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Conditional Tat protein brain expression in the GT-tg bigenic mouse induces cerebral fractional anisotropy abnormalities

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Abstract

Cerebral white matter changes including tissue water diffusion abnormalities detected with diffusion tensor magnetic resonance imaging (DTI) are commonly found in humans with Human Immunodeficiency Virus (HIV) infection, as well as in animal models of the disorder. The severities of some of these abnormalities have been reported to correlate with measures of disease progression or severity, or with the degree of cognitive dysfunction. Accordingly, DTI may be a useful translational biomarker. HIV-Tat protein appears to be an important factor in the viral pathogenesis of HIV-associated neurotoxicity. We previously reported cerebral gray matter density reductions in the GT-tg bigenic mouse treated with doxycycline (Dox) to conditionally induce Tat protein expression. Presently, we administered intraperitoneal (i.p.) Dox (100 mg/kg/day) for 7 days to GT-tg mice to determine whether induction of conditional Tat expression led to the development of cerebral DTI abnormalities. Perfused and fixed brains from eight GT-tg mice administered Dox and eight control mice administered saline i.p. were extracted and underwent DTI scans on a 9.4 Tesla scanner. A whole brain analysis detected fractional anisotropy (FA) reductions in several areas including insular and endopiriform regions, as well as within the dorsal striatum. These findings suggest that exposure to Tat protein is sufficient to induce FA abnormalities, and further support the use of the GT-tg mouse to model some effects of HIV.

Keywords

Diffusion Tensor Imaging; Fractional Anisotropy; GT-tg mouse; HIV; Tat

Introduction

White matter lesions and volume reductions are commonly found with structural magnetic resonance imaging (MRI) in the brains of people infected with Human Immunodeficiency Virus (HIV) [1–12]. Several of these studies reported associations between the degree of

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Conflicts of Interest

The authors report no conflicts of interest.

white matter abnormality and either the severity of clinical course (e.g., viral load, CD4+ cell counts) or of cognitive dysfunction [7–9, 11, 12].

Diffusion tensor imaging (DTI), an MRI technique that detects the diffusion of water between cells thereby reflecting tissue microstructural integrity, also has revealed widespread white matter microstructural abnormalities in HIV patients. These include microstructural integrity reductions within major white matter fiber bundles such as the corpus callosum [5, 13–19] as well as more broadly in whole brain [20] and in areas comprising frontal white matter [21], frontostriatal tracts [22], optic radiation and other major white matter tracts [23]. As with structural MRI assessments of white matter, DTI has been used to document associations between diffusion abnormalities and clinical, cognitive, or other measures of HIV disease progression or severity [15–17, 19, 20, 23, 24].

Diffusion imaging abnormalities also have been reported in several animal models of HIV. Cats infected with feline immunodeficiency disorder (FIV) exhibited increased white matter diffusion [25]. Fractional anisotropy (FA) was found to be reduced in the body of the corpus callosum of HIV-1 transgenic rats [26]. Similarly, reduced FA was reported in whole brain and in the genu of the corpus callosum in Simian immunodeficiency virus (SIV)-infected macaques [27]. Accordingly, diffusion imaging appears to have potential as a noninvasive translational biomarker of white matter microstructural damage associated with HIV and related animal models.

Research suggests that HIV-1 Tat protein may be an important factor in the viral pathogenesis leading to the development and progression of HIV-associated neurotoxicity [28–33]. Conditional expression of Tat protein was previously found to induce glial, neuronal, and behavioral abnormalities including learning and memory deficits and increased anxiety in transgenic mice [34–37]. GT-tg bigenic mice have a “Tet-on” system that is activated transcriptionally when doxycycline (Dox) is administered [38]. This system is integrated into the astrocyte-specific GFAP promoter, facilitating Tat expression in brain. Centrally-targeted Tat expression in these mice has been confirmed by RT-PCR and Southern blots [38] and the degree of Tat brain expression was previously confirmed by our group via Western blots [34, 37]. Using *ex vivo* MRI to conduct voxel based morphometry in this animal model, we previously demonstrated that exposure to Tat protein can cause significant gray matter density reductions in limbic brain regions associated with cognition and affective behavior [39]. In the present study, we used ultra high magnetic field (9.4 Tesla) DTI to assess whether GT-tg mice administered Dox for 7 days to induce Tat expression develop FA abnormalities detectible *ex vivo*, to determine whether this mouse line models the abnormal FA phenotype commonly reported in HIV patients and animal models.

