

Review Article

Tau Hyperphosphorylation and Oxidative Stress, a Critical Vicious Circle in Neurodegenerative Tauopathies?

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Abstract

Hyperphosphorylation and aggregation of the microtubule-associated protein tau in brain, are pathological hallmarks of a large family of neurodegenerative disorders, named tauopathies, which include Alzheimer’s disease. It has been shown that increased phosphorylation of tau destabilizes tau-microtubule interactions, leading to microtubule instability, transport defects along microtubules, and ultimately neuronal death. However, although mutations of the MAPT gene have been detected in familial early-onset tauopathies, causative events in the more frequent sporadic late-onset forms and relationships between tau hyperphosphorylation and neurodegeneration remain largely elusive. Oxidative stress is a further pathological hallmark of tauopathies, but its precise role in the disease process is poorly understood. Another open question is the source of reactive oxygen species, which induce oxidative stress in brain neurons. Mitochondria have been classically viewed as a major source for oxidative stress, but microglial cells were recently identified as reactive oxygen species producers in tauopathies. Here we review the complex relationships between tau pathology and oxidative stress, placing emphasis on (i) tau protein function, (ii) origin and consequences of reactive oxygen species production, and (iii) links between tau phosphorylation and oxidative stress. Further, we go on to discuss the hypothesis that tau hyperphosphorylation and oxidative stress are two key components of a vicious circle, crucial in neurodegenerative tauopathies.

1. Introduction

The tauopathies are a class of neurodegenerative disorders characterized by hyperphosphorylation and aggregation of the microtubule-associated protein tau (MAPT) into paired helical filaments (PHFs) or straight filaments (SFs), forming neurofibrillary tangles (NFTs) in brain. Unlike amyloid-beta (Aβ) aggregation, which is associated with Alzheimer’s disease (AD), tau tangles are found in multiple neurodegenerative disorders such as progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick’s disease, dementia pugilistica, frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), and many other disorders including AD [1]. Mutations in the MAPT gene have been linked with several familial early-onset tauopathies [1]. More than 50 pathogenic mutations have been identified in the MAPT gene [1], providing evidence that tau alterations alone can cause neurodegeneration. It has been shown that abnormal tau hyperphosphorylation impairs its binding to microtubules and its capacity to promote microtubule assembly, resulting in its self-aggregation into NFTs, microtubule disorganization, and impaired transport along axonal microtubules [2, 3].

In addition to tau hyperphosphorylation, a growing body of evidence suggests that oxidative stress (OS) is another component of the pathophysiology of tauopathies. According to the OS theory of aging, which was deduced from genetic studies showing that manipulation of antioxidant defenses affects longevity in several animal models, brain neurons are seen as a crucial target of oxidative attacks. Moreover, OS has been implicated in the disease process in several neurodegenerative disorders, including AD [4]. In AD, the link between the production of toxic Aβ peptide and OS is well documented and has been the subject of several recent
Here, after a brief summary of our knowledge of tau structure and function, we review evidence suggesting that OS is both a late consequence of tau pathology paralleling the course of the disease, and an early cellular response to injuries linked to tau toxicity. Lastly we discuss the hypothesis that tau hyperphosphorylation and OS are the two key elements of a vicious circle, crucial in tau pathology.

2. Structure and Posttranslational Modifications of the Tau Protein

Tau is a highly soluble, natively unfolded, and phosphorylated protein predominantly located in axons of mature neurons [7–9]. Tau is also found in the neuronal somatodendritic compartment [10] and nucleus [11], and to a lesser extent in astrocytes and oligodendrocytes [12]. Six tau isoforms are expressed in the CNS in human adults [13]. The six isoforms are generated via alternative splicing of a single MAPT gene located at 17q21.31 and comprising 16 exons [14]. Tau isoforms range in length from 352 to 441 amino acids, and from 45 to 65 kDa, with exons 1, 4, 5, 7, 9, 11, 12, and 13, translated in all tau isoforms. Tau isoforms differ by the presence of three (3R) or four (4R) carboxy-terminal tandem repeat sequences of 31 amino acids, corresponding to microtubule-binding domains (MBDs). Tau isoforms also differ by the absence (0N) or presence of one (1N) or two (2N) N-terminal repeated sequences encoded by alternative exons 2 and 3. The 3R- and 4R-tau isoforms are found in equal amounts in the adult human brain, while the 0N, 1N, and 2N tau isoforms comprise about 37%, 54%, and 9% of total tau, respectively [15, 16] (Figure 1).

The tau protein found in the PNS is named “big tau,” in reference to its high molecular weight compared with isoforms expressed in CNS neurons. This higher weight stems from the inclusion of exon 4a in the amino-terminal half of the protein [17]. Expression of different tau isoforms is tissue-specific [13] and dynamic during development, probably owing to its key role in cytoskeletal plasticity during embryogenesis and early development. In humans, only the shortest tau isoform (3R/0N) is expressed in fetal brain, whereas all six isoforms are found in adult CNS [18].

