Int J Clin Exp Pathol (2009) 2, 433-443
www.ijcep.com/IJCEP901004

Original Article

HIV-1 Tat Contributes to Alzheimer's Disease-like Pathology in PSAPP Mice

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Received 27 January 2009; Accepted and Available online 30 January 2009

Abstract: Prevalence of HIV-associated cognitive impairment is rising. Amyloid-beta (A-beta) plaque deposition in the brain may be a contributing factor as epidemiological data suggests significant numbers of long-term HIV survivors are at elevated risk of developing Alzheimer's disease (AD). HIV-1 Tat-induced A-beta deposition, tau phosphorylation, and subsequent neuronal death could be risk factors for subsequent AD and/or HIV-related cognitive impairment. To mimic this clinical condition, we generated mice with HIV-1 Tat-induced AD-like pathology. We first performed a short-term Doxycycline (dox) dosing (54, 108, and 216 mg/kg/day) study in transgenic mice whose astrocytes express HIV-1 Tat via activation of a GFAP/dox-inducible promoter. After one week, mouse brains were examined histologically and the expression of Bcl-xL, Bax, and phospho-tau was investigated by Western blotting. We next cross-bred these mice with the PSAPP mouse model of AD. To simulate chronic Tat secretion over periods longer than one week, we used an optimized dose of 54 mg/kg/day on a bi-weekly basis over three months; based on the initial dose ranging study in the Tat transgenic mice. This was followed by antisera detection of A-beta, and Western blot for phospho-tau, Bcl-xL, and Bax. Tat significantly induced neuron degeneration and tau phosphorylation in Tat transgenic mice, dox dependently ($P<0.001$) with the most robust effects at the 216 mg/kg/day dose. In the long term study, similar effects at the chronic 54 mg/kg/day dose were observed in PSAPP/Tat mice induced with dox. These mice also showed significantly more A-beta deposition ($P < 0.05$), neurodegeneration, neuronal apoptotic signaling, and phospho-tau than PSAPP mice ($P < 0.05$). In conclusion, HIV-1 Tat significantly promotes AD-like pathology in PSAPP/Tat mice. This model may provide a framework in which to identify new mechanisms involved in cognitive impairment in the HIV infected population, and possible treatments. Additional works will be needed to fully characterize the mechanism(s) of HIV- induced amyloid deposition, and also to uncover viral mechanisms promoting AD-like pathology in general.
Key Words: HIV-1, Tat, PSAPP, Alzheimer's, Dementia, beta-amyloid

Introduction

The natural history of HIV infection is changing. Advanced age will be an important moderator of clinical outcomes associated with the disease, particularly dementia. Historically there was not much need to consider age-related neurodegenerative disorders, such Alzheimer's disease (AD), as contributing to affective or cognitive disorders in HIV-infected patients, as infection existed virtually only in young adults [1]. This paradigm no longer holds true in the era of highly active antiretroviral therapy (HAART), raising new issues regarding the diagnosis and treatment of HIV-related neurocognitive disorders. Indeed it has been suggested this new course in the HAART era may also signify a phenomenon whereby HIV lowers the threshold (cerebral reserve hypothesis) for the clinical presentation of other neurodegenerative diseases such as AD expanding risk to

younger, middle-aged patients [1].

Because of long-term survival in the HIV-infected population, the epidemic is extending into older age brackets and is commonly characterized by AD-like pathology. Currently, some 60,000 HIV-infected individuals are over the age of 50. The number of HIV+ patients over the age of 65 increased exponentially from some 1,000 to more than 10,000 in the past ten years [2-3]. Furthermore, it is predicted that 50% of prevalent AIDS cases in this country will fall into this older age group by 2015. Several post-mortem studies have revealed a significant incidence of AD-like pathology in the HIV-infected brain including increased brain beta-amyloid (A-beta) deposition [4], increased extracellular amyloid plaques [5], and decreased cerebrospinal fluid (CSF) A-beta levels [6].

Particularly, the HIV-1 transactivator protein (Tat) has been implicated in this AD-like pathology through a variety of mechanisms. However the chronicity of neuropathologies in humans is uncertain due to the fact that all histological data comes from post-mortem samples. Using an *in vitro* system, we have recently shown HIV-1 Tat inhibits microglial phagocytosis of A-beta peptide *in vitro* and that this dysfunction is augmented by the pro-inflammatory cytokine, IFN-γ (interferon-gamma) [7], and opposed by the natural STAT1 (signal transducer and activator of transcription-1) inhibitor from Green Tea, epigallocatechin-3-gallate (EGCG). To date, no *in vivo* model has been created to study HIV-1 Tat-induced AD-like pathology. Such a model is necessary to generate both novel therapeutic targets and also a pharmacological screening platform.

In an effort to generate such a model, we crossed transgenic PSAPP (APPswe, PSEN1dE9) mice [8] and HIV-1 Tat-transgenic mice (GT-tg mice). The latter demonstrates HIV-1 Tat expression from astrocytes under the control of both the astrocyte-specific glial fibrillary acidic protein (GFAP) promoter and a doxycycline (dox)-inducible promoter [9]. We found HIV-1 Tat significantly induced neuron degeneration and tau phosphorylation in Tat transgenic mice dox dependently ($P<0.001$). Compared to both PSAPP mice + dox, and/or PSAPP/Tat mice without dox, PSAPP/GT-tg mice + dox (54 mg/kg/day) showed significantly more A-beta deposition in brain regions examined ($P<0.05$). Western blot analysis confirmed these results indicating dox-induced PSAPP/Tat inducible mice demonstrated significantly more A-beta deposition quantitatively ($P<0.05$). Additionally PSAPP/Tat mice induced with dox displayed significantly more neurodegeneration, neuron apoptosis, and tau phosphorylation than either PSAPP mice + dox or PSAPP/GT-tg mice not induced with dox ($P<0.05$).

