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Research Paper

Evidence for accelerated tauopathy in the retina of transgenic P301S tau mice exposed to repetitive mild traumatic brain injury

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ABSTRACT

Chronic traumatic encephalopathy (CTE) is associated with repetitive mild traumatic brain injury (mTBI) in the context of contact and collision sports, but not all exposed individuals develop this condition. In addition, experiments in animal models in several laboratories have shown that non-transgenic mice do not develop tauopathy after exposure to repetitive mTBI schedules. It is thus reasonable to assume that genetic factors may play an etiological role in the development of CTE. More than 40 mutations in the tau gene are known to confer proneness to aggregation and are thought to cause neurodegenerative diseases including frontotemporal degeneration (FTD). Transgenic mice harboring these mutations can be used to ask the question whether repetitive mTBI can accelerate onset and course of tauopathy or worsen the outcomes of transgenic disease. In this study, we exposed mice harboring the tau P301S transgene associated with FTD to repetitive mTBI schedules by impact acceleration (IA) that we have previously characterized. We explored the progression of tauopathy in the retina and neocortex based on density of neuronal profiles loaded with tau pS422, a marker of advanced tau hyperphosphorylation. We found that the density of tau pS422 (+) retinal ganglion cells (RGCs) increased twenty fold with one mTBI hit, a little over fifty fold with four mTBI hits and sixty fold with 12 mTBI hits. The severity of mTBI burden (number of hits) was a significant factor in tauopathy outcome. On the other hand, we found no association between repetitive mTBI and density of pS422 (+) neuronal profiles in neocortex, a region that is not featured by significant TAI in our repetitive mTBI model. We observed similar, but less prominent, trends in tauopathy-prone transgenic mice harboring all 6 isoforms of wild-type human tau without mouse tau. Our findings indicate that repetitive mTBI accelerates tauopathy under diverse genetic conditions predisposing to tau aggregation and suggest a vulnerability-stress model in understanding some cases of acquired neurodegenerative disease after repetitive mTBI.

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1. Introduction

Chronic traumatic encephalopathy (CTE) is a neurological condition primarily encountered in professional boxers and athletes playing collision sports such as football, and is thought to be caused by repetitive mild traumatic brain injury (TBI) or concussion (McKee et al., 2009, 2013). Because CTE has little in common with classical traumatic neuropathologies such as contusions and diffuse axonal injury and very much in common with neurodegenerative tauopathies, it is assumed that repetitive TBI somehow transforms the injury burden into proteinopathy (Koliatsos and Xu, 2015; Mckee et al., 2015; Stein et al., 2014). Public concern over CTE is growing not only because of the risk among NFL professionals, but also because of the exposure of millions of non-professional athletes playing football and other collision and contact sports including mixed martial arts, hockey, and rugby (Kelly, 1999; Sosin et al., 1996; Iverson et al., 2004; Koh et al., 2003; Guskiewicz et al., 2003).

CTE is featured by hyperphosphorylation and aggregation of the microtubule-associated protein tau in neurofibrillary tangles (NFTs).

Abbreviations: AD, Alzheimer's disease; CTE, chronic traumatic encephalopathy; DAB, 3,3′-diaminobenzidine; FTD, frontotemporal degeneration; IA, impact acceleration; IHC, immunohistochemistry; mTBI, mild traumatic brain injury; NFL, National Football League; NSS, neurological severity score; RGCs, retinal ganglion cells; TAI, traumatic axonal injury.

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How repetitive mild TBI (mTBI) causes tau to undergo such changes is unclear but key biochemical features of tau profiles in CTE are indistinguishable of those found in Alzheimer's disease (AD) (Schmidt et al., 2001). Not all individuals exposed to repetitive mTBI will develop CTE (Baugh et al., 2012; McKee et al., 2013). Consistent with this trend, non-transgenic mice do not appear to develop tauopathy after exposure to repetitive mTBI schedules based on impact acceleration (IA) despite the fact that such TBI schedules produce traumatic axonal injury (TAI) (Xu et al., 2014). Therefore, it is reasonable to assume that genetic factors may play an etiological role in the development of CTE. Tau mutations conferring proneness to aggregation have been identified in other neurodegenerative diseases such as frontotemporal degeneration (FTD) and include more than 40 mutations (Wolfe, 2009; Dujardin et al., 2015). There are several strains of transgenic mice harboring such mutations, including P301S, P301L and etc., all of which recapitulate aspects of FTD and related disorders (Denk and Wade-Martins, 2009; Yoshiyama et al., 2007). Such transgenic mouse models can be used to study the question whether repetitive mTBI can accelerate onset and course of tauopathy or worsen the outcomes of transgenic disease.

Here, we expose P301S mice to a repetitive mTBI schedule based on impact acceleration (IA) that we have recently developed and characterized (Xu et al., 2014) and ask whether exposure to repetitive mTBI accelerates the onset of constitutive neurodegenerative disease. Because the visual tract is among the most prominent pathways implicated in the IA and other animal models of TBI (Koliatsos et al., 2011; Xu et al., 2014; Wang et al., 2011), we focus on the course of tauopathy in the retina. Our findings demonstrate that single, but especially repetitive, mTBI can induce premature hyperphosphorylation of tau in retinal ganglion cells (RGCs) based on pS422 immunoreactivity. This outcome is replicated in wild-type human tau transgenic mice that do not harbor tau mutation but are also prone to tau aggregation. These results suggest that repetitive mTBI accelerates tauopathy under diverse genetic conditions predisposing to tau aggregation.