Posttranslational modifications are a major factor behind the diversity of tau. Besides phosphorylation, the crucial posttranslational modification in tau, this protein also undergoes an array of other posttranslational changes, such as O-glycosylation [19], ubiquitination [20], SUMOylation [21], nitration [22], glycation [23], acetylation [24], cross-linking by transglutaminase [25], isomerization [26], conformational alteration, and proteolytic cleavage [27], all of which are involved in tau regulation and pathology. O-GlcNAcylation (a form of tau O-glycosylation) reduced tau phosphorylation in cerebral cortex and hippocampus in rat [28], and in Alzheimer’s disease, O-GlcNAcylation levels negatively correlate with tau phosphorylation [29]. Tau acetylation at lysine residue 280 increased tau fibrillization and decreased tau-dependent microtubule assembly in vitro. Tau acetylation was also found to be strongly associated with tau hyperphosphorylation and tau inclusions in Alzheimer’s disease, corticobasal degeneration, progressive supranuclear palsy, and in tau transgenic mouse models of tauopathies [24, 30].
However, the precise functions of tau modifications remain enigmatic.

### 3. Functions of the Tau Protein

The main function recognized for tau is promoting microtubule polymerization and stabilization. Microtubules form part of the cytoskeletal framework in all eukaryotes and are composed mainly of heterodimers of α- and β-tubulin forming tubular polymers. Microtubules play a major role in cytoskeletal maintenance and act as highways for intracellular transport of organelles, vesicles, proteins, and signaling molecules [31].

The 4R-tau isoform displays the highest affinity for microtubules and efficiently promotes microtubule assembly, likely due to the presence of the interrepeat sequence located between the first and second MBDs [16]. Binding of tau to microtubules has been visualized by NMR spectroscopy [32] and cryo-EM [33]. However, some studies have challenged the primary function of tau as main regulator of microtubule stability and assembly. In cell culture, tau colocalizes with the most dynamic microtubules [9], those showing the highest basal turnover rate among microtubule populations [34]. Moreover, siRNA-mediated knockdown of tau does not impair microtubule polymerization [35]. In addition, tau, via its MBD, interacts with other proteins such as actin [36], presenilin-1 (PS-1) [37], tau phosphatases [38], RNA [39], and DNA [40], strongly suggesting that tau has multiple functions apart from its putative role as modulator of microtubule dynamics.

The N-terminal domain of tau interacts with Src homology 3 (SH3) domains in a number of signaling proteins [41], such as Fyn tyrosine kinase. Tau also promotes process extension in oligodendrocytes by connecting Fyn to microtubules [12]. Tau also stimulates Src-mediated actin rearrangements after growth factor stimulation [42]. Interestingly, the binding of tau to neuronal plasma membrane components and SH3 domains is affected by its phosphorylation [43, 44]. Other functions of tau include binding and activation of phospholipase C (PLC) γ [45], inhibition of the enzyme histone deacetylase-6 (HDAC-6) [46], modulation of the cellular response to heat shock [47], and adult neurogenesis [48]. Lastly, it was recently shown that the 2N/4R tau isoform plays a role in microglial activation in humans [49]. These data suggest that tau is a scaffolding protein able to (i) bind to at least two signaling proteins, (ii) restrict signaling molecules and transduction pathways to defined cellular compartments, and (iii) regulate several signaling pathways [50].

Notwithstanding these multiple hypothetical functions, the relationship between tau and microtubules is well established. In particular, tau appears to modulate axonal transport via interaction with motor proteins, such as detachment of kinesin from microtubules and directional reversal of dynein, without affecting the speed of transport [51–53]. High levels of tau accumulation are detected in the axonal region near the synapse [54], which may facilitate cargo delivery to presynaptic terminals [51]. Moreover, while several observations suggest that removal or overexpression of tau in vitro or in vivo does not impair axonal transport [55, 56], other studies suggest that overexpression of wild-type or mutant tau in either cell or mice models of taupathy impairs axonal transport [57, 58]. These discrepancies may be due to differences in study design or model systems used.

Four tau knockout (KO) lines have been independently generated in mice [59–62]. All four lines are viable and fertile, with no apparent phenotypes. Harada et al. [59] generated the first mouse tau KO strain. Apart from the absence of an overt phenotype, these mice showed reduced microtubule stability and mild disorganization of small-caliber axons. In another tau KO line, Dawson et al. [60] reported a delay in neuronal maturation in in vitro culture from the tau KO line. However, in both KO lines, microtubule-associated protein (MAP) 1A was found to be elevated during early developmental stages, suggesting that overexpression of MAP1A is able to compensate for the loss of tau. Interestingly, an increased accumulation of MAP1A was also reported in the third tau KO line, which was generated by Fujio et al. [62]. In addition, despite normal levels of MAP1B in tau KO mice generated by Harada et al. [59], cross-breeding of this line with MAP1B-deficient mice resulted in cerebral developmental defects and early lethality [63], suggesting a further functional overlap between tau and this MAP.