Materials and Methods

Animals and housing

Adult male GT-tg bigenic mice, 8–14 weeks of age, were used in these experiments. Mice were bred and housed and cared for in the Northeastern University animal facility in accordance with the 1996 National Institutes of Health Guide for the Care and Use of

Laboratory Animals as approved by the Institutional Animal Care and Use Committee. The creation and development of the GT-tg bigenic mouse and genotype confirmation of the inducible and brain-targeted HIV-1 Tat protein are described in detail [38]. GT-tg bigenic mice are engineered to express the Tat₁₋₈₆ gene upon Dox-mediated activation of a brain-specific promoter. Mice used in this study were obtained from a colony of GT-tg bigenic mice at Northeastern University that was established using two breeding pairs of bigenic mice previously back-crossed seven generations onto the C57BL/6 line.

Chemicals

Doxycycline hyclate (Dox) was obtained from Sigma-Aldrich (St. Louis, MO) and was dissolved in 0.9% saline prior to injection.

Induction of brain-targeted Tat with Doxycycline treatment

To express Tat₁₋₈₆ protein, GT-tg bigenic mice were administered Dox via intraperitoneal (i.p.) injection with a single daily dose (100 mg/kg, dissolved in 0.9% saline in a volume of 0.3 ml/30 mg body weight) for 7 days as found previously to be optimal [34, 37]. Uninduced GT-tg mice were administered 0.9% saline in the same volume/body weight for 7 days and served as controls in this study.

Magnetic Resonance Imaging

After 7 days of Dox or saline treatments, mice were perfused with 10% buffered formalin containing the magnetic resonance contrast agent ProHance (Gadoteridol, Bracco Diagnostics, Inc) in a 20:1 volume ratio. Fixed brains were manually extracted and processed according to Cyr [40] for *ex vivo* diffusion imaging, which facilitates longer scans enabling higher spatial resolution than typically possible with *in vivo* imaging protocols. Structural MRI and diffusion imaging scans were acquired within 72 hours after perfusion/fixation with a Varian Direct Drive 9.4 T (horizontal bore) scanner (Agilent Technologies, Santa Clara, CA) at McLean Hospital, using a micro imaging gradient (outer/inner diameters of 115/60 mm, gradient strength of 1000 mT/m, and a 150 μ sec rise time), and a Varian 1H (400 MHz) surface coil with an inner diameter of 25 mm.

Structural MRI and Diffusion imaging—*Ex vivo* high resolution structural MRI images were acquired using the following scan parameters: Repetition Time (TR) = 3033 ms, echo time (TE) = 25.9 ms, echo train length (ETL) = 8, matrix size = 512 $\times$ 512, FOV = 20 $\times$ 20 mm, number of averages = 64, slice number = 28, slice thickness = 0.5 mm, 0 mm gap, in plane resolution = 39 $\times$ 39 μ m, scan time = 3.33 hours. Next, high resolution diffusion imaging (DTI) was performed using a fast spin-echo multi-slice protocol subsequent to structural imaging, utilizing previously established methods [41, 42]. DTI scans were performed on a subset of brains having undergone structural imaging for a voxel based morphometry analysis [39]. DTI data were acquired volumetrically using the following parameters: TR = 4000 ms, effective TE = 27 ms, ETL = 8, matrix size = 256 $\times$ 256, FOV = 20 $\times$ 20mm, number of averages = 8, diffusion gradients were applied at 6 noncollinear directions, diffusion gradient amp = 18G/cm, duration = 5 ms, separation = 11.5 ms, giving an equivalent b-value of 1140 mm²/s, scan time = 1.75 h.