Material and Methods

Mice

The creation and genotyping of the inducible and brain-targeted HIV-1 Tat transgenic (GT-tg) mice was previously described [9]. A founder pair of GT-tg mice was generously provided by Dr. Johnny He (Indiana University). Transgenic PSAPP (APPswe, PSEN1dE9) mice were obtained from the Jackson Laboratory. These animals were housed and maintained at the College of Medicine Animal Facility of the University of South Florida (USF) Health Sciences Center, and all experiments were in compliance with protocols approved by the USF Institutional Animal Care and Use Committee (IACUC).

Short Term Dose Ranging Study in Tat-Transgenic Mice

To express Tat for the initial dose-ranging study, a total of 18 GT-tg mice were used divided evenly between males and females and administered one of three doses of dox (Sigma, Louis, MO; 54, 108, or 116 mg dox/mg/day) in drinking water based on the previous works of Kim *et al* [9]. After 7 days mice were euthanized with overdose of isofluorane and transcardially perfused with ice-cold physiological saline containing heparin (10 U/ml). Brains were rapidly isolated and quartered using a mouse brain slicer (Muromachi Kikai, Tokyo, Japan). The 1st and 2nd anterior quarters were homogenized for Western blot analysis and ELISA, and the 3rd and 4th posterior quarters were fixed in 4% paraformaldehyde in PBS at 4°C overnight and routinely processed for hematoxylin/eosin (H/E) staining as immunohistochemistry. Empty vehicle treatment was included as a control (data not shown).

Long Term HIV-1 Tat Expression in PSAPP Mice

PSAPP mice with administered dox (PSAPP/dox) were compared to PSAPP/Tat mice without dox administration, and PSAPP/Tat mice administered with dox (PSAPP/Tat/Dox). The first two groups are both negative controls as little to no HIV-1 Tat expression occurs in the absence of dox. The third group, PSAPP/Tat/Dox, represents the novel model of HIV-1 Tat-induced, AD-like pathology. Twelve mice (6 males and females) at 10 months of age were used per group. The mice were divided evenly between males and females and administered this minimum dose of dox (54 mg/kg/day, every-other-week) in drinking water. In order to mimic a chronic secretion of Tat in the brain, as seen in clinical cases of HIV-1 infection, we chose to lower the dose of dox to 54 mg/kg , daily, on a biweekly basis (administered every other week) for three months based on our short term dose ranging study. We find that longer periods of Tat expression, at this lower dose, closely mimics the HAD-like damage seen in the 1 week dose ranging study at the higher dox dose of 216 mg/kg/day. After three months mice were euthanized with overdose of isofluorane and transcardially perfused with ice-cold physiological saline containing heparin (10 U/ml). Brains were prepared for pathologic examination as above.

Histological Examination

Five coronal sections from each brain (10 μm thickness) were cut with a 150 m interval. Sections were routinely deparaffinized and hydrated in a graded series of ethanol before pre-blocking for 30 min at ambient temperature with serum-free protein block (Dako Cytomation, Carpinteria, CA). Nuclear pyknosis suggestive of chromatin condensation was examined by H&E stain. Ten micrometer sections were fixed with 4% paraformaldehyde in phosphate-buffered saline solution (PBS: 10 mM KH2PO4, 37 mM Na2HPO4, 87 mM NaCl, 53 mM KCl, pH 7.4) for 1 hr, washed with distilled water for 2 min, then incubated with Mayer's hematoxylin (diluted 1:10) for 4 min at room temperature. The slices were then rinsed with EtOH for 2 min, followed by a dH_20 rinse for 2 min, covered with a 0.5% eosin solution for 5 min, and then rinsed twice with water. Sections were then examined under light microscopy.

Immunohistochemistry

Immunohistochemical staining was performed using anti-human A-beta antibody (clone 4G8; Sigma) in conjunction with the VectaStain Elite ABC kit (Vector Laboratories, Burlingame, CA) coupled with diaminobenzidine substrate. 4G8-positive A-beta deposits were examined under bright field using an Olympus (Tokyo, Japan) BX-51 microscope. To study neurofibrillary tangle-like structures, conventional immunofluorescence staining using mouse PHF-1 monoclonal antibody, which recognizes the dually phosphorylated Ser396 and Ser404 epitope of tau peptides [10] was utilized. The PHF-1 antibody was kindly provided by Dr. Peter Davies (Albert Einstein College of Medicine; Bronx, NY). Incubation with PHF-1 antibody (1:60) took place overnight at 4ºC, followed by 1 hr at RT. One control section was processed identically except that the primary antibody was omitted from the incubation buffer. After rinsing, sections were incubated in the presence of Cy3-conjugated goat, anti-rabbit secondary antibody (1:200) or goat anti-mouse antibody (1:100) (Sigma; St. Louis, MO) for 3 hrs at room temperature. Sections were then thoroughly rinsed in PBS. For the same-section comparisons in this study, this step was immediately followed by the thioflavin-S staining procedure as previously described [11]. Sections were stained by immunohistochemistry for A-beta using 4G8 monoclonal antibody.

Western Blot Analysis and Immunoprecipitation

Mouse brains were lysed in ice-cold lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% v/v Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM α-glycerolphosphate, 1 mM NaVO, 1 ug/ml leupeptin, 1 m PMSF), and an aliquot corresponding to 50 g of total protein was electrophoretically separated using 10% Tris-gels. Electrophoresed proteins were then transferred to polyvinylidene difluoride membranes (Bio-Rad, Richmond, CA), washed in dH_2O, and blocked for 2 hrs at room temperature in Tris-buffered saline (TBS; Bio-Rad) containing 5% (w/v) nonfat dry milk. After blocking, membranes were hybridized for 2 hrs at room temperature with various primary antibodies. Membranes were then washed 3 x for 5 min each in dH_2O and incubated for 1 hr at ambient temperature with the appropriate HRP-conjugated secondary antibody (1:1000;

Pierce Biotechnology, Rockford, IL). All antibodies were diluted in TBS containing 5% (w/v) nonfat dry milk. Blots were developed using the luminol reagent (Pierce Biotechnology). Densitometric analysis was done using a FluorS Multiimager with Quantity One software (Bio-Rad). Immunoprecipitation was performed for detection of phosphorylated Tau by incubating 200 µg of total protein of each sample with monoclonal antibody AT270 (1:400; Pierce Biotechnology) or anti-phospho-tau antibody that recognizes human phosphorylated tau at Ser199/202 overnight with gentle rocking at 4°C. Then 10 L of 50% protein A-Sepharose beads were added to the sample (1:10; Sigma) before gentle rocking for an additional 4 hrs at 4°C. After washes with 1 x cell lysis buffer, samples were subjected to Western blot analysis as described above. Antibodies used for Western blot analysis included anti-Bcl-xL antibody (1:1,000, AnaSpec), anti-Bax antibody (1:1,000, AnaSpec), and anti-actin antibody (1:1,500 an internal reference control; Roche).

