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.

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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; Rasmussen 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...
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; Nunnan 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 neurit thread (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 have 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
unexpected death in epilepsy (Nashef, 1997; Black and Graham, 2002) in the remaining 3 cases.

**Immunohistochemistry**

Immunohistochemistry (IHC) was performed on serial paraffin-embedded sections with investigators blinded to the cases’ clinical history. Using a well-characterized panel of antibodies, we evaluated one antibody per slide for each case, resulting in the evaluation of a total of 15–20 slides per case. We chose to use several antibodies that targeted different binding sites of each protein of interest in order to provide a more comprehensive picture of the proteins present. Single-labeled IHC was carried out using the primary antibodies listed in Table 1, followed by incubation with the appropriate secondary antibody and the ABC kit (Vector Laboratories, Inc, Burlingame, CA). Visualization was achieved using DAB (Vector Laboratories, Inc, Burlingame, CA) and counterstaining with hematoxylin (Uryu et al., 2003). To evaluate the co-accumulation of proteins, we used double- or triple-labeled fluorescence immunohistochemistry (FIHC) as described elsewhere (Uryu et al., 2003; Chen et al., 2003). To evaluate the co-accumulation of proteins, we used double- or triple-labeled fluorescence immunohistochemistry (FIHC) as described elsewhere (Uryu et al., 2003; Chen et al., 2004). Briefly, tissue was incubated with a combination of primary antibodies (Table 1) followed by the appropriate fluorescent-conjugated secondary antibody. Omission of the primary antibody or application of control serum instead of the primary antibody was performed on selected sections of tissue to provide a negative control. Paraffin-embedded sections from pathologically confirmed human AD and Parkinson disease brain tissue served as positive control for tau, Aβ and α-syn staining.

**Table 1**

Summary of antibodies used for immunohistochemical analysis

<table>
<thead>
<tr>
<th>Protein</th>
<th>Antibody</th>
<th>Host</th>
<th>Recognition site</th>
<th>Dilution</th>
<th>Provider</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tau</td>
<td>17026</td>
<td>Rabbit</td>
<td>Pan Tau</td>
<td>1:10k</td>
<td>CNDR</td>
</tr>
<tr>
<td>PHF-1</td>
<td>pS396/pS404</td>
<td>Mouse</td>
<td></td>
<td>1:1000</td>
<td>Davies*</td>
</tr>
<tr>
<td>PHF-6</td>
<td>pT231</td>
<td>Mouse</td>
<td></td>
<td>1:1000</td>
<td></td>
</tr>
<tr>
<td>α-syn</td>
<td>syn202</td>
<td>Mouse</td>
<td>Pan synuclein</td>
<td>1:20,000</td>
<td></td>
</tr>
<tr>
<td>NF</td>
<td>NF-L</td>
<td>Mouse</td>
<td>Conformational a-syn</td>
<td>1:5000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RMO44</td>
<td>Mouse</td>
<td>Rod domains of NF-M</td>
<td>1:500</td>
<td>CNDR</td>
</tr>
<tr>
<td></td>
<td>RMO217</td>
<td>Mouse</td>
<td>P+ NF-H side arm, C-terminus</td>
<td>1:5</td>
<td>CNDR</td>
</tr>
<tr>
<td>APP</td>
<td>LN39</td>
<td>Mouse</td>
<td>APP</td>
<td>1:50</td>
<td>CNDR</td>
</tr>
<tr>
<td></td>
<td>Donkey</td>
<td>APP/N-terminal</td>
<td>1:800</td>
<td>Greenberg**</td>
<td></td>
</tr>
<tr>
<td>Aβ</td>
<td>BC05</td>
<td>Mouse</td>
<td>Aβ 1–42/43</td>
<td>1:10,000</td>
<td>Suzuki***</td>
</tr>
<tr>
<td></td>
<td>BAN27</td>
<td>Mouse</td>
<td>Aβ 1–40</td>
<td>1:10,000</td>
<td>Suzuki</td>
</tr>
<tr>
<td></td>
<td>13335</td>
<td>Rabbit</td>
<td>Aβ 1–42</td>
<td>1:1000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NAB288</td>
<td>Mouse</td>
<td>pan Aβ</td>
<td>1:20,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amy117</td>
<td>Mouse</td>
<td>Amyloid — 100 kDa protein present</td>
<td>1:20,000</td>
<td></td>
</tr>
<tr>
<td>BACE</td>
<td>BACE</td>
<td>Rabbit</td>
<td>BACE</td>
<td>1:500</td>
<td>Alpha Diagnostics</td>
</tr>
<tr>
<td></td>
<td>BACE</td>
<td>Rabbit</td>
<td>BACE/N-terminal</td>
<td>1:10000</td>
<td>CNDR</td>
</tr>
<tr>
<td>PSI</td>
<td>PS1</td>
<td>Goat</td>
<td>N-terminal</td>
<td>1:100</td>
<td>Chemicon</td>
</tr>
</tbody>
</table>

CDNR=Center for Neurodegenerative Research (University of Pennsylvania), NF=neurofilament, APP=amyloid precursor protein, Aβ=amyloid beta, α-syn=alpha synuclein, BACE=beta amyloid cleaving enzyme, PS1=presenilin-1, AMY117=amyloid 100 kDa protein, Aβ=amyloid beta, α-syn=alpha synuclein.