2. Materials and methods

2.1. Subjects and experimental design

In these experiments we used 5-week old hemizygous P301S tau transgenic mice (P301S mice, B6;C3-Tg[Prnp-MAPT*P301S]PS19Vle/J, Jax # 08169; The Jackson Laboratory, Bar Harbor, Maine) (Yoshiyama et al., 2007) of both genders. Homozygous human tau mice which have all 6 wild-type human tau isoforms including both 3R and 4R forms (B6.Cg-Mapt$^{tm1(EGFP)Klt}$ Tg(MAPT)8cPdav/J, Jax # 005491; The Jackson Laboratory, Bar Harbor, Maine) (Andorfer et al., 2003) were used to compare between two strains of mice prone to tauopathy. Animal handling and experimentation was approved by the Animal Care and Use Committee of the Johns Hopkins Medical Institutions in accordance with the relevant NIH Guide (publication 86–23).

To evaluate the course of tauopathy, three mTBI regimens were used for P301S mice: single injury (1×), a 4-injury regimen (4×) and a 12-injury regimen (12×) as described (Xu et al., 2014) ($n = 4$ for each injury or control regimen). To explore, in a comparative fashion, the course of tauopathy in mice expressing wild human tau, the 4× repetitive mTBI regimen was used. Control animals in all TBI regimens received sham injuries as described (Xu et al., 2014). To eliminate variation on expression of tau transgene among litters, injured P301S and human wild-type tau mice were paired with sham-injured mice of same sex from the same litter.

2.2. Model of repetitive mTBI-injury induction and schedules

The animals were subjected to mTBI using the IA method of Marmarou (Marmarou et al., 1994) as modified for use in mice (Xu et al., 2014). The impact acceleration setting was 40 g of impact weight dropped from 1 m vertically (Xu et al., 2014). Procedures were carried out with gas anesthesia (isoflurane:oxygen:nitrous oxide = 1:33:66) under aseptic conditions. Briefly, a steel disc (3.2 mm in diameter) was glued on saline-washed, air-dried mouse cranium between bregma and lambda under microscopic guidance. The mouse was placed prone on a foam bed (4–0 spring constant foam, Foam to Size Inc., Ashland, VA) under a hollow Plexiglass tube, and secured in place with strapping tape. Injury was induced by releasing the column of brass weights (impactor) through the Plexiglass tube from a distance of 1 m onto the disc. The foam bed was moved quickly right after the impact to avoid secondary rebound injury. To imitate real-life concussion, we did not use mechanical ventilation. After righting itself, the mouse was briefly re-anesthetized and helmet was removed. Animals with skull fractures were excluded from the study. Sham-injured animals were subjected to same procedures except the weight drop.

In the 4× schedule animals were injured once daily at days 0, 1, 3 and 7; in the 12× schedule, animals were given 3 injuries daily at days 0, 1, 3 and 7. In order to evaluate the acute and chronic outcomes of repeat injury, animals were euthanized 7 days or 10 weeks after single TBI or the final injury of the repeat-TBI regimen. Neurological functions were also evaluated before injury using the neurological severity score (NSS), a standardized abbreviated 10-point scale system developed to assess neurobehavioral outcomes in rodents after TBI (Flierl et al., 2009).

2.3. Histology, histochemistry, immunohistochemistry, and microscopy

Animals were perfused with freshly depolymerized 4% neutral-buffered paraformaldehyde. The brain and eyes with associated optic nerves were dissected and immersed in the same fixative overnight at 4 °C. All tissues were cryoprotected and then stored at −80 °C for further processing. The eyes were dissected further and retinal whole mounts were prepared. Brain tissues were sectioned at the sagittal plane (40 μm). Lesions were first surveyed on brain sections with Gallyas silver staining of injured/degenerating axons and terminals, using a commercially available kit (Neurosilver kit II; FD Neurotechnologies, Ellicott City, MD) as described (Koliatsos et al., 2011).

As a pilot to experiments based on immunohistochemistry (IHC), a series of antibodies targeting major phosphorylation sites of tau protein (AT8, PHF1, CP13 and pS422) were evaluated on retinal whole mounts and brain sections of P301S mice and transgenic mice with wild-type human tau, under sham and experimental IA conditions. The mouse monoclonal antibody AT8 (MN1020, 1:200, Thermo Scientific Inc., Rockford, IL) is directed against tau pS202; the mouse monoclonal antibody PHF1 is directed against tau pS396 and pS404 (1:200) and the mouse monoclonal CP13 is directed against tau pS202 and pT205 (1:200). The rabbit polyclonal antibody pS422 (pS422, 1:600, Genetex, Irvine, CA) is directed against a phosphorylated tau epitope at S422. Antibodies PHF1 and CP13 were provided by Dr. Peter Davies (Albert Einstein College of Medicine, Bronx, NY). Non-transgenic mice do not show immunostaining with any of these antibodies using IA schedules identical to the ones employed here (Xu et al., 2014). For transgenic mice, AT8 and CP13 immunoreactivities were too extensive in both sham and injured animals to allow the appreciation of any differences between the two groups and, in the case of cortex, they produced a diffuse staining of the cytoplasm but did not stain tau tangles. PHF1 IHC produced inconsistent results in our hands. Therefore, the main experiments reported here were based on IHC for pS422, an epitope known to become phosphorylated in neurodegenerative tauopathies (Bussiere et al., 1999; Collin et al., 2014; Pennanen and Gotz, 2005; Guillozet-Bongaarts et al., 2006). Whole mount retinas were taken for dual-label immunofluorescence using general methods published from our group (Koliatsos et al., 2011; Xu et al., 2009). Specifically, retinal whole mounts were rinsed with PBS and then dually labeled with antibodies against γ-synuclein (1:600, Genetex, Irvine, CA) to identify surviving RGC bodies and tau pS422 (see above). Retinas were first

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