ELISA

Mouse brains were isolated under sterile conditions on ice and placed in ice-cold lysis buffer. Brains were then sonicated on ice for a total of 1.5 min (30 second sonification separated by 30 second rest period, cycled over three minutes), allowed to stand for 15 min at 4°C, and centrifuged at 15,000 rpm for 15 min. Total A (including A1–40, 42) species were detected by acid extraction of brain homogenates in 5 M guanidine buffer, followed by a 1:10 dilution in lysis buffer. Soluble total A-beta was directly detected in brain homogenates prepared with lysis buffer described above by a 1:4 or 1:10 dilution, respectively. A-beta was quantified in these samples using the total A-beta ELISA kits [11]. Total A-beta species were detected by acid extraction of brain homogenates according to our previous methods [11]. Data are expressed as pg/mg A1-x, mean ± SD.

Image Analysis

Quantitative image analysis (conventional "A burden" analysis) was performed for A immunohistochemistry in PSAPP mice receiving dox, and all PSAPP/Tat groups. Images were obtained using an Olympus BX-51 microscope and digitized using an attached MagnaFire imaging system (Olympus). Briefly, images of five 5 µ sections (150 µ apart) through each anatomic region of interest (hippocampus or cortical areas) were captured, and a threshold optical density was obtained that discriminated staining from background. Manual editing of each field was used to eliminate artifacts. Data are reported as a percentage of immunolabeled area captured (positive pixels) divided by the full area captured (total pixels). Quantitative image analysis was performed by a single examiner (M.T.) blinded to sample identities.

Statistical Analysis

All data were normally distributed; therefore, in instances of single mean comparisons, Levene's test for equality of variances followed by t-test for independent samples was used to assess significance. To examine for gender differences, we employed analysis of variance (ANOVA) followed by pairwise comparison of the means using a post hoc Newman-Keuls test. In instances of multiple mean comparisons, analysis of variance was used, followed by post hoc comparison using Bonferonni's method. Alpha levels were set at 0.05 for all analyses. The statistical package for the social sciences release 10.0.5 (SPSS Inc., Chicago, Illinois) was used for all data analysis.

Results

HIV-1 Tat Dose Dependently Increases Neuronal Damage and tau Phosphorylation in Tat Transgenic Mice (GT-tg)

Administration of dox is positively associated with increased nuclear H&E staining, suggesting that HIV-1 Tat protein expression induces increased neurodegeneration (**Figure 1A**). As an additional confirmatory measure, Western blotting analysis for the apoptotic repressor protein, Bcl-xL, shows that dox dose dependently decreases its ratio to actin (**Figures 1B** and **1C**) ($P<0.005$). As shown in **Figure 1D**, tau phosphorylation was significantly and positively correlated with the dosage of dox administration. As a validation, we examined HIV-1 Tat expression in the mice after dox administration using Western blotting analysis and found that expression of HIV-1 Tat protein occurred dox dependently (data not shown) as has also been demonstrated in previous studies [9]. In addition, a minimum dosage of dox, 54 mg/kg/day, was determined