Precise investigations of tau KO mice generated by Harada et al. [59] revealed progressive behavioral impairments and motor deficits in older individuals. These phenotypes comprised muscle weakness, impaired balance, hyperactivity, and learning deficits from 12 months of age onward [64]. More recently, complex motor deficits linked to dopaminergic neuronal loss and increased iron accumulation in neurons in substantia nigra were reported in another tau KO line [65]. Tau KO mice also showed deficits in neuronal circuit formation as revealed by electrophysiology [66]; alongside these negative consequences, tau KO mice are more resistant to epileptogenesis, excitoxicity, and beta-amyloid toxicity [67, 68].

Tau overexpression in neuronal cell cultures results in an increased number of neurites per cell [69]. Moreover, while insertion of human MAPT gene in mice leading to expression of all six tau isoforms does not induce severe neuropathology [70], expression of this transgene in a mouse tau KO context resulted in tau hyperphosphorylation and accumulation of insoluble 3R tau [71]. These mice also develop neurodegeneration and age-dependent memory loss [72, 73]. By contrast, hind-limb clasping and spinal cord abnormalities accompanied with somatodendritic distribution of the human tau protein were detected in human 2N4R tau cDNA mouse, while endogenous mouse tau was present [70]. A transgenic line with KI insertion of human 2N4R tau cDNA in the first exon of the mouse MAPT gene displayed increased neuronal survival accompanied by better performance in a novel object recognition task [74]. Apart from different types of transgenes and the presence or absence of endogenous mouse tau yielding different phenotypes, the promoter type also profoundly affected the observed phenotypes. Severalfold overexpression of the shortest human tau isoform to endogenous mouse tau resulted in axonal degeneration and progressive motor weakness [75].
### Table I: Major pathogenic tau mutations.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Type</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P301L</td>
<td>Substitution</td>
<td>Neuronal impairment, NFT formation</td>
<td>Vogelsberg-Ragaglia, et al., 2000 [94]</td>
</tr>
<tr>
<td>P301S</td>
<td>Substitution</td>
<td>Tau hyperphosphorylation and aggregation</td>
<td>Bugiani, et al., 1999 [95]</td>
</tr>
<tr>
<td>ΔK280</td>
<td>Deletion</td>
<td>Tau aggregation and decreased microtubule affinity, neural loss</td>
<td>Vogelsberg-Ragaglia, et al., 2000 [94]</td>
</tr>
<tr>
<td>VPR triple mutant (R406W, P301L, and V337M)</td>
<td>Substitution</td>
<td>Tau hyperphosphorylation, aggregation, and decreased microtubule affinity</td>
<td>Tatebayashi, et al., 2002 [96]</td>
</tr>
<tr>
<td>N296N</td>
<td>Silent</td>
<td>Greater 4R to 3R ratio (splicing)</td>
<td>Spillantini, et al., 2000 [98]</td>
</tr>
</tbody>
</table>

The precise mechanism of hyperphosphorylated tau-mediated neurotoxicity remains to be elucidated, but two major mechanisms have emerged to date: (i) toxic loss of function, with loss of physiological tau function held responsible for tau pathology [76–78] and (ii) toxic gain of function with highly phosphorylated tau displaying ill-defined toxic effects in neuronal cells [72, 79, 80]. Interestingly, it was shown that hyperphosphorylated tau relocates to dendritic spines, where it may exert its toxic effect by impairing trafficking and/or synaptic anchoring of glutamate receptors [81].

More than 50 mutations of the MAPT gene have been identified to date that are associated with neurodegenerative tauopathies [1]. Table I lists the most important pathogenic tau mutations. Many of these mutations are missense mutations or small deletions, which modify tau sequence. In particular, several missense mutations such as G272V, P301L, P301S, V337M, G389R, and R406W decrease the in vitro affinity of tau to microtubules, resulting in deficits in microtubule assembly and stability [15, 82, 83]. By contrast, missense mutations S305N and Q336R slightly increase the ability of tau to promote microtubule assembly [84, 85]. In addition, most of these missense mutations enhance tau aggregation [86, 87]. Lastly, several mutations impair binding of tau to protein phosphatase 2A, one of the major tau phosphatases in brain neurons [88]. Other mutations are located in either intronic sequences close to the 5’ splice site of exon 10 (positions +3, +11, +12, +13, +14, +16, +19, and +29) or exonic sequences, also impairing exon 10 splicing (N279K, ΔK280, L284I, N296H, ΔN296, P301L, P301S, G303V, and S305N). All these mutations disrupt the physiological 1:1 ratio of 4R to 3R tau isoform in the adult human brain. In turn, the increased production of 4R tau leads to its assembly onto NFTs and ultimately neurodegeneration [89].

