conjugated secondary antibodies (Jackson). Densitometric values were normalized versus β-actin.

Statistical analysis

Data were expressed as the mean $\pm$ SEM of the indicated numbers of experimental replicates. Differences among groups were evaluated for statistical significance using one-way ANOVA with a Student's t test. Statistical significance was considered to be $p < 0.05$.

RESULTS AND DISCUSSION

Accumulating evidence indicates that HAND can be found in increasing segments of populations at risk for the incidence of AD-like neurocognitive problems in older HIV-1-infected individuals and that levels of Aβ are significantly increased in postmortem brain samples from patients infected with HIV-1 (5, 6). HIV-1 Tat protein was implicated as a causative agent in the pathogenesis of HAND (20, 21) and AD-like pathology in HIV-1 infected patients (22, 23).

To determine the potential roles of extracellular HIV-1 Tat in the development of AD-like neuropathological features in older HIV-1-infected individuals, we exposed the rat hippocampal slices to biologically active recombinant Tat1-86 protein for 3 days to measure the levels of APP accumulation, Aβ generation, and Tau phosphorylation in the hippocampus. Immunoblotting analysis of total slice extracts was performed with the 22C11 antibody to identify membrane APP or soluble form of APP derivatives. As shown in Fig. 1A and B, data demonstrated that relatively longer exposure to HIV-1 Tat significantly augmented the level of APP accumulation in the hippocampus, the brain region most commonly damaged in HAD. Furthermore, Tat promoted the level of Aβ1-40 generation in the slice culture media as measured by a specific Aβ ELISA assay (Fig. 1C). Importantly, Tat1-86 treatment resulted in a prominent increase in the level of pTau identified by AT8 antibody, which binds to abnormally hyperphosphorylated Tau in the hippocampus (Fig. 2A and C), compared with the vehicle-treated samples under the same experimental conditions (Fig. 2A and B). These findings together suggest that HIV-1 Tat treatment evoked increased levels of APP accumulation, Aβ generation, and Tau phosphorylation in the hippocampus.

Infection with HIV-1 causes degeneration of neurons leading to motor and cognitive dysfunction in AIDS patients. One of the key viral regulatory proteins, the HIV-1 Tat protein, which is released by infected macrophages or microglia, can be taken up by various uninfected cells including neurons. HIV-1 Tat is known to induce neuro-
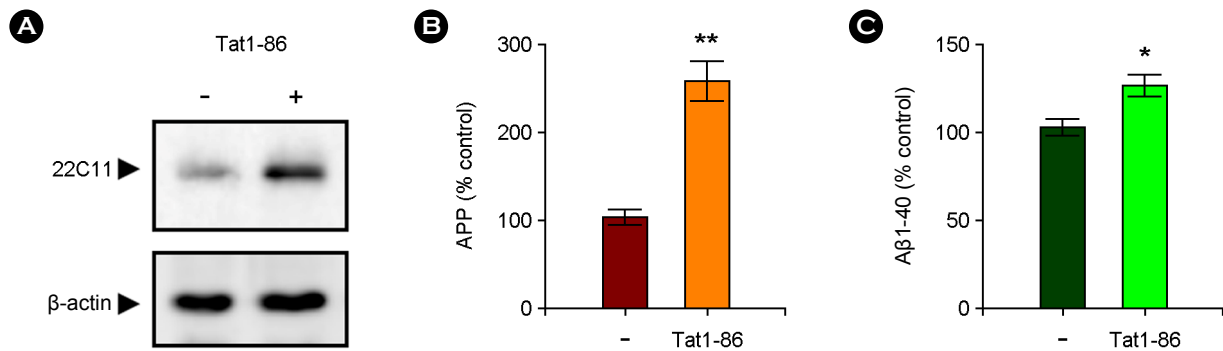
Figure 1. Tat1-86 increased the level of APP accumulation and Aβ1-40 generation in the hippocampus. The hippocampal slices were treated for 3 days with Tat1-86 (1 μM) or vehicle only. (A) Western blots showing the Tat-induced increase of APP accumulation in hippocampal slices. Total lysates were analyzed via immunoblotting with 22C11 antibody to identify membrane APP or soluble form of APP derivatives. The blots were stripped and developed with anti-β-actin for equal protein loading. The data represent three independent experiments. (B) A normalized densitometric quantification of APP against β-actin for equal protein loading. (C) The levels of Aβ1-40 released in the slice culture media were assessed by using a specific Aβ ELISA kit. The results of triplicate experiments are expressed as the means ± SEM. *$p < 0.05$ versus vehicle-treated samples.
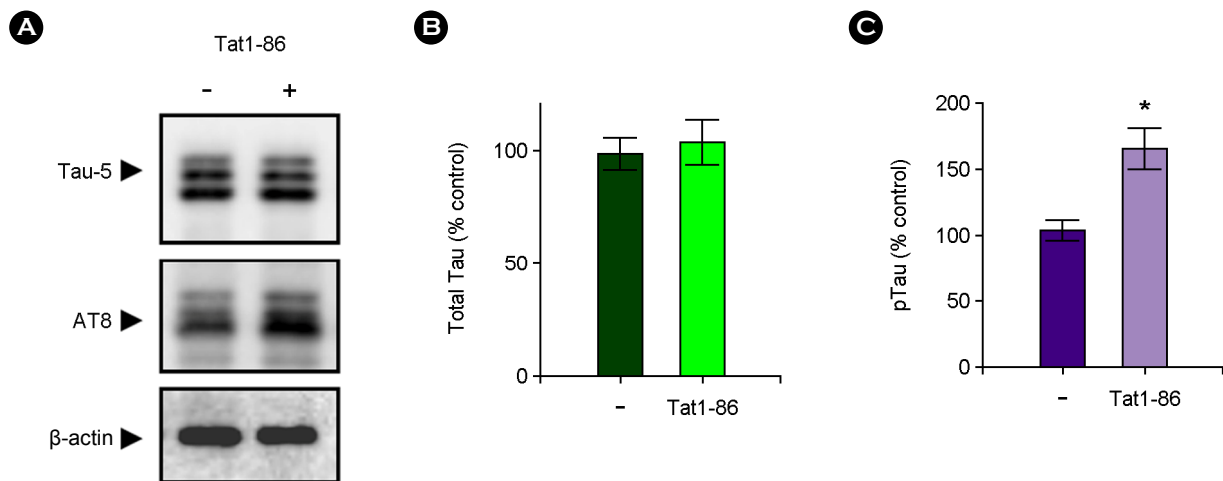