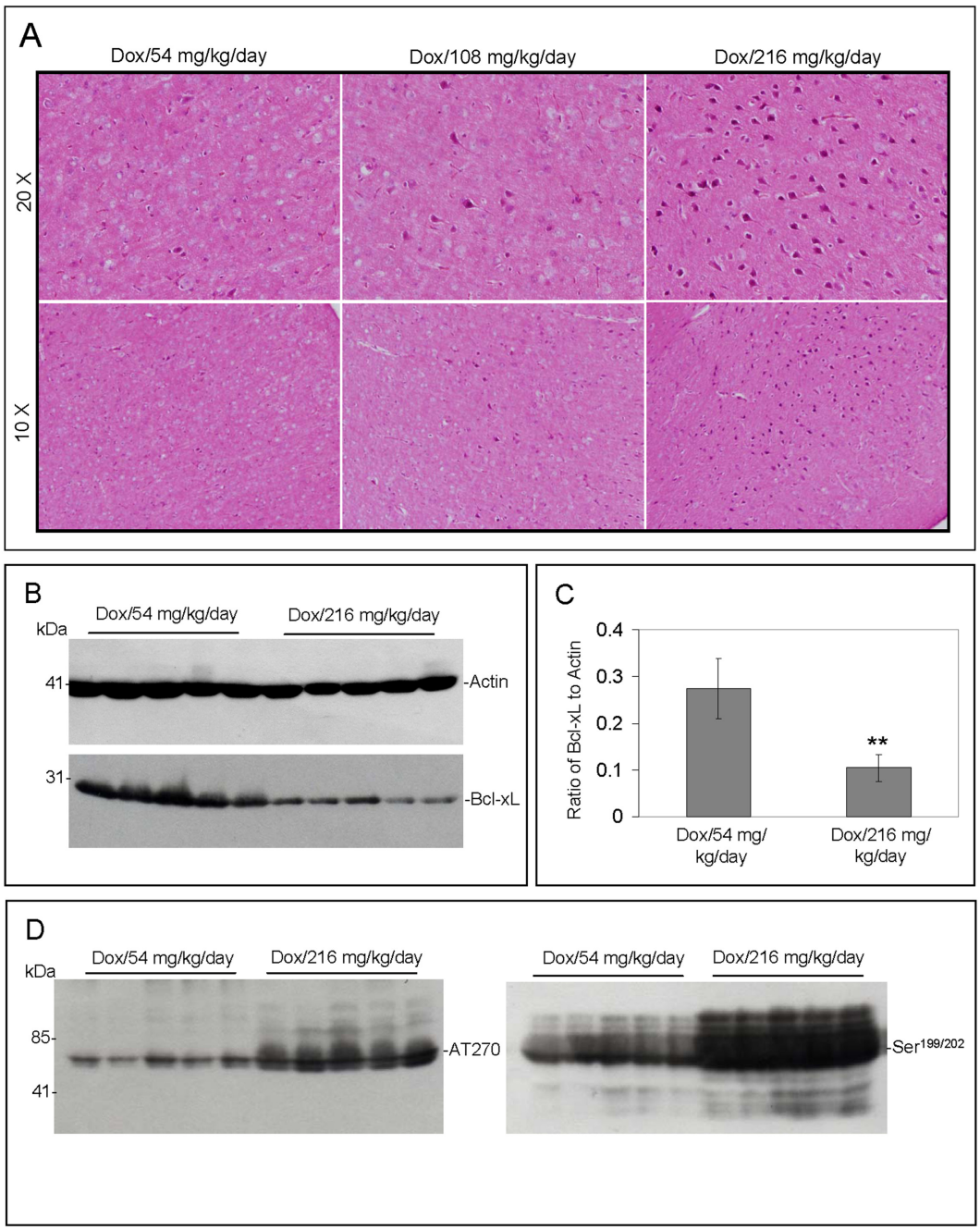

Figure 1 Oral administration of dox results in increased neuronal damage and tau phosphorylation with decreased Bcl-xL expression dose dependently in GT-tg mice. **A.** Mouse brain coronal frozen sections were stained with H&E. Left column indicates PSAPP mice receiving dox, middle column indicates PSAPP/Tat mice without dox and right column indicates PSAPP/Tat mice receiving dox. **B.** Bcl-xL expression was analyzed by Western blotting analysis of the mouse brain homogenates with anti-Bcl-xL antibody. **C.** Densitometry analysis shows the band density ratio of Bcl-xL to actin. **D.** Western blot analysis by antibody AT270 (left) or Ser199/202 (right) shows phosphorylated tau protein.

for the following study where PSAPP and GT-tg transgenic mice were cross-bred and subjected to a long term study of brain HIV-1 Tat secretion. Furthermore, these data suggest that dox-induced HIV-1 Tat expression significantly results in neurodegeneration in the mice.