Data Analysis

The diffusion tensor calculation was performed using the TrackVis DTI software (<http://trackvis.org>, Martinos center for Biomedical Imaging, Massachusetts General Hospital). The linear least-square fitting method was used for diffusion tensor calculation. Whole brain fractional anisotropy (FA) maps were generated from the calculated diffusion tensor map. The FA maps were compared using MRICro analysis software (freeware, [43]), and significant differences identified with cluster corrected *t*-tests with a threshold set at $p = 0.05$. The C57BL/6J mouse brain atlas from the Mouse Brain Library (URL: <http://www.mbl.org/atlas/atlas.php>) was used as a reference to identify the brain regions where significant differences were observed.

Results

Treatment with Dox resulted in a significant reduction of microstructural integrity (FA reductions) in the brains of GT-tg mice compared to saline-administered control littermates (Figure). Areas exhibiting FA reductions in the Dox-induced compared to saline-treated GT-tg mice included white and gray matter near the insula and endopiriform nucleus and gray matter within the dorsal striatum (corrected $p = 0.05$, two-tailed *t*-test).

Discussion

In the present study, we tested the hypothesis that Tat protein expression in adult male mice for 7 days would induce brain microstructural changes detectable postmortem using *ex vivo* DTI. Tat expression significantly reduced FA in brain areas near the insula, endopiriform nucleus, and within the dorsal striatum. While the specific etiology of these microstructural abnormalities is undetermined, there is pathologic evidence that white matter FA reductions represent reduced myelin content [44] and/or axonal membrane integrity [45]. Notably, recent electron microscopy investigations using a similar transgenic mouse model demonstrated Tat-induced perturbations in striatal myelination [35]. Collectively, these findings show that exposure to Tat protein by itself is sufficient to induce cerebral gray and white matter microstructural abnormalities, which may result in abnormal brain structural connectivity.

Although diffusion imaging typically is used to assess white matter microstructure, striatal (gray matter) FA increases have been reported as a consequence of normal aging and may be mediated by iron accumulation [46]. Cortical gray matter FA increases also have been reported in a rodent model of controlled cortical impact to simulate traumatic brain injury (TBI), and were interpreted to represent cortical gliosis [47]. A subsequent study of humans with mild TBI also reported increased brain FA in gray matter regions proximate to the tissue injury [48]. The present finding of a striatal FA *decrease* in Tat-expressing mice thus could thus reflect a gray matter glial reduction or other dysfunction. White matter glial damage is thought to be a basis for white matter diffusion abnormalities in HIV and in animal models of the disorder (e.g., [26]), and perhaps gray matter glia also are vulnerable to Tat, an issue that will be addressed in future studies. The striatal FA finding in Tat expressing mice is consistent with previous reports of striatal abnormalities in animal models of HIV and the human disorder including a report of striatal damage in mice induced

to express Tat protein [35], reports of striatal damage induced by exogenous administration of Tat protein [49, 50], and reports of striatal damage and dysfunction in people with HIV [51–53].

The functional significance of the striatal FA abnormality remains to be determined, although it could be related to the learning and memory abnormalities previously reported in Tat-expressing GT-tg mice [34]. Interestingly, correlations between striatal FA integrity and either memory or overall cognitive dysfunction have been reported in people with HIV [24]. In combination with our earlier behavioral study, the striatal FA finding in Tat-exposed GT-tg mice could reflect a similar phenomenon. The striatum also is involved in drug reward [54, 55] and a striatal FA abnormality could enhance risk for the development of addiction disorders. Supporting this possibility, we recently reported that exposure to Tat protein both potentiated cocaine conditioned place preference and induced reinstatement of an extinguished conditioned place preference [56]. These effects could in part be mediated by dopamine transporter expression abnormalities, as Tat infusions reduce ventral striatal (nucleus accumbens) dopamine uptake [57] and in part be mediated by increased medial prefrontal cortex excitability via over-activation of L-type calcium channels [58].

