

Progressive supranuclear palsy

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Abstract

This review concentrates on the molecular pathology of the protein τ , including its expression in various regions of the brain. The role of post-translational modifications in fibrillogenesis is also discussed, particularly hyperphosphorylation and glycation. However, the initiating event that causes aberrant τ processing remains unclear.

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Progressive supranuclear palsy (PSP; also called Steele–Richardson–Olszewski syndrome) occurs most commonly in middle to late age and is a neurodegenerative, hypokinetic movement disorder, with dementia a common feature in end stage disease. Steele, Richardson, and Olszewski first described PSP in 1964 as a heterogeneous system degeneration involving the brainstem, basal ganglia, and cerebellum.¹ Subsequently, neuronal degeneration was described in the perirhinal, inferior temporal, and prefrontal cortex.^{2–4} The midbrain atrophy is accompanied by dilatation of the aqueduct of Sylvius and depigmentation of the substantia nigra, which appears to be orange, discoloured, and shrunken, with severe loss both of pigmented and non-pigmented neurones.⁵ The locus coeruleus less often shows decreased pigment. The subthalamic nucleus may be noticeably smaller than expected and the superior cerebellar peduncle may be small. External examination of the cerebral cortex may be normal, but mild atrophy in the frontal lobes, extending to the pre-central gyrus is not uncommon.^{6,7}

The neurodegeneration is characterised by the presence of neurofibrillary tangles (NFTs), neuropil threads (NTs), and fibrillary gliosis, resulting from the aggregation of hyperphosphorylated τ protein filaments, thereby causing PSP to be considered as “tauopathy”. NFTs are mildly basophilic and globose (spool-like), and appear to consist of finely entwined filaments often arranged in parallel, so that their structure resembles a ball of string. Under the electron microscope, the tangles in PSP appear as straight unpaired filaments with a diameter of 15–18 nm and comprising at least six protofilaments 2–5 nm in diameter.^{8,9} Apart from τ proteins in various stages of proteolytic breakdown, NFTs contain other molecules that may induce filament polymerisation or promote filament stability. Flame shaped tangles, characteristic of Alzheimer’s disease (AD), may also occur in PSP and have been found to consist of a coarse rope-like texture of

fibrils.⁷ NTs are scattered thread-like structures. In PSP, they occur in both neuronal processes but also, in part, in oligodendrocytic processes. To distinguish them from neuronal threads, these glioneuronal threads are also known as interfascicular threads.¹⁰

The National Institute of Neurological Disorders and Stroke criteria for the diagnosis of PSP require a high density of NFTs and NTs to be present in at least three of the following sites: pallidum, subthalamic nucleus, substantia nigra, or pons, and a low to high density in at least three of the following sites: striatum, oculomotor complex, medulla, or dentate nucleus. Neuronal loss and gliosis are variable. Amyloid deposits and neuritic plaques are notably absent.^{3,11} The diagnosis of PSP also requires the exclusion of other disorders, including large or numerous infarcts, pronounced diffuse or focal atrophy, Lewy bodies, oligodendroglial argyrophilic inclusions, Pick bodies, diffuse spongiosis, or prion P positive amyloid plaques.¹²

Verny *et al* found that τ positive cortical lesions were constant in PSP, affecting mostly the precentral and angular gyri; NFTs were mainly located in the deepest cortical layers, affecting both large and small neurones.¹³ Statistical analysis has suggested that both cortical and subcortical NFTs are linked to the pedunculo-pontine nucleus, which may play a prominent role in spreading the lesions.¹² Threads are much less numerous in the cerebral cortex and are restricted, if present, to the precentral and postcentral gyri, where thick and winding threads are arranged parallel to the direction of the axonal bundles in the cortical gyrus.¹⁰ Cortical pathology adds an important dimension to the clinical manifestation of PSP. When patients with dementia were compared with those without cognitive defects, significantly more cortical, neurofibrillary τ was present in those with dementia, suggesting a strong causal link.¹⁴