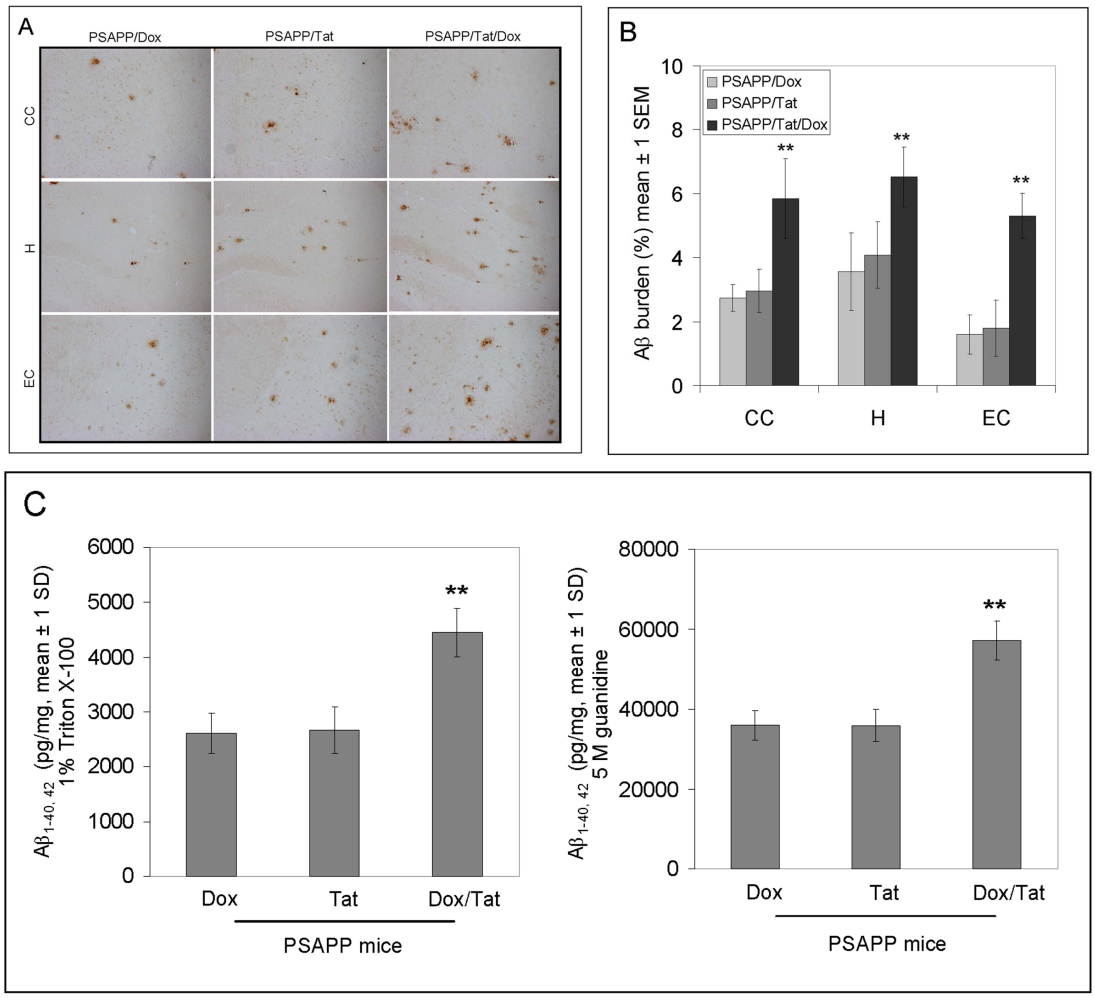

Figure 2 PSAPP/Tat mice receiving oral dox show increased A-beta plaques compared to PSAPP mice. **A.** Mouse brain coronal frozen sections were stained with rabbit polyclonal anti-human A antibody. Left column indicates PSAPP mice receiving dox, middle column indicates PSAPP/Tat mice without dox and right column indicates PSAPP/Tat mice receiving dox. As indicated, the top panels are from the cingulate cortex (CC), the middle panels are from the hippocampus (H), and bottom panels are from the entorhinal cortex (EC). **B.** Percentages of A antibody-immunoreactive A plaque (mean ± SD) were calculated by quantitative image analysis. **C.** Soluble A1-40, 42 (left) and insoluble A-beta1-40, 42 (right) prepared with 5 M guanidine were analyzed by ELISA. Data are presented as (pg/mg protein) of A1-40 or A1-42 separately.

Oral Administration of Dox Increases A-beta Plaque Formation in PSAPP/Tat Mice

Most interestingly, as determined by chronic dox administration, the PSAPP/Tat mice shows significantly increased A-beta deposits in the brain regions examined (**Figure 2A**) ($P<0.05$). Image analysis of micrographs from A-beta antibody stained sections reveals that plaque burdens are significantly increased in the cingulate cortex (CC), hippocampus (H), and entorhinal cortex (EC) by 47%, 55%, and 30%, respectively (**Figure 2B**). To verify the findings from these coronal sections we found dox-induced HIV-1 Tat expression markedly increases both in soluble and insoluble forms of A-beta 1-40, 42 by ELISA (**Figure 2C**) ($P<0.05$). No significant differences in pathology were noted between males and females of each group ($P>0.05$).

Oral Administration of Dox Increases tau Hyperphosphorylation and Neurodegeneration in PSAPP/Tat Mice

Given that the induced HIV-1 Tat expression

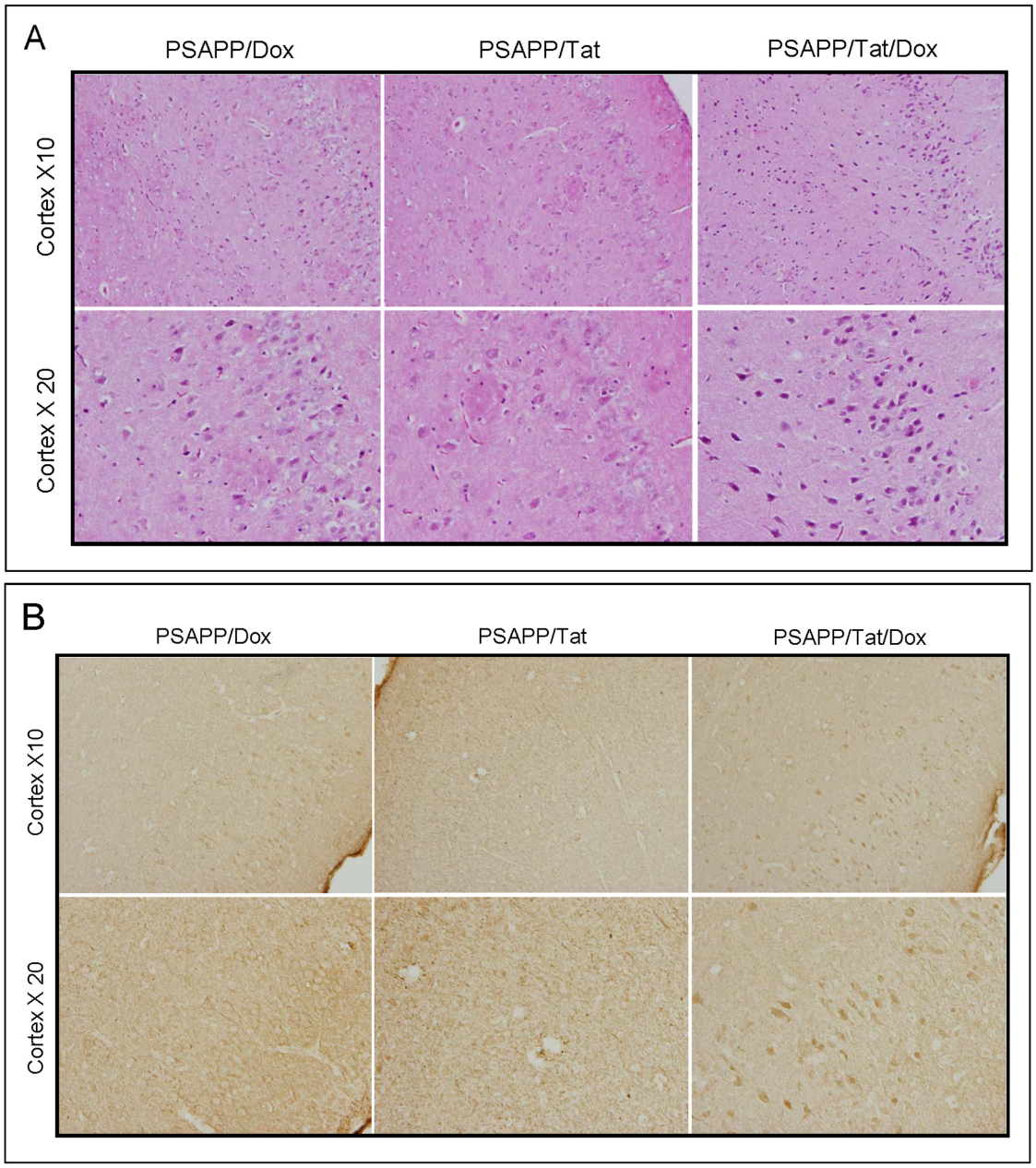

Figure 3 PSAPP/Tat mice receiving oral dox show increased neuronal degeneration and tau-phosphorylation after oral administration of dox compared to PSAPP mice. Left column indicates PSAPP mice receiving dox, middle column indicates PSAPP/Tat mice without dox and right column indicates PSAPP/Tat mice receiving dox. Mouse brain coronal frozen sections were stained with H&E (**A**) or with phospho-tau antibody (AT270) (**B**).