Other mutations in intronic sequences adjacent to the stem-loop structure in exon 10 increase accumulation of soluble 3R tau isoform through alteration of MAPT gene splicing. Interestingly, these mutations lead to neuronal apoptosis and increased accumulation of tau degradation products, but not the formation of NFTs [90]. Finally, several pathologic tau mutations are located in exon 10, such as ΔK280, ΔN296, and N296H, and induce tau protein accumulation and affect MAPT RNA levels [83, 91–93].

Although these results point to tau as a key factor essential for proper microtubule assembly and dynamics, they also highlight the complex and still poorly understood function of this protein.

### 4. Phosphorylation, Oligomerization, Aggregation, and Propagation of the Tau Protein

The activity of the phosphoprotein tau is mainly regulated by phosphorylation. Similar to the expression of different tau isoforms, tau phosphorylation is also developmentally regulated, which is important for cytoskeletal plasticity during early development. Tau phosphorylation also influences the structure, distribution, and function of the protein in neurons [99].

Tau phosphorylation is increased in both physiological and pathological contexts, but it is still unclear whether the mechanisms involved in physiological and pathological tau hyperphosphorylation overlap. Tau phosphorylation is markedly increased during embryonic development [100], likely due to the increased need for neuronal plasticity. Several cellular stress conditions such as OS [101–103], heat stress or hypothermia [103, 104], and even starvation [105] modulate tau phosphorylation. Hyperphosphorylated tau is also identified as the main component of NFTs [106]. High levels of hyperphosphorylated tau have been detected in the cerebrospinal fluid (CSF) of patients suffering from tauopathies. Levels of hyperphosphorylated tau in CSF also correlate with hippocampal atrophy in prodromal AD, also called “mild cognitive impairment” (MCI) [107, 108]. Tau is hyperphosphorylated in all tauopathies, but hyperphosphorylation states differ among and within disorders [109]. Importantly, no single phosphorylation site is specific for tauopathies, and hyperphosphorylation is characterized by an overall increase in tau phosphorylation at multiple residues. Also, tau hyperphosphorylation is defined as an increase in either the number of phosphorylated sites per tau molecule or the fraction of tau molecules phosphorylated at a given site. Thus 1.9 moles of phosphate per mole of tau is found in a healthy human brain, against 6 to 8 moles of phosphate per mole of tau in an AD brain [110]. However, the precise phosphorylation state of tau is difficult to define in postmortem biopsy material, due to the labile nature of phosphorylated tau, which quickly becomes dephosphorylated after excision [111].
Nearly 45 phosphorylated sites have been identified on tau extracted from AD brain, that is, more than half of all the 85 theoretical phosphorylatable residues on the longest tau isoform (2N4R) (Figure 2). This situation contrasts with the approximately 10 and 18 phosphorylated residues found on soluble tau extracted from adult human brain and fetal rat brain, respectively. Most hyperphosphorylated residues in AD are clustered in the C-terminal and the proline-rich domains of tau, and very few sites are located within the N-terminal region and MBD. Most phosphorylated sites have been identified by mass spectrometry or Edman degradation, and a few sites located mostly at the N-terminal region have been identified only by phosphospecific antibodies [112].

Tau hyperphosphorylation is thought to result from an imbalance in the function of several protein kinases and phosphatases [109]. While tau is phosphorylated by a large number of kinases in vitro [112], the identity of the kinases, which are responsible for physiological or pathological phosphorylation of tau in vivo, has so far remained elusive. In particular, as no single kinase is able to phosphorylate all pathological tau residues, several kinases may be involved in tau hyperphosphorylation. Tau kinases fall into the two main groups of proline-directed and non-proline-directed kinases. Proline-directed tau kinases GSK3β and CDK5 are important tau kinases that phosphorylate tau at a large number of serine and threonine residues and play an important role in tau pathology in AD [113, 114]. Furthermore, GSK3β activation and CDK5 overexpression were shown to induce tauopathy-related phenotypes in mouse models [115, 116]. Activity of nonreceptor tyrosine kinases (such as Fyn and c-Abl) have also been linked to AD pathology [117]. It has been proposed that tau phosphorylation by different kinases likely follows a sequential pattern, in which phosphorylation of given residues facilitates that of other phosphorylation sites. Such a sequential pattern of kinase activities has been suggested by in vitro observations of the effect of DYRK1A and CDK5 kinases on GSK3β-mediated tau phosphorylation [118, 119]. In addition, the activation of one kinase may activate a second

