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Multiple proteins implicated in neurodegenerative diseases accumulate in axons after brain trauma in humans

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Abstract

Studies in animal models have shown that traumatic brain injury (TBI) induces the rapid accumulation of many of the same key proteins that form pathologic aggregates in neurodegenerative diseases. Here, we examined whether this rapid process also occurs in humans after TBI. Brain tissue from 18 cases who died after TBI and from 6 control cases was examined using immunohistochemistry. Following TBI, widespread axonal injury was persistently identified by the accumulation of neurofilament protein and amyloid precursor protein (APP) in axonal bulbs and varicosities. Axonal APP was found to co-accumulate with its cleavage enzymes, beta-site APP cleaving enzyme (BACE), presenilin-1 (PS1) and their product, amyloid-β (Aβ). In addition, extensive accumulation of α-synuclein (α-syn) was found in swollen axons and tau protein was found to accumulate in both axons and neuronal cell bodies. These data show rapid axonal accumulation of proteins implicated in neurodegenerative diseases including Alzheimer's disease and the synucleinopathies. The cause of axonal pathology can be attributed to disruption of axons due to trauma, or as a secondary effect of raised intracranial pressure or hypoxia. Such axonal pathology in humans may provide a unique environment whereby co-accumulation of APP, BACE, and PS1 leads to intra-axonal production of Aβ as well as accumulation of α-syn and tau. This process may have important implications for survivors of TBI who have been shown to be at greater risk of developing neurodegenerative diseases.

Keywords: Traumatic brain injury; TBI; Axonal injury; Amyloid β; APP; BACE; PS-1; α-Synuclein; Tau

Introduction

It has become increasingly accepted that traumatic brain injury (TBI) results in pathophysiological changes similar to those seen in neurodegenerative diseases. Several investigations have suggested a link between a history of TBI and the subsequent development of Alzheimer's disease (AD) (Mortimer et al., 1985; Rasmusson et al., 1995; Schofield et al., 1997; Nemetz et al., 1999; Guo et al., 2000; Lye and Shores, 2000; Plassman et al., 2000). Likewise, TBI is an epidemiological risk factor for the development of sporadic Parkinson's disease (PD) (Nayernouri, 1985; Factor and Weiner, 1991; Stern, 1991; Ben-Shlomo, 1997; Lees, 1997; Goldman et al., 2006).

Pathologically, AD is characterized by Aβ-containing plaques and neurofibrillary tangles comprised of tau protein (Braak and Braak, 1991; Selkoe, 2001; Forman et al., 2004). To a lesser extent, both dystrophic neurites and Lewy bodies containing α-synuclein protein (α-syn) are also observed in AD. Lewy bodies and α-syn immunoreactivity are also hallmark pathological features of PD and other synucleinopathies such as dementia with Lewy bodies (DLB) and multi-system atrophy (MSA) (Smith et al., 2003a,b,c; Norris et al., 2004). As with neurodegenerative diseases, protein accumulation is also a feature of TBI. Most notably, Aβ plaque formation and the accumulation of neurofilament proteins, tau and α-syn have

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been found in brain tissue of humans within hours to days following TBI (Grady et al., 1993; Roberts et al., 1994; Graham et al., 1995; Newell et al., 1999; Smith et al., 2003a,b,c; Abrahamson et al., 2006). The mechanism underlying this rapid protein build-up after TBI remains unknown, as does its contribution to the later development of neurodegenerative disease.

Aβ peptide is generated via the trans-membrane cleavage of amyloid precursor protein (APP) by the β- and γ-secretases. More specifically, its anabolism is mediated by beta-site APP cleaving enzyme (BACE) and the catalytic component of β-secretase, presenilin-1 (PS1) (De Strooper et al., 1998; Vassar et al., 1999; Nunan and Small, 2000; Selkoe and Wolfe, 2000; Esler and Wolfe, 2001). Mounting evidence suggests that this process may also occur within the axonal membrane compartment. Large accumulations of Aβ have been found in swollen axons after TBI in a pig model of head rotational acceleration (Smith et al., 1999; Chen et al., 2004), in rodent models of brain contusion (Iwata et al., 2002; Stone et al., 2002; Chen et al., 2004), and in humans (Roberts et al., 1994; Smith et al., 2003a,b,c). Axonal accumulations of Aβ were frequently found near diffuse, extracellular AD-like Aβ plaques in both the pig and in humans at the earliest survival timepoints measured (3 days and 18 h respectively). This suggests a potential link between axonal pathology and Aβ plaque formation (Smith et al., 1999, 2003b). More recently, extensive co-accumulations of Aβ with APP, BACE, and PS-1 were identified at sites of axonal injury and disconnection after TBI in the pig (Chen et al., 2004). Thus, disruption of axonal transport after TBI may create an environment whereby large accumulations of APP are processed to form Aβ, potentially leading to subsequent neurodegeneration. Indeed, other recent studies have demonstrated the intra-axonal generation of Aβ in both central and peripheral nerve axons (Kamal et al., 2000, 2001). Similarly, in a transgenic mouse model of AD, interrupted axonal transport and axonal swelling was shown to promote Aβ generation (Stokin et al., 2005).