Recently, it has been noted that τ positive glial inclusions are a consistent feature in the brains of patients with PSP; indeed, many of the cortical lesions seen result from glial pathology. Glial inclusions were originally referred to as glial fibrillary tangles, but they now seem to be more heterogeneous than previously thought. Currently, they are classified according to their cellular origin; astrocytic—subdivided into fibrillary or protoplasmic—and oligodendrocytic.¹⁰ Astrocytes possessing protoplasmic τ inclusions may appear as tufts of abnormal fibres.¹⁵ On Gallyas-Braak staining, tufts are seen as an aggregation of conglomerated, fine, or thick processes in a concentric arrangement. They exhibit tree shaped branching without collaterals. Fibrillary inclusions

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give rise to thorn shaped astrocytes often possessing eccentric nuclei. They are limited to subpial and subependymal areas, occasionally extending to deep white matter, and are usually embedded in areas of fibrillary gliosis. Ultrastructurally, the cells consist of 15 nm diameter straight τ filaments and amorphous material, coexisting with densely packed 7–10 nm diameter glial fibrils.¹⁰

Tau immunohistochemistry has also revealed small round cells in the white matter of areas affected by PSP; these have been shown to be oligodendrocytic inclusions. The cells are also referred to as “coiled bodies” and their oligodendrocytic origin has been confirmed with immunoelectron microscopy. Coiled bodies contain enlarged, lucent nuclei and are typically fine and branching in PSP. They are predominantly found in interfascicular oligodendrocytes in the white matter, but may also occur in satellite cells of the grey matter. In PSP, they are limited to the precentral cortex, internal capsule, pencil fibres in the lenticular nuclei, midbrain (particularly in the tectum and red nuclei), and tegmentum of the pons and medulla. Tau aggregates also occur in oligodendrocytic processes ensheathing damaged axons, where they appear as threads. Some of the thread-like processes in white matter are found within both the inner and outer surfaces of myelin sheaths, corresponding to the inner and outer loops of oligodendrocytes.^{6–10}

Some of the most interesting recent pathological findings have come from the comparison of PSP and Parkinson’s disease (PD). Henderson *et al*, in a detailed investigation of thalamic pathology, show a selective loss of caudal intralaminar nuclei in both diseases. They suggest that the resultant loss of glutaminergic regulation, together with the loss of dopaminergic control from the degeneration of the substantia nigra pars compacta, may account for the similar parkinsonian symptoms in PSP and PD.¹⁶

Molecular pathology

The human τ gene is located over 100 kb on the long arm of chromosome 17q21 and contains 16 exons. In the adult human brain, different isoforms of τ exist as a result of alternative splicing of the mRNA transcript. Exons 2, 3, and 10 are alternatively spliced, allowing six different combinations of τ isoforms.¹⁷ The τ variants differ from each other by the presence or absence of 29 or 58 amino acid inserts located in the N-terminal part (that is, exon 3 only or exons 2 and 3 incorporated, respectively) and a 31 amino acid repeat located in the C-terminal part. In the absence of the latter, which is encoded by exon 10, the spliced products give rise to three τ isoforms with three repeats (3R). The three other τ isoforms contain this 31 amino acid repeat and thus have four repeats (4R). These repeats and their adjacent domains constitute the microtubule binding domains of τ .^{17–18}

In normal brain, 3R τ isoforms are slightly more predominant than 4R isoforms. In PSP, hyperphosphorylated τ isoforms with their sequence encoded by exon 10 (4R) are

abundant and are found to aggregate into filaments, whereas τ isoforms without exon 10 (3R) are not detected.¹⁹ The typical electrophoretic profile of pathological τ proteins obtained using phosphorylation dependent monoclonal antibodies is that of a doublet, corresponding to isoforms of 64 kDa and 69 kDa, with a minor 74 kDa band also detected. Similar findings are found in corticobasal degeneration (CBD) and some forms of frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17).^{17–20} One difference between CBD and PSP is that, in the highly insoluble fraction of τ aggregates, a 37 kDa band is seen in PSP, whereas in CBD there is a doublet with bands of 40–42 kDa, indicating a degree of differential processing in the two diseases.²¹

