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Is phosphorylated tau unique to chronic traumatic encephalopathy? Phosphorylated tau in epileptic brain and chronic traumatic encephalopathy

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ABSTRACT

Repetitive traumatic brain injury (rTBI) is one of the major risk factors for the abnormal deposition of phosphorylated tau (PT) in the brain and chronic traumatic encephalopathy (CTE). CTE and temporal lobe epilepsy (TLE) affect the limbic system, but no comparative studies on PT distribution in TLE and CTE are available. It is also unclear whether PT pathology results from repeated head hits (rTBI). These gaps prevent a thorough understanding of the pathogenesis and clinical significance of PT, limiting our ability to develop preventative and therapeutic interventions. We quantified PT in TLE and CTE to unveil whether a history of rTBI is a prerequisite for PT accumulation in the brain. Six *postmortem* CTE (mean 73.3 years) and age matched control samples were compared to 19 surgically resected TLE brain specimens (4 months-58 years; mean 27.6 years). No history of TBI was present in TLE or control; all CTE patients had a history of rTBI. TLE and CTE brain displayed increased levels of PT as revealed by immunohistochemistry. No age-dependent

Abbreviations: CNS, central nervous system; CTE, chronic traumatic encephalopathy; CTX, cortex; MVA, motor vehicle accident; NFL, national football league; PT, phosphorylated tau protein; rTBI, repetitive traumatic brain injury; TLE, temporal lobe epilepsy

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changes were noted, as PT was present as early as 4 months after birth. In TLE and CTE, cortical neurons, perivascular regions around penetrating pial vessels and meninges were immunopositive for PT; white matter tracts also displayed robust expression of extracellular PT organized in bundles parallel to venules. Microscopically, there were extensive tau-immunoreactive neuronal, astrocytic and degenerating neurites throughout the brain. In CTE perivascular tangles were most prominent. Overall, significant differences in staining intensities were found between CTE and control ($P<0.01$) but not between CTE and TLE ($P=0.08$). pS199 tau analysis showed that CTE had the most high molecular weight tangle-associated tau, whereas epileptic brain contained low molecular weight tau. Tau deposition may not be specific to rTBI since TLE recapitulated most of the pathological features of CTE.

1. Introduction

The controversy surrounding the significance of PT in neurological diseases is one of the hottest topics in current neuroscience research. The growing interest in tau can be ascribed to a variety of reasons, not all of them necessarily related to the pathological significance of tau itself. For example, the possibility of an etiologic link between PT and CTE has been popularized by the discovery of phosphorylated tau protein in brain specimens of deceased NFL players as well as in victims of other forms of blast-induced or closed head injury (Daneshvar et al., 2011; Peskind et al., 2013). The conclusion from many of these studies is that a history of repetitive head impacts is necessary for the deposition of PT and of subsequent neurodegeneration (McKee et al., 2009a; Goldstein et al., 2012a).

Tau protein is one of the first microtubule associated proteins to be characterized (for review, see (Mandelkow and Mandelkow, 2012)). In *Homo sapiens*, tau is encoded by a single gene, MAPT but six splice variants have been described (Morris et al., 2011). In the brain, tau stabilizes axonal microtubules but tau's affinity for microtubules is decreased when tau is phosphorylated. Tau phosphorylation targets three amino acids serine, threonine, and tyrosine; the extent of phosphorylation can have small or major inhibitory effects on the binding of tau to microtubules depending on the number of repeats and the region of the protein. The normal phosphorylation of tau regulates microtubule assembly while its hyperphosphorylation destabilizes microtubules and alters axonal transport. However, tau-deficient mice have no significant phenotypic alterations suggesting that loss of function is not the main trigger of tauopathies (Morris et al., 2011).

The term “chronic traumatic encephalopathy” describes the neurodegenerative disease in athletes with histories of repeated head injuries and their sequelae (Smith et al., 2013). CTE is considered a tauopathy with phosphorylated tau-positive deposits in characteristic locations. Although a connection between repetitive head trauma and CTE has been proposed, a causal role of pathological tau deposition has not yet been shown. Many athletes with rTBI never develop CTE while on the other hand pathologies other than CTE, especially seizures, have been shown to be characterized by PT in neurons of affected individuals (Smith et al., 2013; Sen et al., 2007; Thom et al., 2011; Hazrati et al., 2013). These observations call into question the etiologic role of rTBI in the genesis of PT deposition. An important caveat of all these studies is the fact that most if not all specimens derived from postmortem brain. This was true for samples from football players, blast TBI patients or epileptic subjects (McKee et al., 2009a; Thom et al., 2011; Hazrati et al., 2013). Since a postmortem analysis is required for the evaluation of brain phosphorylated tau in CTE, a positive correlation with a given pathology has been difficult. Finally, limited evidence suggests that poorly controlled or multiple drug resistant seizures may result in PT deposition (Sen et al., 2007; Thom et al., 2011). A direct comparison between brain samples from athletes with rTBI and patients with refractory epilepsy is lacking.

The objective of the current study was to determine the specificity of rTBI for PT deposition by comparing the immunohistochemical profile of phosphorylated tau deposits in brain samples from athletes with a history of rTBI and diagnosed with CTE to patients with TLE. We also investigated the presence of specific tau phosphorylation sites in post-traumatic vs. aged or epileptic human brain.

2. Results

We examined the most typical macro- and microscopic features of temporal lobe epilepsy and CTE in sections of temporal lobes from 6 CTE and 19 epileptic subjects, as summarized in Table 1. We also used temporal lobes from six postmortem control brain specimens. From each brain sample, we used at least 7 sections for a total of 195 stained slices. Of these, most were stained with AT8 or with CP-13; other samples were stained for total tau, IgGs or albumin. In addition, 6 CTE, 2 epileptic and 6 control brain samples were analyzed by Western blotting of frozen brain samples. These were tested with CP13, AT8 and total tau (Tau 5) antibodies. Details on antibodies used are described in Section 4 and Supplemental Table 1.

The overall appearance of CTE brain samples, when examined en bloc, was otherwise unremarkable and undistinguishable from epileptic brain or controls. CTE and