**Semiquantitative analysis**

Examination of tissue was conducted by two individuals who were both blind to the clinical circumstances of the cases. Determination of the frequency of pathological profiles containing neurofilament (NF), APP, BACE, PS1, Aβ, tau, or α-syn proteins was achieved by reviewing microscopic sections stained with specific antibodies recognizing the respective molecules. We followed the pathological diagnostic criteria for AD as described previously by Mirra et al. (1993). This is an observational study where sections were examined at 100× magnification and profiles were ranked semi-quantitatively as no occurrence (−), low occurrence (+), moderate occurrence (++) or frequent occurrence (+++). Examination of tissue was conducted by two individuals who were both blind to the clinical circumstances of the cases. Determination of the frequency of pathological profiles containing neurofilament (NF), APP, BACE, PS1, Aβ, tau, or α-syn proteins was achieved by reviewing microscopic sections stained with specific antibodies recognizing the respective molecules. We followed the pathological diagnostic criteria for AD as described previously by Mirra et al. (1993). This is an observational study where sections were examined at 100× magnification and profiles were ranked semi-quantitatively as no occurrence (−), low occurrence (+), moderate occurrence (++) or frequent occurrence (+++) in a 100 μm² field. The staining results for each protein of interest were in complete agreement, regardless of antibody recognition site; either all were positive for the protein or all were negative. Moreover, the number of profiles observed for each antibody was consistent across the tissue sections observed. Therefore, the findings presented in Table 2 are based on the consideration of all slides stained for a particular protein of interest.

**Results**

**Recognition of axonal injury**

Axonal pathology, characterized by axonal bulbs and/or swellings, was identified using antibodies to NF protein and APP.
Axonal bulbs had the appearance of discrete spherical profiles surrounded by a halo and were morphologically distinct from the varicose swellings of the axon. The bulbs ranged in size from 5 to 100 μm. Only one subject in the uninjured group exhibited positive staining for NF and APP, while 15 of the 18 injured cases had extensive immunoreactivity to APP in the white matter. However, only about one-third of these cases showed positive staining for NF, an additional marker of axonal pathology (Table 2) (Figs. 1a–d).

Accumulation and co-localization of APP, Aβ, BACE and PSI

In the control group, only one subject showed APP accumulation; however, no additional protein deposits were noted. As mentioned above, almost all cases subject to TBI displayed evidence of APP accumulation within axons. In addition, BACE and PSI staining was observed within axonal bulbs in a majority of these cases. With the exception of 2 cases, the number of profiles appeared to increase in those cases survived between

Fig. 1. Bright-field photomicrographs showing pathological protein accumulation associated with axonal pathology in humans. Neurofilament and amyloid precursor proteins (APP) were both found in axonal swellings and bulbs following TBI (a–d). Enzymatic agents of APP cleavage (BACE and PSI) were also present, although to a lesser extent (e, f). Antibodies specific for Aβ revealed axonal swellings (g) and bulbs (g, inset) positive for Aβ_{42/43} throughout the white matter of subjects and within the brainstem of several young cases (h). A limited number of swellings and bulbs stained positive for Aβ_{40}. Infrequent amyloid deposits in subjects over 70 years of age were seen with Aβ_{40} (i). Scale bars = 50 μm.
8 days and 4 weeks (Figs. 1e,f). Aβ\textsubscript{1-42} immunoreactivity within axons was noted in 13 of the total 18 injured cases (Fig. 1g). Aβ IHC revealed axonal bulbs positive for Aβ\textsubscript{1-42} but negative for Aβ\textsubscript{1-40}. Interestingly, brain tissue from four young cases (Cases K, L, N, O) indicated rather strong axonal Aβ staining in their brainstem (Fig. 1h). When detected, Aβ plaques were limited in number and restricted to cases aged 70 years or more (Fig. 1i).