Genetic associations of PSP

Genetic influences are increasingly being implicated in the aetiology of PSP. Although most cases of PSP are considered to be sporadic, familial cases such as those reported by De Yebenes and co-workers²² have a pattern of inheritance consistent with an autosomal dominant disorder. More recently, a clinical genetic study of familial PSP suggested that hereditary PSP is more frequent than previously thought, and that the scarcity of familial cases may be related to the lack of recognition of the variable phenotypic expression of the disease.²³ The link between a genetic predisposition to PSP and the differential isoform expression of the τ gene may be the key to explaining the pathogenesis of PSP.

Recent studies have revealed a genetic polymorphism in the τ gene associated with a greater than chance frequency with PSP. Conrad *et al* first identified a polymorphic dinucleotide repeat sequence in intron 9 (between exons 9 and 10) of the τ gene in a white population with PSP.²⁴ The most common allele in the normal population has a TG repeat number of 11, and is designated as A0; alleles with a TG repeat number of 12 are designated A1, those with 13 repeats as A2, and those with 14 repeats as A3. They described a significant over representation of A0 in patients with PSP, particularly those with the homozygous genotype A0/A0. These data have recently been confirmed by several studies in white populations,^{25–27} although these differences were not seen in Japanese populations.²⁸

This polymorphism may be a predisposing factor to PSP, which, similarly to the apolipoprotein E ϵ 4 allele in AD, increases the risk of developing PSP but is in itself neither necessary nor sufficient to cause the disease. Although this polymorphism lies before an alternatively spliced sequence, encoding exon 10 of the τ gene, it is unknown whether this polymorphism, or an adjacent linked area, has an effect on the alternative splicing of τ , and whether this is important in the pathogenesis of the disease. These data were extended to a haplotype including several polymorphisms in linkage disequilibrium with A0, named H1.²⁹ Essentially, two forms of the τ gene have been defined, H1 and H2; the H1 haplotype

corresponds to the A0 polymorphism, and has numerous single nucleotide polymorphisms along the entire τ gene, and one intronic 238 bp deletion flanking exon 10. Most cases of typical PSP have the H1 haplotype, and a surprisingly large number of patients with PSP are H1/H1 homozygous. The percentage of individuals with a H1/H1 genotype was 88.9% in a group of 63 patients with PSP compared with 65.2% in 66 control subjects. However, neither the A0 nor H1 genotypes were correlated with disease onset, severity of symptoms, or survival.³⁰ Exactly how the A0 genotype and the polymorphisms occurring in the H1 haplotype predispose an individual to develop PSP is unclear, but this appears to be related to exon 10 splicing. Over representation of the A0 and H1 haplotypes occurs in other neuropathologies, particularly CBD, underlining the common basis of the two conditions.^{20–31}

Stanford *et al* recently identified a novel silent mutation (S305S) in the τ gene in a subject with cellular pathology characteristic of PSP; the mutation was also present in two affected sisters with differing clinical presentations, one of whom had clinically and neuropathologically confirmed PSP.³² The mutation was located in exon 10 of the τ gene and formed part of a stem loop structure at the 5' splice donor site. Although the mutation did not give rise to an amino acid change in the τ protein, functional exon trapping experiments showed that it produced a significant 4.8 fold increase in the splicing in of exon 10, resulting in the presence of 4R τ . This mutation defined the first molecular lesion shown to be responsible for PSP.