results in increased A-beta deposition, we wondered whether or not HIV-1 Tat could also contribute to other A-beta associated pathology. Since it has been shown that A-beta plaques and neuronal damage are associated with phosphorylated tau-immunoreactive structures in AD transgenic mice [12], we investigated neuronal injury (**Figure 3A**) and tau hyperphosphorylation (**Figure 3B** and **3D**). Clearly, in the PSAPP/Tat mice receiving dox, significantly higher number of positive cortical neuronal cells for anti-phospho-tau antibody (AT270) was found. To further determine if tau hyperphosphorylation would be involved in this Tat-induced neuronal damage, we examined Bcl-xL and Bax expression in these mice using

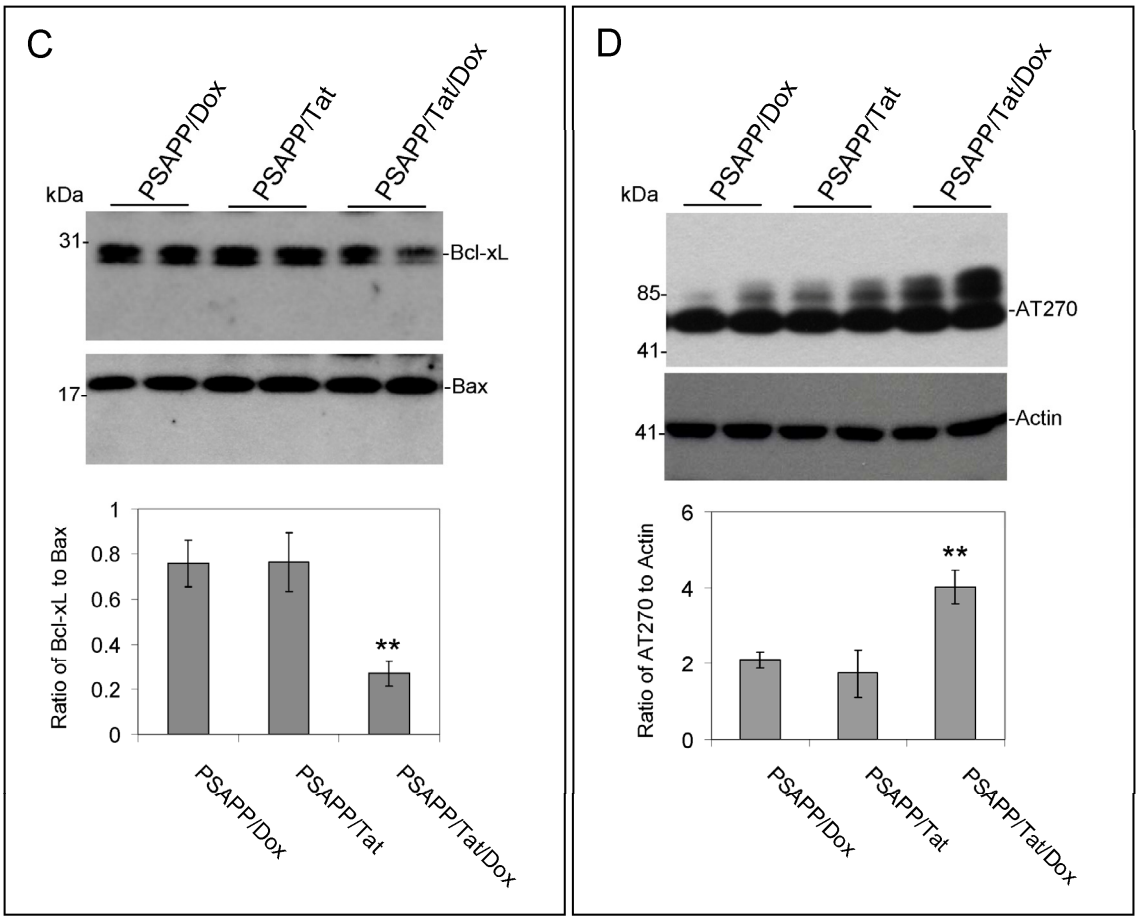

Figure 3 (cont.) PSAPP/Tat mice receiving oral dox show increased neuronal degeneration and tau-phosphorylation after oral administration of dox compared to PSAPP mice. Mouse brain homogenates were prepared from these mice and subjected to Western blot analysis for Bcl-xL, Bax; and phosphor-Tau. Compared to PSAPP mice receiving dox or PSAPP/Tat mice without dox, PSAPP/Tat mice receiving dox demonstrate a significantly increased ratio of Bax to Bcl-xL (**C**) as well as Tau protein (phospho-AT270) to actin ratio by densitometry analyses (**D**).

Western blot. As shown in **Figure 3C**, the ratio of Bcl-xL to Bax was significantly decreased ($P<0.001$). No significant differences in pathology were noted between males and females of each group ($P>0.05$). Similar results were obtained in the same samples using Ser199/202 antibody (data not shown).

Discussion

HIV-infected patients are either aging with HIV infection or becoming newly infected at older ages. This, compounded by the amyloidogenic effects of Tat protein, and possibly the lipodystrophic effects of HAART medications seem to promote the AD-like pathology seen in some 50% of the HIV-1 infected population [4]. Given that this type of pathology may contribute to the neurobehavioral morbidity of HIV-1 infection, we created a mouse model of HIV-1 Tat-induced AD-like pathology by crossing PSAPP and HIV-1 Tat (GT-tg) transgenic mice. This effectively mimics age-related changes in A-beta deposition [8] with superimposed brain targeted Tat expression in the PSAPP/Tat mouse model [9].