Figure 2. Tat1-86 promoted the level of phosphorylated Tau (pTau) in hippocampal slices. The slices were treated for 3 days with Tat1-86 (1 μM) or vehicle only. (A) Western blots showing the Tat-induced increase of highly phosphorylated Tau (pTau) in hippocampal slices. Total lysates were analyzed via immunoblotting with Tau-5 antibody to recognize total Tau protein or with AT8 antibody to detect hyperphosphorylated Tau. (B) A normalized densitometric quantification of total Tau against β-actin for equal protein loading. (C) A normalized densitometric quantification of pTau against β-actin for equal protein loading. The results of triplicate experiments are expressed as the means ± SEM. *$p < 0.05$ versus vehicle-treated samples.

toxicity in rat fetal primary neuronal cell cultures prepared from hippocampus, cortex, or midbrain (24) and extracellular Tat is known to induce caspase activation in primary cultures of rat fetal neurons (25, 26). Consistently, our recent study has shown that longer exposure of hippocamal slices to 1 μM Tat1-86 for 3 days clearly induced a decline of cell viability (27), thus supporting a possible role of this protein in the progression of HAND.

Importantly, accumulating evidence suggests that Tat plays an important role in the processing and accumulation of Aβ in the brains of AIDS patients. First, HIV-1 Tat was shown to elevate Aβ in fetal human brain spheres in culture and Aβ was elevated in postmortem brain tissues of patients with HAND (23). Second, addition of recombinant Tat

protein inhibits the uptake of Aβ by primary mouse microglial cells, suggesting that Tat regulates the level of Aβ by inhibiting microglial phagocytosis (28). Third, the induction of Tat in astrocytes increases neuronal damage, Tau phosphorylation, and Aβ plaque formation in APP/ presenilin-1 (PS1) transgenic mice (22). In addition, HIV-1 Tat increased endolysosome accumulation of Aβ precursor protein and neuronal Aβ production (29). Consistently, our study indicates that that biologically active recombinant Tat protein promoted the levels of APP accumulation, Aβ generation, and pTau in the hippocampus, which undergo Tat-induced cytotoxicity (27). These findings together support the notion that HIV-1 Tat plays an important role in the development of HAND and thereby contributes to the development of AD-like pathology in HIV-1-infected individuals. Recent study demonstrating that stereotaxic injection of a lentiviral Tat expression construct into the hippocampus of APP/presenilin-1 (PS1) transgenic mice resulted in increased Tat-mediated production and processing of Aβ *in vivo* (30), further supporting one common pathway in HAND and AD pathogenesis.

In conclusion, our data strongly suggest that HIV-1 Tat protein might play an important role in APP accumulation, Aβ generation, and Tau phosphorylation in the hippocampus, which is primarily linked to cognitive function, and thereby contribute directly to the development of AD-like pathology in HIV-1-infected patients. Our findings also implicate that blocking HIV-1 Tat protein could be a potential therapeutic target against HAND, although further study remains to examine this possibility, and also to uncover viral mechanisms promoting AD-like neuropathology and common pathways with AD.

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