With regard to the other brain areas exhibiting Tat-induced FA abnormalities, the insula has been implicated in emotional processing [59], anxiety [60], and drug craving [61], and enhanced insular cortex activity has been reported in anxiety disorders [62]. As noted above, GT-tg mice exposed to Tat protein demonstrate a greater magnitude of cocaine reward [56] and they also exhibit increased levels of anxiety while performing several behavioral tasks [37]. Likewise, the endopiriform nucleus is a highly connected structure, receiving inputs from olfactory cortex and several other cortical areas including infralimbic cortex [63, 64]. Infralimbic cortex is involved in a number of physiological and pathological processes and can disinhibit basolateral amygdala, thereby modulating contextual cue memories [65]. Thus, endopiriform area FA abnormalities might enhance amygdala activity and increase both drug seeking and anxiety behavior. This effect could, via reduced connectivity with infralimbic cortex, modulate extinction learning capacity [66] and lead to sustained reactivity to fear-inducing or drug reward-related cues.

It is of note that FA reductions were localized primarily to the right-hemisphere. While this effect could be a result of our sample size, right-lateralized brain damage has been associated with behavioral disinhibition syndromes (see [67] for review) as well as increased sensitivity for stress-induced depression or anxiety [68]. Behavioral phenotypes associated with right hemisphere damage/dysfunction thus appear similar to those exhibited by Tat-expressing GT-tg mice [34, 35, 37, 56, 69].

The anatomical distribution of Tat-induced FA abnormalities differs in one key way from that of prior human and animal studies in that corpus callosum involvement was not detected. This apparent discrepancy could be due to a number of factors, including the relatively small sample size in this study, which might have been underpowered to detect some effects of Tat expression. It also is possible that *ex vivo* diffusion imaging (versus *in vivo* diffusion scans conducted in prior studies) may have been less sensitive to callosal abnormalities. This possibility does not seem likely as callosal FA in mice scanned at high

magnetic field is similar whether brains are scanned *in vivo* or *ex vivo* after rapid perfusion/fixation [70]. It is conceivable that some brain (and behavioral) changes in Dox-induced GT-tg mice evolve quite rapidly within 7–10 days of Dox exposure (present study, [34, 37, 39, 56, 69]) while others, such as callosal abnormalities, may take longer to develop. Indeed, both longitudinal and cross-sectional DTI studies suggest that callosal FA and mean diffusivity (MD) abnormalities in people with HIV evolve and worsen over time as a function of disease duration [15, 19], and in the transgenic rat HIV model, callosal MD abnormalities also worsened over time [26].

The present study must be considered in light of another important limitation: the utilization of a between-subjects cross-sectional design not only is static in nature but also can be confounded by cohort effects. Obviously, this is required when imaging is conducted *ex vivo*, which was done presently to obtain higher anatomical resolution (via longer scanner times and by eliminating respiratory motion). One of the major advantages of MRI, DTI, and related techniques is that they are noninvasive and nondestructive. Future longitudinal studies will exploit this capability by prospectively assessing, within subjects, effects of prolonged Tat induction on FA and other brain effects, as well as effects of agents that may prevent or mitigate Tat-induced brain structural and functional abnormalities.

Notwithstanding the limitations discussed, the present findings are generally consistent with prior reports of FA abnormalities in people with HIV as well as in animal models of HIV. This finding supports our working hypothesis that diffusion and other imaging studies of Dox-induced GT-tg mice may be useful for developing both basic insights on the relationship of Tat to HIV and for testing novel HIV treatments, including hormone-related therapeutics such as progesterone [69] or other agents such as didehydro-Cortistatin A (dCA), which directly binds to and prevents some effects of Tat [71].

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Figure. Conditional Tat Protein Expression Induces White and Gray Matter FA Reductions
 Dox-induced GT-tg mouse brains exhibited significant white and gray matter microstructural abnormalities manifest as Fractional Anisotropy (FA) reductions, versus saline administered GT-tg control mouse brains. FA reductions were evident near the insula (1), endopiriform nucleus (2), and within the striatum (3). These areas are illustrated as colorimetric overlays on an FA image. (The anatomical locations shown are from +2.22 mm to -4.20 mm Bregma, $n = 8/\text{group}$, areas in color = $p < 0.05$, cluster-corrected t -test). The left side of the figure is the right side of the brain.