As expected in our initial dose ranging study in Tat transgenic mice, brain-targeted expression was sufficient to cause a dose dependent increase in neuronal degeneration (**Figure 1A**), and tau phosphorylation (**Figure 1D**) while causing a decrease in the anit-apoptotic protein, Bcl-xL (**Figures 1B** and **1C**). The microtubule-associated protein tau is abundantly expressed in neurons, and is deposited in cells in an abnormally phosphorylated state as fibrillar lesions in

many neurodegenerative diseases, particularly AD. In neurons, the protein plays a crucial role in binding and stabilizing microtubules, and regulating axonal transport. Both are controlled by site-specific phosphorylations. There is growing evidence that disruption in the normal phosphorylation state of tau followed by conformational changes plays a key role in the pathogenic events that occur in AD [15]. HAD patients have been shown to have significantly increased total and phopshorylated-tau concentrations similar to AD [6]. Aberrant phosphorylation of tau protein might be responsible for the breakdown of microtubules in affected neurons classically seen in AD not only because the altered protein has greatly reduced microtubule-promoting ability but also because it interacts with normal tau, making the latter unavailable for promotion of tubulin assembly into microtubules [15]. Past *in vitro* studies demonstrated HIV-1 Tat directly modulates polymerization of microtubules; a process which positively correlates with apoptosis. Thus the decrease in the anti-apoptotic Bcl-xL which positively correlated dose dependently with dox administration (and thus Tat expression) is in accord with previous studies. This effect of Tat on the self-association of tubulin may be of interest for studies on the mechanism of microtubule formation in the HIV-infected brain and could be used in the design of new agents aimed at protecting neuronal microtubules [16]. Furthermore Campbell and colleagues demonstrated this Tat-mediated effect on microtubules occurs without the full-length protein. Indeed peptides from its central glutamine-rich and basic regions are involved in Tat-mediated apoptosis [17].

Based on previous studies [18] as well as the dox dose-dependent decrease in Bcl-xL and increase in neurodegeneration, we hypothesize that HIV-1 Tat is able to induce apoptosis through both the release of cytochrome c, removal of the Bcl-xL suppression of Bax, and/or an increase in intracellular Ca^{2+} via the phospholipase C pathway [19]. First, it has previously been shown that Tat is able to directly trigger cytochrome c release, a central event in the mitochondrial apoptotic pathway. A recent study also implicates Ca2+ uptake into mitochondria in Tat-dependent toxicity [20]. Macho *et al* [21] show that, in lymphocyte cultures under low serum conditions, Tat accumulates at the mitochondria and positively correlates with disruption of the mitochondrial membrane potential [21]. This then leads to the release of pro-apoptotic factors such as cytochrome c. Regarding exitotoxic effects and Tat-mediated cell death, induction of the phospholipase c pathway by Tat confers a rapid increase in neuronal intracellular Ca2+ in primary neuron cultures, presumably causing phospholipase C and inositol phosphate-3 release, and a subsequent response via a plasma membrane glutamate receptor [19, 22].

In this novel PSAPP/GT-tg model of Tat-induced AD-like pathology, amyloid burden, neurodegeneration, and apoptotic signaling were significantly enhanced by Tat expression. Moreover, superimposed Tat expression appears to over-ride gender differences seen in plaque loads between male and female PSAPP mice. Previous investigations [9] have shown that neurodegeneration and apoptosis are positively correlated with the level and spatial distribution of Tat mRNA and/or protein expression [9] and occurred only when dox is administered but not without dox, or in dox-treated wild-type normal mice. Therefore, these findings did not result from an insertional mutation, or other unknown nonspecific effects.

Several studies have focused on Tat-mediated induction of amyloidosis *in vitro*. We previously demonstrated Tat inhibits microglial uptake of A-beta$_{1-42}$ peptide, a process that is enhanced by interferon-gamma (IFN-γ) and rescued by the STAT1 inhibitor (-)-epigallocatechin-3-gallate (EGCG) [7]. It is hypothesized that reduced A-beta uptake occurs through IFN-γ mediated STAT1 activation, which promotes a phenotype switch from a phagocytic to an antigen presenting phenotype in microglia. Additionally, we showed that HIV-1 Tat significantly disrupts apolipoprotein-3 (Apo-E3) promoted microglial A-beta uptake [7]. As microglial are a primary means for A-beta removal from brain parenchyma, this process may be involved in the enhanced amyloid burden in the PSAPP/GT-tg model compared to the PSAPP model. Tat protein has also been shown to directly interact with the neuronal low density lipoprotein (LRP) receptor and thus inhibit the uptake of its ligands including apolipoprotein E4 (Apo-E4) and A peptide [23]. A similar inhibition of LRP may occur in microglia. Moreover Daily and colleagues [24]

have demonstrated that Tat competitively and reversibly inhibits the extracellular A-beta degrading enzyme, neprilysin. However it was also found that both Tat peptides and Tat protein were slowly hydrolyzed by neprilysin. Thus although the accumulation of Tat-derived proteolytic fragments may serve to inhibit neprilysin and increase amyloid beta peptide levels, one would suspect that there is also some level of compensatory destruction of Tat protein also, by neprilysin. Therefore the mechanisms involved in enhanced amyloid deposition mentioned above may be acting in concert in this model.

Future studies using this model will be required to delineate the molecular steps most important in HIV-1 Tat-induced AD-like pathology including amyloid burden and tau phosphorylation. Combined with behavioral testing this should lay the foundation to isolate new mechanistic underpinnings and treatment targets. Additionally the model may simulate some prognostic implications for newly HIV-infected patients in terms of brain pathology and future development of neurobehavioral deficits.

Acknowledgements

We thank Wei Zhu (Indiana University) for assisting with acquisition of founder pairs of Tat transgenic mice. Additionally we thank and Kazuhiro Ando (University of California, Los Angeles) for extensive help in providing human brain specimens. Also we thank Dr. Peter Davies (Albert Einstein College of Medicine; Bronx, NY) for kindly providing the PHF-1 antibody. This work was supported by two NIH/NIMH grants: Clinical Scientist Career Development Award (1K08MH082642-01A1) (BG) and NIH/NIMH (5R21MH080168-02) (JT).