Functions of τ

Tau is one of the principal microtubule associated proteins (MAPs) in the mammalian nervous system. It is found mainly in neurones, although non-neuronal cells usually have trace amounts—for example, τ proteins can be expressed in glial cells (mainly in pathological conditions), and it is also possible to detect τ mRNA and proteins in several peripheral tissues, including the heart, kidney, lung, muscle, pancreas, testis, and fibroblasts.¹⁷

In the normal human brain, τ proteins are distributed mainly in axons and to a lesser extent in neuronal and glial soma and dendrites.¹⁰ Tau is a heat stable protein that is natively unfolded and has domains with high potential for protein–protein interaction. One such protein is tubulin, which polymerises to form microtubules within cells. Microtubules are responsible for neurite extension and serve as the “tracks” for intraneuronal transport. The role of τ in neuronal maturation was illustrated in τ knockout mice. Embryonal hippocampal cultures from these animals showed a significant delay in maturation. However, when the knockout animals were crossed with ones expressing normal human τ , the normal pattern of axonal growth and neurite extension was restored.³³

MAPs play important roles in the assembly of microtubules, crosslinking of microtubules to each other or to other filaments or

organelles, and in transport functions. Tau proteins bind to spectrin and actin filaments; through these interactions, they restrict the flexibility of microtubules and allow them to interconnect with other cytoskeletal components such as neurofilaments.¹⁷ The interaction of τ with MAP1B was explored in double knockout mice ($\tau^{-/-}$; MAP1B $^{-/-}$). Primary cultures of hippocampal neurones from these animals showed severe axonal elongation and cerebellar neuronal cultures exhibited suppressed neurite elongation, illustrating the cooperative function of τ and MAP1B in microtubule organisation in vivo.³⁴ Tau proteins also interact with cytoplasmic organelles and such interactions may allow for binding between microtubules and mitochondria.³⁵ The N-terminal projection domain also permits interactions with the neural plasma membrane. Thus, it was thought that τ might act as a mediator between microtubules and the plasma membrane, and this has recently been shown to involve interactions between the N-terminal part of τ and the SH3 domains of a member of the Src family receptor tyrosine kinases, such as fyn. Tau and fyn have been colocalised just beneath the plasma membrane and an association between τ –fyn complexes and the actin cytoskeleton has been described.^{17–36} These data suggest a role for τ proteins in the Src family tyrosine kinase signalling pathway that might modify cell shape by acting on the submembranous actin cytoskeleton. The N-terminal region of τ proteins is probably involved in the interaction with phospholipase C γ isoenzymes, enhancing the activity of these enzymes in the presence of unsaturated fatty acids, such as arachidonic acid.¹⁷ Thus, τ is capable of modulating the assembly, dynamic behaviour, and spatial organisation of microtubules in neurones and probably glial cells. By supporting cytoskeletal structure and sustaining axonal transport, τ plays a fundamental role in the maintenance of neuronal survival.

In normal neurones, τ is soluble, binds reversibly to microtubules, and has a rapid turnover. However, the forms of τ found in many neurodegenerative diseases have lost their affinity for microtubules, collect as insoluble aggregates (in the form of straight filaments (SFs) in PSP), and resist proteolysis.²⁴ Although the aetiology, clinical symptoms, pathological findings, and the biochemical composition of inclusions in tauopathies are different, there is emerging evidence suggesting that the mechanisms involved in the aggregation of normal cellular proteins to form various inclusions are comparable.

Post-translational modifications of τ

A key feature appears to be initial alterations in the conformation of normal cellular proteins, which initiate the generation of nuclei or seeds for filament assembly. This process can be influenced by the post-translational modification of normal proteins, by mutation or deletion of certain genes, and by factors that bind to normal proteins and thus alter their conformation.³⁷

Tau proteins are found in all cell compartments, but in different phosphorylation states. There are 79 putative Ser or Thr phosphorylation sites on the longest brain τ isoform, and phosphorylation seems to affect several sites simultaneously.¹⁷ Phosphorylation, in combination with the type of isoform, may significantly modulate the properties of τ proteins. An example of this is seen in microtubule assembly, where phosphorylated τ proteins are less effective than their non-phosphorylated counterparts upon microtubule polymerisation. In particular, phosphorylation of Ser 262 (within the microtubule binding domain) dramatically reduces the affinity of τ for microtubules *in vitro*.³⁸ Nevertheless, this site alone, which is present in fetal τ , adult τ , and in hyperphosphorylated τ proteins found in NFTs, is insufficient to eliminate τ binding to microtubules.³⁹ Phosphorylation even outside the microtubule binding domains can also influence tubulin assembly by modifying τ -microtubule affinity.¹⁹ Microtubule binding is also influenced by the type of τ isoform, with 4R isoforms being more effective than 3R ones.^{17 19}