Figure 2: Schematic representation of the distribution of tau phosphorylation sites on the longest tau isoform (441 amino acids). The two amino-terminal inserts are demonstrated by E2 and E3. The microtubule-binding domains are represented with R(1–4). Physiologic tau phosphorylation comprises approximately 10 phosphorylated residues. The physiologically phosphorylated residues are shown in purple and cluster in the proline-rich domain (PRD) and in the C-terminal region. The number of phosphorylated residues rises to 45 on the longest tau isoform from Alzheimer brain with the appearance of phosphorylated residues shown in red. The two amino-terminal inserts and repeat regions are not physiologically phosphorylated in adult human brain. A number of phosphorylation sites are detected in both regions on the longest tau isoform from Alzheimer brain.
one through a “kinase cascade,” as what was observed by the stimulatory effect of CK1 and c-Abl kinases on CDK5 [120, 121].

Phosphatases dephosphorylating tau in vitro include PPI, PP2A, PP2B, and PP5 [122]. PP2A displays the strongest dephosphorylation effect on tau [123] and may be an important regulator of tau phosphorylation in vivo [124]. A decrease in PP2A activity mediates hyperphosphorylation of tau in hypothermia [104]. Studies also suggest that PP2A expression and activity are markedly decreased in AD [125, 126]. Furthermore, an AD-like pathology has also been observed secondary to treatment with okadaic acid, a potent inhibitor of phosphatases 1 and 2A in rats [127, 128].

Increased tau phosphorylation decreased its affinity for microtubules: pathologic phosphorylation of tau at Ser396 and Ser404 decreases its binding affinity for microtubules [129]. Similarly, phosphorylation at S214 and T241 in the proline-rich domain reduces the binding of tau to microtubules [2].

Aggregation-prone tau species display toxicity in cell culture [130] and in a transgenic mouse model [131]. However, whereas it was previously thought that aggregated tau was toxic, more recent studies suggest that soluble and prefibrillar tau species are more likely to be implicated in neurodegenerative processes [132]. In particular, several observations suggest that tau oligomers, but not tau aggregates, are the toxic species. In AD patients, neuronal loss exceeds NFT number by at least one order of magnitude [133], and some familial tau mutations associated with frontotemporal dementia cause very few aggregated tau inclusions [134]. Tau oligomers and truncated tau species display toxicity in vitro [135, 136]. Following expression of the aggregation-prone tauΔK280 construct in N2a neuroblastoma cells, the toxic tau species were identified as prefibrillar forms of tau before the β-sheet containing aggregates were detected [130]. Moreover, tau overexpression in several Drosophila models induces neurodegeneration without NFT formation [137, 138]. However, it must be borne in mind that the lifespan of Drosophila neurons is one order of magnitude lower than that of mammalian neuronal cells. Lastly, accumulation of soluble forms of the tau protein correlates with neuronal and synaptic dysfunction and toxicity in several mouse models [80, 81, 139], in two different conditional mouse models of tauopathies, transgene silencing resulted in memory recovery while NFTs were still present [80, 139].

In vitro, recombinant full-length and nonphosphorylated tau is able to form filaments following interaction with negatively charged compounds such as sulfated glycosaminoglycans, RNA, or fatty acids [39, 140–142]. Moreover, both wild-type and mutant tau bearing G272V, ΔK280, P301L, P301S, S305N, V337M, or R406W mutations do not form filaments in vitro in the absence of heparin or other negatively charged compounds. However, the exact mechanism by which heparin induces formation of tau fibrils remains a matter of debate.

Interestingly, in a given tauopathy, tau aggregation propagates in a sequential and predictable fashion from one brain region to another, in a manner similar to that described for prion proteins. This stereotypical spatiotemporal spreading of tau aggregation has been described in AD [143] and argyrophilic grain disease (AGD) [144], suggesting that tau pathology spreads along defined neuronal pathways. In vitro studies have demonstrated intercellular transfer of tau inclusions in cultured cells [145]. Sulfated glycosaminoglycans at the cell surface are required for internalization of aggregated tau [146]. This phenomenon has also been observed in vivo in mice expressing wild-type human 4R tau isoform, following injection of brainstem extracts from mice expressing human tauP301S [147]. Also, formation of tau inclusions is accelerated when filaments assembled from recombinant human tauP301S were injected into the brains of young tauP301S transgenic mice before the formation of tau aggregates [148]. In addition, transgenic mice overexpressing human tauP301L in restricted areas of the entorhinal cortex and subiculum showed a propagation of the pathology in other synaptically connected brain regions [149, 150]. The data suggest that filamentous tau can convert soluble tau to fibrillar tau, forming tau inclusions.