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References

[1] Valcour V and Paul R. HIV infection and dementia in older adults. *Clin Infect Dis* 2006; 42:1449-1454.

[2] Levy-Dweck S. HIV/AIDS fifty and older: A hidden and growing population. *J Gerontol Soc Work* 2005;46:37-50.

[3] Stoff DM., Khalsa JH, Monjan A and Portegies P. Introduction: HIV/AIDS and aging. *AIDS* 2004;18(Suppl 1):S1-2.

[4] Green DA, Masliah E, Vinters, HV, Beizai P, Moore DJ and Achim CL. Brain deposition of beta-amyloid is a common pathologic feature in HIV positive patients. *AIDS* 2005;19:407-411.

[5] Achim C, Masliah E, Vinters H, Schindelar J and Green D. Beta-amyloid in the HIV brain. Society of Neuroscience 2004 (Washington, DC).

[6] Brew BJ, Pemberton L, Blennow K, Wallin A and Hagberg L. CSF amyloid beta42 and tau levels correlate with AIDS dementia complex. *Neurology* 2005;65:1490-1492.

[7] Giunta B, Zhou Y, Hou H, Rrapo E, Fernandez, F and Tan J. HIV-1 Tat inhibits microglial phagocytosis of Aβ peptide. *Int J Clin Exp Pathol* 2008;1:260-275.

[8] Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S P Jantzen P, Wright K, Saad I, Mueller R, Morgan D, Sanders S, Zehr C, O'Campo K, Hardy J, Prada C, Eckman C, Younkin S, Hsiao K and Duff K. Accelerated alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med* 1998;4: 97-100.

[9] Kim BO, Liu Y, Ruan Y, Xu ZC, Schantz L and He JJ. Neuropathologies in transgenic mice expressing human immunodeficiency virus type 1 tat protein under the regulation of the astrocyte-specific glial fibrillary acidic protein promoter and doxycycline. *Am J Pathol* 2003; 162:1693-1707.

[10] Otvos L Jr, Feiner L, Lang E, Szendrei GI and Goedert M. Monoclonal antibody PHF-1 recognizes tau protein phosphorylated at serine residues 396 and 404. *J Neurosci Res* 1994;39:669-673.

[11] Rezai-Zadeh K, Shytle D, Sun N, Mori T, Hou H, Jeanniton D, Ehrhart J, Townsend K, Zeng J, Morgan D, Hardy J, Town T and Tan J. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. *J Neurosci* 2005;25:8807-8814.

[12] Noda-Saita K, Terai K, Iwai A, Tsukamoto M and Shitaka Y. Tg2576 mice suggest a mechanism of senile plaque formation and progression of neuritic dystrophy in Alzheimer's disease. *Acta Neuropathol* 2004;108:435-442.

[13] Rempel HC and Pulliam L. HIV-1 tat inhibits neprilysin and elevates amyloid beta. *AIDS* 2005;19:127-135.

[14] Esiri MM, Biddolph SC and Morris CS. Prevalence of alzheimer plaques in AIDS. *J Neurol Neurosurg Psychiatry* 1998;65:29-33.

[15] Mi K and Johnson GV. The role of tau

phosphorylation in the pathogenesis of alzheimer's disease. *Curr Alzheimer Res* 2006; 3:449-463.

[16] de Mareuil J, Carre M, Barbier P, Campbell G, Lancelot S, Opi S, Esquieu D, Watkins JD, Prevot D, Braguer D, Peyrot V and Loret EP. HIV-1 tat protein enhances microtubule polymerization. *Retrovirology* 2005;2:5.

[17] Campbell GR, Pasquier E, Watkins J, Bourgarel-Rey V, Peyrot V, Esquieu D, Barbier P, Mareuil J, Braguer D, Kaleebu P, Yirrell DL and Loret EP. The glutamine-rich region of the HIV-1 tat protein is involved in T-cell apoptosis. *J Biol Chem* 2004;279:48197-48204.

[18] Giunta B, Obregon D, Hou H., Zeng J, Sun N, Nikolic V, Ehrhart J, Shytle RD, Fernandez F and Tan J. EGCG mitigates neurotoxicity mediated by HIV-1 proteins gp120 and tat in the presence of IFN-gamma: Role of JAK/STAT1 signaling and implications for HIV-associated dementia. *Brain Res* 2006;1123: 216-225.

[19] Haughey NJ, Holden CP, Nath A, Geiger JD. Involvement of inositol 1,4,5-trisphosphate-regulated stores of intracellular calcium in calcium dysregulation and neuron cell death caused by HIV-1 protein tat. *J Neurochem* 1999;73:1363-1374.

[20] Langford D, Grigorian A, Hurford R, Adame A, Crews L and Masliah E. The role of mitochondrial alterations in the combined toxic effects of human immunodeficiency virus tat protein and methamphetamine on calbindin positive-neurons. *J Neurovirol* 2004;10:327-337.

[21] Macho A, Calzado M, Jimenez-Reina L, Ceballos E, Leon J and Munoz E. Susceptibility of HIV-1- Tat transfected cells to undergo apoptosis. Biochemical mechanisms. *Oncogene* 1999;18:7543-7551.

[22] Jajoo S, Mukherjea D, Brewer GJ and Ramkumar V. Pertussis toxin B-oligomer suppresses human immunodeficiency virus-1 tat-induced neuronal apoptosis through feedback inhibition of phospholipase C-beta by protein kinase C. *Neuroscience* 2008;151: 525-532.

[23] Liu Y, Jones M, Hingtgen CM, Bu G, Laribee N, Tanzi RE, Moir RD, Nath A and He JJ. Uptake of HIV-1 tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. *Nat Med* 2000;6:1380-1387.

[24] Daily A, Nath A and Hersh LB. Tat peptides inhibit neprilysin. *J Neurovirol* 2006;12:153-160.