Tau proteins are normally found in axons, but in neurodegenerative disorders they accumulate in somatodendritic neuronal compartments. Because τ trafficking is phosphorylation dependent, abnormal phosphorylation of τ proteins may lead to aberrant cell trafficking and τ aggregation.¹⁷ It is unclear whether increased kinase or decreased phosphatase activity is responsible for the hyperphosphorylation of τ proteins associated with PSP, and there are few reports on this issue. Although more is known about the phosphorylation of τ in AD, the matter is still unresolved. Numerous kinases have been implicated in AD, including glycogen synthase kinase 3 and protein kinase A,⁴⁰ although these are controversial candidates. Mitotic protein kinases may play a role, because many mitosis specific epitopes are found in NFT.⁴¹ Stress activated protein kinases have also been shown to phosphorylate τ proteins (JNK/SAPK1, p38/SAPK2, SAPK3).⁴² Conversely, some data suggest that phosphatase activities may be decreased in AD brains.¹⁷ As a possible means of elucidating the role of hyperphosphorylation, Eidenmuller *et al* developed a model that mimics phosphorylation, in which the serine and threonine residues that are phosphorylated were replaced by glutamate residues. The resultant τ proteins bound to microtubules, but could be displaced by wild-type τ .⁴³ Whether this is an effective model that overcomes some of the difficulties encountered with attempts to induce phosphorylation *in vitro* remains to be seen.

Until recently, it was thought that an abnormal phosphorylation of τ proteins was responsible for their aggregation in AD. However, normal τ proteins are also phosphorylated in fetal and adult brain and they do not aggregate to form filamentous inclusions. Moreover non-phosphorylated recombinant τ proteins form filamentous structures under physiological conditions *in vitro*, when sulphated glycosaminoglycans and/or other polyanions are

present, suggesting that, in addition to phosphorylation, other mechanisms may be involved in the formation of pathological τ filaments.^{17 37} Because hyperphosphorylation of τ proteins may only occur once τ proteins are already aggregated into filaments, rather than be the cause of aggregation, other post-translational processes may be involved in the disruption of τ biochemistry. Those principally implicated in PSP are glycation and transglutamination. Polyubiquitinylation and subsequent proteolysis have been relatively under investigated in PSP. However, one report by Fergusson *et al* has indicated that globose NFTs in PSP are strongly positive for ubiquitin BB1, and they infer that partially degraded polyubiquitinylated τ is important in tangle formation in a manner analogous to that in AD.⁴⁴ In relation to this, Kuusisto *et al* reported the presence of the ubiquitin binding protein p62 in both neuronal and glial inclusions in several tauopathies. They did not include PSP in their investigation, but it would be interesting to see whether p62 is also present in PSP.⁴⁵

Glycation is the reaction between the amino part of a side chain of an amino acid and the carboxyl part of a glucose residue or other reducing sugar. This post-translational modification, also known as non-enzymatic glycosylation, leads to the formation of heterogeneous products called advanced glycation end products (AGEs). AGEs have recently been detected immunohistochemically in NFTs from PSP, and also in AD, amyotrophic lateral sclerosis, and Pick's disease.⁴⁶ Insolubility of aggregated τ may be related to glycation because a crosslinking reaction leads to the formation of insoluble aggregates of proteins and is often described as a consequence of protein glycation. In addition, potential glycation sites (lysine residues) in the longest τ isoform are located in a tubulin binding motif of τ ; modification of these regions could result in decreased interaction of τ with microtubules.⁴⁷ Finally, AGE τ generate oxygen free radicals that could in turn induce oxidative stress, which might also contribute to the pathogenesis of disease, as in AD.⁴⁸