5. Neurodegenerative Tauopathies

Tauopathies are heterogeneous disorders with partially overlapping clinical, neuropathological, and genetic characteristics, forming a spectrum of disorders. These diseases are characterized by microtubule-associated tau protein abnormalities [1]. Tau inclusions occur in neurons, astrocytes, and oligodendrocytes [151, 152].

Intraneuronal tau inclusions display different morphological features in tauopathies, ranging from NFTs and neurofilamentous threads to dystrophic neurites and Pick bodies. Tau aggregates are also differentiated by the phosphorylation and isoform content of tau, allowing a molecular classification of tauopathies. A biochemical classification based on the electrophoretic profile of tau discriminates four distinct classes [153].

Class I tauopathies are characterized by three major electrophoretic tau isoforms at 60, 64, and 69 kDa and a minor isoform at 72/74 kDa. This profile corresponds to the aggregation of the six tau isoforms in the human brain and characterizes AD, Down syndrome, and several other tauopathies. Class II forms are characterized by two major electrophoretic isoforms at 64 and 69 kDa and a minor band at 74 kDa. This profile characterizes several tauopathies such as CBD, ADG, and PSP. The profile of Class III tauopathies displays two major bands at 60 and 64 kDa that correspond to tau isoforms lacking exon 10 encoded sequences (3R tau). This profile is found in Pick’s disease and FTD-17. Lastly, in Class IV tauopathies, a major band at 60 kDa and two minor bands at 64 and 69 kDa are found that correspond to tau isoforms devoid of sequences encoded by exons 2, 3, and 10. This class comprises type I and type II myotonic dystrophy.

While many familial tauopathies are caused by mutations in the MAPT gene [154], other forms are caused by specific environmental factors. In particular, tau aggregations are found in chronic traumatic encephalopathy (CTE), a neurodegenerative disease first described in boxers and termed dementia pugilistica. In this case, the etiology of the disease
is linked to repetitive blast and/or injury to the brain. CTE is characterized by progressive neurodegeneration with widespread deposition of hyperphosphorylated tau as NFTs.

Brain trauma is thought to induce the dissociation of tau from microtubules via mechanisms such as intracellular calcium influx, glutamate receptor-mediated excitotoxicity, and kinase activation, leading to hyperphosphorylation of intracellular tau [155–157]. In addition, a sporadic tauopathy, endemic to the island of Guadeloupe, has been linked to the consumption of annonacin, a naturally occurring toxin with mitochondrial complex I inhibitor properties [158]. Low nanomolar concentrations of the toxin have been shown to cause neurodegeneration and induce redistribution of tau from axons to the somatodendritic compartment in neuronal culture [159]. In vivo, the toxin causes an increase in both tau levels and tau phosphorylation. This toxin also increases neuronal somatodendritic accumulation of hyperphosphorylated tau in transgenic tauTg406W mice following a brief 3-day exposure [160].

Initial causative events and precise pathological processes remain largely elusive in tauopathies. Mutations in the MAPT gene have been identified in a large number of rare familial tauopathies, but environmental factors such as injuries and toxins, most of which remain to be identified, are also suspected to play a role in the far more frequent sporadic forms of the disease. In particular, multiple lines of evidence point to OS as an important agent in the pathophysiology of neurodegenerative tauopathies.

6. Oxidative Stress Is a Common Feature of the Pathophysiology of Neurodegenerative Diseases

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen, such as superoxide anion ($O_2^-$), hydroperoxyl radical (HO$_2$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (OH$^-$). Under normal conditions, cells permanently produce limited amounts of ROS by multiple biochemical processes. The "professional" ROS producers, such as mitochondria, peroxisomes, and endoplasmic reticulum, are the major sources. In mitochondria, ROS are produced permanently as a byproduct of ATP production by the electron transport chain. It is well documented that elevated levels of ROS are highly toxic to cells, in which they damage essential macromolecules, such as DNA, RNA, proteins, and membrane lipids. Importantly, it has been proposed that accumulation of ROS and oxidative damage on macromolecules contribute not only to the physiology of ageing, but also to the pathophysiology of many diseases, including various types of neurodegenerative disorders.

Cells have developed several strategies to manage ROS. In particular, they synthesize several enzymes that display antioxidant properties. Among this large family of antioxidant proteins are catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD), which protect against the damaging effects of reactive oxygen species (ROS). In particular, the chief defense against superoxide anion produced in the course of respiration is mitochondrial SOD2. However, ROS detoxification is not 100% efficient, and residual superoxides and peroxides persist in cells. In addition, it has been shown that microglial cells produce ROS, either in an attempt to eliminate pathogens or during the first steps of neuroinflammatory processes. Several recent studies have established a direct, albeit complex relationship between neurodegenerative diseases of the AD/tauopathy types and microglial activation [49, 161, 162]. However, this novel and very interesting field of investigation lies outside the scope of this review.