The other classic pathological findings in AD are neurofibrillary tangles (NFTs) and neuropil threads (Braak and Braak, 1991; Selkoe, 2001; Forman et al., 2004). These intracellular structures are found to contain abnormal forms of the microtubule associated protein tau. NFTs with similarly abnormal tau are found in the brains of patients with dementia pugilistica; a progressive dementing disorder resulting from repetitive head trauma (Schmidt et al., 2001). Following a single episode of TBI in humans, hyperphosphorolyated tau has been demonstrated in brain tissue as well as elevated levels of the protein in cerebrospinal fluid (Newman et al., 1995; Zemlan et al., 1999). Additionally, excessive tau protein accumulation has been found in swollen axons in a pig model of TBI (Smith et al., 1999).

α-syn is a small, highly soluble protein believed to play a role in synaptic maintenance (Norris et al., 2004). In the context of AD and the synucleinopathies, this protein is found as abnormal, highly insoluble, filamentous perikaryal aggregates (Trojanowski and Lee, 2002; Forman et al., 2004). It appears that the α-syn found in disease states is pathologically altered due to ubiquitination, oxidation/nitration, phosphorylation and/or conformational modification (Giasson et al., 2000; Duda et al., 2002; Fujiwara et al., 2002). α-syn accumulation has also been demonstrated in neurons and axons following a single episode of TBI in humans (Newell et al., 1999; Ikonomovic et al., 2004) as well as in patients with dementia pugilistica (Schmidt et al., 2001). Accumulation of nitrated and conformationally modified α-syn in axons has also recently been found after TBI in transgenic mice (Uryu et al., 2003).

Here, we examined whether the findings in animal TBI models of rapid axonal accumulation of proteins found in neurodegenerative diseases also occurs in human TBI. In particular, we evaluated protein accumulation similar to that seen in AD and the synucleinopathies, including the accumulation of NF, APP, BACE, PS-1, Aβ, tau, and α-syn.

Materials and methods

This study was approved by the Ethics Committee of the Southern General Hospital, South Glasgow University Hospitals NHS Trust, UK.

Case material and preparation

Brain tissue from 18 cases following a single incident of fatal head injury was secured after full diagnostic autopsy using standardized techniques (Adams et al., 1980) by the Department of Neuropathology, Southern General Hospital, Glasgow, UK. Superficial and deep grey and white matter from the frontal lobe, temporal lobe, and brainstem was examined; however, the specific location of the tissue was unknown. None of the cases investigated had a prior history of TBI or other neurodegenerative disease. The mean±standard deviation age of TBI cases was 45.7±24.0 years. The survival time from TBI ranged from 4 h to 5 weeks and the post-mortem delay time was 50.2±33.6 h.

The cause of injury was a fall in 8, a road traffic accident in 7 and assault in 3. A skull fracture was present in 14, contusions in 17 and there was an intracranial haematoma in 9. Diffuse axonal injury (Adams et al., 1989) was identified in 11 (grade 3 in 4 cases; grade 2 in 2 and grade 1 in 5). Hypoxic damage was present in 15 (Graham et al., 1989) and graded as severe in 5, moderately severe in 3 and mild in 7. Brain swelling was present in 10 — unilateral in 5 and bilateral in 5, and there was internal herniation in 12 (Adams and Graham, 1976). The cause of death was raised intracranial pressure in 11, pneumonia in 5, multiple injuries in 1 and systemic hypoxia in 1.

The brain of each case was collected and fixed in 10% neutral buffered formalin, then cut into slices 10 mm thick and processed for paraffin embedding. Serial sections of 6 μm were cut on a Leitz rotary microtome and mounted on poly-L-lysine-coated slides for histological study.

Controls

Tissue was also secured from 6 control cases from the same institution. The mean±standard deviation age of control cases was 37.8±22.1 years and post-mortem delay time was 59.2±41.3 h. These cases had no prior history of head injury or had any evidence of structural brain damage due to pre-existing disease or injury; the cause of death in 3 was septicemia and sudden