Using double- or triple-label fluorescence IHC, co-localization of Aβ and its precursor protein, APP, as well as co-factors of APP processing, BACE and PS1, was examined. APP was observed to co-localize with Aβ (Figs. 2a–c) or BACE and PS1 (Figs. 2d–g) within axonal bulbs following injury. Aβ was also noted to co-localize with BACE and PS1 in axonal bulbs (Figs. 2h–k).

**Tau and α-syn**

None of the tissue from uninjured control cases stained positive for tau or α-syn proteins, and few of the injured cases stained positive for tau (Fig. 3a) and bulbs (Fig. 3b) following injury, and, to a lesser extent, in glial cells (Fig. 3c). α-syn was also present within axonal swellings (Fig. 3d) and bulbs (Fig. 3e) in a majority of the injured subjects. Scale bars=50 μm.

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**Fig. 2.** Multiple immunofluorescent staining showing co-accumulation of APP, BACE, PS1, and Aβ in axons following TBI. Double-labeling revealed co-accumulation of APP and Aβ\textsubscript{1-42} in multiple axon bulbs (a–c). Further immunohistochemical staining showed co-accumulation of BACE and PS-1 with APP (d–g) and Aβ\textsubscript{1-42} (h–k). Scale bars=50 μm.

**Fig. 3.** Bright-field photomicrographs showing accumulation of tau and α-syn proteins in axonal swellings and bulbs. Of the 18 TBI cases examined, only 2 stained for tau. Tau staining was observed in axonal swellings (a) and bulbs (b) following injury, and, to a lesser extent, in glial cells (c). α-syn was also present within axonal swellings (d) and bulbs (e) in a majority of the injured subjects. Scale bars=50 μm.
stained positive for tau. However, a majority of the tissue from injured cases showed positive staining for α-syn. PHF-1 positive phosphorylated tau was noted in a small number of swollen axons and clusters of neuronal cell bodies in the cerebral cortex (Figs. 3a,b). In the same area, reactive astrocytes occasionally showed tau positive staining (Fig. 3c). Neuronal tau tended to be more intensely stained than glial tau. α-syn protein was observed mostly in swollen, undulating axons as well as in bulbs, with little reactivity in the neuronal soma (Figs. 3d,e).

Summary of principal findings

Cases with a history of traumatic brain injury demonstrated axonal pathology in the majority of cases as shown by increased immunoreactivity to APP or NF. This compares to controls where just one case demonstrated minimal immunoreactivity. Immunoreactivity to BACE, PS-1 and Aβ were all found to be increased in TBI cases versus controls.

Co-immunoreactivity between BACE, PS-1 and APP was found within axonal bulbs. In addition, within these bulbs, Aβ was also found co-accumulating with APP, BACE and PS-1. α-syn immunoreactivity was found in two-thirds of the cases, predominantly in association with axonal pathology. Only 2 cases were positive for tau which was found in both neurons and nearby reactive astrocytes. No controls had immunoreactivity for either α-syn or tau.

It is important to note that while protein accumulation was seen in many of the injured cases we evaluated, the number and location of samples that can be evaluated in an autopsy study is limited. As such, some cases graded as negative may have actually had pathologic changes in areas not examined.

Discussion

There is increasing evidence that the brains of TBI cases display many of the same pathologies associated with several neurodegenerative diseases. TBI can also induce the rapid accumulation of several proteins that compose similar pathologic aggregates found in neurodegenerative diseases such as AD, of which the most widely studied include NF proteins, APP, Aβ, and α-syn. Here, the accumulation of multiple proteins and their anabolic agents implicated in neurodegenerative diseases were found within damaged axons up to 5 weeks after TBI in humans. This evidence suggests that damaged axons provide a key source of proteins implicated in neurodegenerative processes.

Traumatic axonal injury is a common and important pathology resulting from TBI in humans (Adams et al., 1989, 1991; Smith and Meaney, 2000) and is frequently observed after motor vehicle accidents, falls, and assaults (Adams et al., 1982, 1989; Pilz, 1983; Gennarelli, 1993). In DAI, the axoskeleton can be severely damaged, resulting in impaired axonal transport, build-up of transported proteins, axonal swellings and bulbs (Povlishock and Becker, 1985; Maxwell et al., 1997).