Thus while controlled amounts of ROS are permanently made as a byproduct of cellular metabolism and inflammatory processes under physiological conditions, a pool of antioxidant molecules is permanently produced to detoxify toxic oxygen species. In this context, oxidative stress refers to an imbalance between ROS levels and available antioxidant molecules. In particular, it is well established that sustained oxidative stress can be very damaging to cells, such conditions leading to cell apoptosis as the consequence of activation of the JNK pathway [163, 164].

Several lines of evidence suggest that the balance between ROS and antioxidant defenses is particularly fragile in brain neurons. More importantly, perturbations of this delicate equilibrium are suspected to play a crucial role in many neurodegenerative diseases, first in Alzheimer's disease and more recently in other tauopathies. We know that neuronal cells display unique features and properties: they are permanently postmitotic cells that also show high energy and oxygen consumption, suggesting an elevated rate of ROS production. Brain neurons also contain high levels of transition metals, which can adopt at least two oxidation states, including a reactive one. Iron and copper are tightly linked with the cellular redox state. Fe$^{2+}$ can generate the hydroxyl radical through the fenton reaction [165]. Moreover, the brain has low levels of antioxidants relative to other organs [166].

7. Brain Neurons Undergo Oxidative Stress in Tauopathies

A large body of evidence indicates that Aβ deposition in both AD patients and transgenic animal models is associated with an accumulation of OS markers. Similarly, 20 years ago, the first evidence for OS was detected in Pick’s disease and CBD patients. Increased levels of heme oxygenase-1 (HO-1), a putative marker of oxidative injury, were detected in Pick bodies in Pick's disease patients and neuropil threads and glial inclusions in patients with CBD [167]. More recently, OS was also found to be associated with tauopathies of the FTLD spectrum of disorders [168] and PSP types [169]. Several OS markers are notably increased in these tauopathies, such as malondialdehyde (MDA), which is formed following the reaction of ROS on polyunsaturated lipids, or 4-hydroxynonenal (HNE) and thiobarbituric acid reactive substances (TBARS) produced by peroxidation of intracellular lipids [168, 170, 171]. Oxidative damage of the glycolytic enzymes fructose bisphosphate aldolase A (aldolase A) and phosphoglycerate kinase-1 (PGK-1) was also detected in the frontal cortex in PSP cases [168].
In addition to OS markers, several studies have reported an activation of antioxidant defenses in several tauopathies. In particular, in several PSP patients, an increased level of SOD2 was detected in the subthalamic nucleus [172], and high levels of Cu/Zn-SOD were found in cerebrospinal fluid [173]. Interestingly, increased levels of SOD, Glutathione peroxidase (GPx), and HNE-conjugated GPx were found in PSP patients that correlated positively with age and thus disease progression [173].

The overexpression of both OS markers and antioxidant enzymes in both patients with different types of tauopathies and animal models strongly suggests that OS is a key component of the pathophysiology of these diseases. However, these data also raise the important question of the role of OS in tau pathologies, a fundamental issue for the development of efficient therapeutic strategies. In other words, is OS an early causal factor in the pathophysiological process or is it merely a consequence of the different cell injuries induced by tau hyperphosphorylation.

8. Oxidative Stress, an Early Marker of Tauopathies

Several studies performed on various cellular or animal models of tauopathies have established that overexpression of mutant forms of human tau underlying various types of dominantly inherited tauopathies increases both the expression of OS markers and the sensitivity of neurons to oxidant stress of wild-type tau in N2a neuroblastoma cells, an increased production of OS markers and the sensitivity of neurons to oxidant stress in tauR406W individuals, correlating with the age-specific increase in susceptibility to ROS, resulting in a dramatic increase in mortality upon exposure [178]. These findings suggest that truncation of tau is an early event that precedes OS in this model.

OS and mitochondrial dysfunction accompanied by behavioral deficits were detected prior to tau hyperphosphorylation and NFT accumulation in transgenic mice expressing human tauP301S protein, which underlies another autosomal dominant form of FTDP-17 [177]. In this model, a decreased expression of MnSOD was detected from 7 months of age onward, while tau hyperphosphorylation and tangle formation were only detected 3 months later and were associated with GSK3β activation. In these mice also, at 7 and 10 months of age, elevated protein carbonyl levels (an indicator of protein oxidation) were detected in the cerebral cortex, suggesting that ROS production occurs early in the disease process. Importantly, while markers of lipid peroxidation and protein oxidation were markedly elevated at 12 and 24 months of age, ROS levels in isolated brain cortex mitochondria were only mildly increased at the same stages. This result indicates that mitochondria are not the major source of ROS in these transgenic tauP301S mice [177].