Tissue transglutaminase (TGase) is a calcium activated enzyme that catalyses the formation of bonds between glutamine residues and primary amines included in peptide bound lysine residues or polyamines. TGase crosslinks specific substrate proteins into insoluble, protease resistant, high molecular weight complexes.¹⁷ Because of this ability, TGase has been implicated in τ aggregation.⁴⁹ Indeed, a TGase induced crosslinking of τ proteins has been suggested to lead to NFT formation in AD⁵⁰ and PSP.⁵¹ In support of this concept, large amounts of isopeptide bond crosslinked τ have been found in the globus pallidus and pons in PSP.⁵² In assembling proteins to allow such crosslinking to occur, the presence of other amino acid residues near the amine donor sites capable of forming non-covalent bonds (such as arginine) may be important.⁵³

The mechanism of τ fibrillogenesis

FIBRILLOGENESIS IN VITRO

The microtubule binding domain of τ is not only important for microtubule assembly, it is also essential for τ self interactions to form polymers. Filaments can be generated in vitro by the incubation of τ fragments containing the microtubule binding domain. The formation of filaments is faster with fragmented τ than with full length τ .⁵⁴ Phosphorylation affects the potential of τ to form aggregates, producing either stimulatory or inhibitory effects, presumably depending on the site of phosphorylation. However, it is important to remember that most reported studies used τ proteins present at 3–5 mg/ml—a concentration much higher than that found in the cytoplasm under physiological conditions.³⁷

In vitro assembled τ filaments resemble paired helical filaments (PHFs) or SFs in morphology, depending on the experimental conditions and τ isoforms used: 4R τ isoforms tend to form SFs, as in PSP, whereas 3R τ isoforms form PHFs, characteristic of AD.³⁷ The process of assembly into PHFs can be accelerated by the presence of crosslinked τ , generated through oxidation at Cys 322 (located in repeat 3).⁵⁵ Intermolecular disulphide bridges involving the Cys 322 residue form between two τ proteins under oxidative conditions, and are probably important in its polymerisation into PHFs, indicating that τ is sensitive to the redox state of the cell and that phosphorylation may not be necessary for aggregate formation. Likewise, in the case of 4R τ constructs, repeats 2 and 3 may be linked via an intramolecular disulphide bridge involving the residues Cys 291 and Cys 322, respectively, allowing τ proteins to form compact monomers.¹⁷

MOLECULES STIMULATING τ FILAMENT FORMATION

The presence of 10–20 mmol/litre of arachidonic acid was found to stimulate τ (2–5 mmol/litre) to form SFs.⁵⁶ Incubating brain τ with TGase formed SFs of variable diameters.⁵⁷ In the presence of glycosaminoglycans or RNA, SF formation from 4R wild-type τ is enhanced. Of note, both glycosaminoglycans and RNA are polyanions that may be involved in forming dimers of τ through disulphide bridges at oxidised Cys 322 and Cys 291 residues in 4R τ isoforms.⁵⁸ Studies of chemically crosslinked, heparin treated τ indicate that heparin treatment induces conformational changes in the τ protein (heparin is polyanionic, it is an analogue of the glycosaminoglycan, heparan sulphate, but more highly charged).⁵⁹

Taken together, the in vitro data suggest that: (1) the competency of τ to form polymers and the morphology of these polymers can be affected by the presence of non- τ molecules; (2) the microtubule binding domain is important for the assembly of τ filaments; and (3) the formation of τ filaments requires conformational changes in τ .³⁷

Although several post-translational modifications of τ have been proposed to trigger the

aggregation, including phosphorylation, glycation, proteolysis, oxidation, or interaction with polyanions, their role in vivo has remained ambiguous. Therefore, Friedhoff *et al* used in vitro studies to map out the parameters that govern the assembly process of τ .⁵⁸ They found that even in oxidative conditions and in the presence of polyanions, which allowed the formation of τ dimers, the assembly of PHFs was extremely slow at typical concentrations of neuronal τ , implying that there was a rate limiting step ensuring that this process does not occur in normal healthy cells. Kinetic studies showed the rate limiting step to be a nucleation reaction, which controls the polymerisation of τ dimers.