Axonal pathology in humans has been identified by the accumulation of NF in damaged axons from 6 h onwards after head trauma in both animals and humans (Grady et al., 1993; Christman et al., 1994). In cases of death very shortly following injury, accumulation of NF is very limited, if present at all, in our experience. This is likely due to the fact that NF is slowly transported and accumulation is insufficient for detection in this early phase. In addition, we also note that in humans, NF immunoreactivity has a tendency to increase as survival time post-trauma elapses. This explains why in this sample we see axonal pathology as identified by NF accumulation in both fewer cases and specifically those with a longer survival period. As such, immunohistochemical detection of the fast transport APP has become a standard method of diagnosing axonal injury in human brain tissue, where axonal swellings can be identified within 1 h of injury (Adams et al., 1980, 1989; Otsuka et al., 1991; Sherriff et al., 1994; Lambri et al., 2001; Gorrie et al., 2002; Reichard et al., 2003). The presence of acute axonal pathology is confirmed here. However, we note that within our heterogeneous population, there are cases who ultimately died secondary to raised intracranial pressure (RICP) and the vascular complications of internal herniation. Therefore, in many cases of fatal TBI it is possible to identify axonal pathology that is both traumatic and non-traumatic (infarction, related to hematomas and contusions) in origin. Axonal swellings are also seen in many other non-traumatic conditions and as part of the aging process. As so, it is appropriate to suggest that such pressure/vascular complications may have independently contributed to the neuropathological findings described (Geddes et al., 2000). Our findings also demonstrate that APP accumulation may be far more than a simple marker of axonal pathology in humans; it also may be the primary substrate for posttraumatic Aβ formation. In particular, in damaged axons, we observed APP accumulation along with its catalytic enzymes, BACE and PS1. To a lesser extent, other AD-associated proteins, tau and α-syn, were observed. Thus, extensive axonal damage may serve as a key reservoir of proteins implicated in neuro-pathologic processes. Lysis or release of accumulate proteins from damaged axons may lead to plaque formation or toxicity.

The present histopathologic findings in human TBI are consistent with findings in previous animal studies. Extensive co-accumulation of APP with Aβ has been found in swollen axons in a swine model of diffuse axonal injury induced by rotational acceleration (Smith et al., 1999). More recently, in the same model, axonal Aβ has been found to co-localize with its precursor protein, APP, along with the catalytic enzymes BACE and PS1 necessary to cleave Aβ from APP (Chen et al., 2004).

A number of studies suggest that axons and their terminals may be a critical site of Aβ production. Firstly, Aβ plaques have been shown to develop in close relationship with axonal terminals in AD brains (Van Hoesen and Hyman, 1990; Kamal et al., 2000; Schonheit et al., 2004). It has also been shown that APP is transported along axons by direct binding to kinesin in mouse sciatic nerves (Kamal et al., 2000). This study demonstrates that APP operates as a kinesin-1 receptor and mediates the transport of its cleavage enzymes PS-1 and β-secretase. This in turn permits the intra-axonal generation of Aβ. However, this mechanism remains controversial and is contradicted by other studies (Lazarov et al., 2005). If such were the case, it is reasonable to assume that disruption of axonal transport may lead to abnormal Aβ generation intraxonally and potentially deposition. Indeed, Stokin et al., using an APP transgenic mouse model, demonstrated that both Aβ levels
and deposition after disruption of axonal transport (Stokin et al., 2005). In addition, inter-axonal Aβ deposits formed in APP transgenic mouse brain could be reduced by anti-Aβ antibody therapy (Brendza et al., 2005). These studies suggest that axons can play an important role in APP processing and Aβ formation both intra-axonally and in the generation of extracellular Aβ plaques. Thus, the extensive axonal damage found in TBI may provide a unique environment in which unusually concentrated co-accumulation of APP, BACE, and PS1 occurs, providing the tools to produce Aβ. In turn, this intra-axonal process may play a critical role in rapid Aβ plaque formation.

Accumulation of both tau and α-syn proteins were also found in the cases we examined; although tau was observed in fewer cases than α-syn. Based on the antibodies used, tau protein appeared to be abnormally phosphorylated and the α-syn protein was conformationally changed. These findings are consistent with the pathologies that are observed in AD brain lesions and other diseases characterized by α-syn accumulation (Fujikawa et al., 2002; Norris et al., 2004). Thus, it seems as though TBI may initiate similar processes leading to the pathological modification of these proteins that occurs in neurodegenerative disease. It is also interesting to note that both tau and α-syn are observed in the grey matter in AD brains (Forman et al., 2004); yet here, we observed both proteins within the axons and axonal bulbs. Additionally, tau protein appeared in far fewer injured cases than did α-syn. If damaged axons provide a source of this protein, the pathological accumulation may occur over a more protracted time course than was observed here.

The present study illustrates the potential contribution of axonal injury to creating pathological protein accumulation in human brain within 4 h–5 weeks following TBI. Thus, it is possible that axonal injury associated with pathological protein accumulation may contribute to AD-related pathogenesis. A further understanding of the mechanistic aspects of the long-term pathophysiology of AD-related proteins in the injured brain may aid the development of interventions to halt possible TBI induced neurodegeneration.

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References