Tau fibrillogenesis in pathological conditions

All reported in vitro studies of τ filament assembly have used a single τ isoform and have not included other cytoplasmic proteins. It remains to be determined whether molecules such as glycans, RNA, and fatty acids are effective initiators of τ polymerisation in the presence of multiple τ isoforms or other proteins. Even though the precise mechanisms are unclear, τ proteins obviously have a tendency to form pathological fibrils. Mutations of the τ gene in FTDP-17 favour this process by reducing fibrillar degradation or accelerating fibril formation⁵⁸; because mutations of τ have not been detected in PSP, it is most likely that τ is pathologically modified by post-translational processes. The best studied modification of τ is phosphorylation.

Abnormal phosphorylation of τ has been recognised as an early event in the development of neurofibrillary pathology, but several attempts to generate τ filaments in cultured cells expressing wild-type τ by manipulating the state of τ phosphorylation have been unsuccessful. Overexpression of τ in cells transfected with kinases did not lead to the accumulation of τ filaments, suggesting that phosphorylation alone is not sufficient to induce τ polymerisation.⁶⁰ Heparan sulphate immunoreactivity is present in NFTs from AD brains, making these molecules (or related ones) strong candidate inducers of τ filament formation.⁶¹ Apart from their inhibitory effect on microtubule assembly and stimulatory effect on τ filament assembly, heparan sulphate and other glycans may play a role in the pathogenesis of disease through their effect on protein phosphorylation and degradation. This is supported by in vitro studies in which heparan sulphate inhibited τ degradation by the calcium dependent protease calpain⁶² facilitated the phosphorylation of τ by glycogen synthase 3,⁶³ and altered the site specificity of cyclin dependent kinase 2-like kinase.⁶⁴ However, the role of heparan sulphate (if any) in the pathogenesis of PSP remains to be elucidated. TGase has been implicated in the formation of NFTs in PSP,⁵¹ and may play a crucial role in the aggregation of τ by altering its conformation, making digestion sites inaccessible to proteases such as calpain, and thereby promoting the resistance of abnormal filaments to proteolysis.¹⁷

In summary, immunochemical and histochemical studies have demonstrated the presence of molecules other than τ and glycans in NFTs.³⁷ Some of the molecules may serve to induce conformational change in τ and thus facilitate the formation of fibrillary inclusions. The identification and characterisation of factors essential for the initiation or facilitation of τ filament formation in disease conditions would be important for the development of therapeutic agents to interfere with the progression of tauopathies.

Mechanism of neuronal cell death in PSP

The pathogenesis of PSP is unknown but, as in many neurodegenerative diseases, a multifactorial causation is implicated. Lesions are found in neurones and glia but, as yet, the sequence of events leading to neurodegeneration remains to be elucidated, although many accept that the disruption of biochemical pathways as a result of the aggregation of abnormal τ filaments is a key event in the process, and several hypotheses have been put forward.⁶⁵

These include decreased neurotrophic factors promoting accelerated neuronal necrosis or apoptosis. It was proposed that abnormalities of mitochondrial ATP production would cause free radicals to accumulate.⁶⁶ Using a cybrid cell model (mitochondria from patients with PSP implanted into a mitochondrial deficient cell), complex I activity was reduced significantly, implying a role for oxidative stress in the aetiology of the disease.⁶⁷ It remains to be determined whether τ positive glia contribute to the pathogenesis or progression of disease. The formation of τ positive inclusions in glia may be an epiphenomenon or an adaptive or even protective phenomenon. The initiating insult that leads to increased or aberrant τ expression in glial cells is unclear. Nevertheless, the conformational change in τ and its subsequent accumulation may directly contribute to cellular death and trigger oxidative stress. These stresses may exaggerate neuronal cell damage.¹⁰

Several hypotheses have been proposed to explain the role of glial cells in neuronal cell death. First, they may play a role in complement mediated neurotoxicity. Numerous oligodendrocytes possessing the C3d, C4d, and amyloid P component epitopes have been identified in PSP, and termed complement activated oligodendroglia.¹⁰ They are present in relatively unaffected cortical areas and are considered to represent an early stage of degeneration. It is believed that the accumulation of abnormal τ protein may damage the cell bodies or processes of oligodendrocytes and activate the classical complement pathway, leading in turn to the activation of microglia (cells with a phagocytic role in response to neuronal injury). Such activation could lead to neurotoxicity. However, no direct evidence has been obtained to support the hypothesis.

A second hypothesis involves glutamate or free radical mediated neurotoxicity. The accumulation of abnormal τ may disrupt the excitatory amino acid transporter (EAAT), leading to insufficient uptake of extracellular glutamate.⁶⁸

The resulting glutamate excess activates calcium influx, leading to superoxide anion (O_2^-) production. Superoxide dismutase (SOD) scavenges O_2^- , but under certain conditions, the non-enzymatic reaction of O_2^- and nitric oxide (NO) produces peroxynitrite (ONOO⁻), which is highly cytotoxic.⁶⁹ Enhanced expression of nitric oxide synthase (NOS) in astrocytes may also cause excessive NO production, again leading to increased peroxynitrite production.¹⁰ SOD reacts with peroxynitrite to form a stable adduct, nitrotyrosine.⁶⁹ In PSP, the intensity of EAAT1 and EAAT2 immunoreactivity was significantly decreased in the subthalamic nucleus and globus pallidus.¹⁰ In addition, strong immunoreactivities to inducible NOS and manganese SOD (present in human mitochondria) are detected in τ positive astrocytes.⁷⁰ The inducible NOS positive cytoplasmic area is significantly greater than that in protoplasmic astrocytes in controls. Nitrotyrosine, which indicates the presence of peroxynitrite, has also been detected in glial cells and neurones in PSP.⁷⁰ Taken together, these findings indicate that τ positive astrocytes may exert neurotoxicity through the overproduction of NO in excess of the detoxification capability of SOD.

AGEs, potential mediators of cytotoxicity in neurodegeneration and aging, have been identified in senile plaques and NFTs in AD, and NFTs in PSP.¹⁰ Using antibodies against N-carboxymethyllysine and pentosidine, AGE immunoreactivity was also identified in astrocytes in PSP.¹⁰ AGE- τ are formed after the glycation of τ —a process that may be related to increased insolubility of τ proteins, decreased interaction of τ with tubulin, and the generation of oxygen free radicals—and contribute to oxidative stress. These observations lend support to previous studies indicating that oxidative stress contributes to neuronal cell death in neurodegenerative disorders.¹⁷

Conclusions

In PSP, an imbalance of τ isoforms or post-translational modifications of τ proteins may result in the accumulation of abnormal τ . The finding of a polymorphism in intron 9 of most white patients with PSP, and more recently, a silent functional mutation in exon 10, also lends support to this hypothesis. The initiating insult that leads to increased or aberrant τ expression in neuronal and glial cells is unclear; indeed, the answer to this may help explain the late onset of disease, which is characteristic of PSP. Tau proteins provide the microtubule with its identity and physical characteristics (rigidity, length, stability, and interactive capacity with other organelles). The ability of τ to regulate microtubule assembly also provides a fundamental role in modulating the functional organisation of the neurone, particularly axonal morphology, growth, and polarity. Conformational changes in τ and its subsequent accumulation may directly contribute to cellular death. Possible free radical mediated cytotoxicity after abnormal τ accumulation secondarily induces oxidative stress and may contribute to neuronal and glial cell

damage. Thus, not only τ aggregation but also secondary cytotoxic events may be appropriate targets for therapeutic intervention.

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