In a recent study, several lines of evidence suggest that OS is a primary and causal player in the neurotoxicity induced by tau mutations, through induction of both apoptosis and dysregulated cell cycle activation [179]. First, using a Drosophila model that overexpresses human mutant tauR406W associated with FTD, Dias-Santagata et al. [179] showed that genetic downregulation of the antioxidant enzymes SOD2 or thioredoxin reductase (Trxr) increases neurodegenerative phenotypes induced by tauR406W expression. In close agreement with these data, treatment of tauR406W flies with 30 mM paraquat leads to an increased mortality rate, underlining the elevated sensitivity of these flies to OS. Next, using a transgenic line expressing another tau construct, designated tauE14, in which 14 S/T phosphorylation sites were mutated to glutamate to mimic phosphorylation, it was shown that flies overexpressing tauE14 and heterozygous for either Sod2Δ14 or TrxrΔ14 mutations displayed an increased number of apoptotic cells. Importantly, in these flies, levels of tau hyperphosphorylation were not modified by the downregulation of the antioxidant enzymes. These results clearly show that heterozygous Sod or Trxr mutations enhance the toxicity of tauE14 independently of tau phosphorylation levels. As the JNK signaling pathway is one of the best characterized responses to oxidative damage [163, 180], the authors investigated JNK activation in tauE14 individuals and tauE14 flies heterozygous for either Sod2Δ14 or TrxrΔ14. Interestingly, compared with levels seen in tauE14 individuals, a 6- or 9-fold increased activation of the JNK pathway was detected in tauE14 flies heterozygous for either Sod2Δ14 or TrxrΔ14, respectively, suggesting that JNK pathway activity induced by ROS leads to induction of apoptosis. Moreover, in these flies, activation of the JNK pathway was only detected in brain neurons, the sole brain cell type showing significant levels of apoptosis. Finally, while proliferating brain neurons are extremely uncommon in adult wild-type flies, approximately 40 and 100 PCNA and phosphorylated histone 3 (PH3) expressing neurons were detected, in tauR406W individuals.
and tauR406W flies heterozygous for either Sod2n283 or TrxrA1, respectively. Thus this study identified both activation of JNK pathway-mediated apoptosis and cell cycle dysregulation as pathogenic processes directly induced by ROS produced as a consequence of the pathophysiological processes induced by tau hyperphosphorylation.

Altogether, these studies identify OS as an early cellular dysfunction in the pathophysiology of tauopathies, which induces both JNK-mediated apoptosis and a dysregulated cell cycle in brain neurons. These data also suggest that either tau hyperphosphorylation per se, microtubule disorganization, or some other not yet identified consequences of abnormal tau accumulation promote ROS production and thereby OS in brain neurons. However, the source of ROS in the different models used, that is, mitochondria versus other producers such as microglia, and the precise role of oxidant molecules in the pathologic processes are two key issues that remain unresolved.

9. Oxidative Stress Can Promote Tau Hyperphosphorylation and Aggregation

Although the results described above clearly establish that expression of tau mutants underlying tauopathies in either human patients or animal models induces OS in neurons, another line of evidence also suggests that accumulation of ROS can directly stimulate tau hyperphosphorylation and aggregation.

Several studies showed that OS leads to increased tau phosphorylation in neuronal cultures [181, 182]. Moreover, carbonyl-4HNE facilitates aggregation of phosphorylated tau in vitro [183] and induces tau hyperphosphorylation [184, 185]. Oxidative events also produce oxidized fatty acids, which have been shown to stimulate tau polymerization in vitro [186]. Moreover, in mice deficient in mitochondrial SOD2, an increased tau hyperphosphorylation parallels mitochondrial dysfunction and OS [187]. More recently, because folate deficiency has been linked to neurological disorders [188], a transgenic zebrafish line deficient in folate was produced through overexpression of γ-glutamylhydrolase (γ-GH), an enzyme that converts active polyglutamyl folates into inactive monoglutamyl folates. Interestingly, this zebrafish transgenic line develops OS associated with tau hyperphosphorylation and aggregation and Aβ plaque formation [189].

However, the mechanism by which OS affects tau phosphorylation remains controversial. In rabbits, clear evidence of mitochondrial injury, OS, increased levels of tau phosphorylation and nuclear translocation of GSK3β was observed following intracisternal injection of aggregated Aβ42. Moreover, in the same model, treatment with lithium chloride (LiCl), an inhibitor of GSK3β, did not prevent mitochondrial DNA damage or tau hyperphosphorylation, suggesting that the translocation of GSK3β may represent an additional event unrelated to tau hyperphosphorylation [190]. GSK3β has been identified as one of the major serine/threonine kinases involved in tau phosphorylation/hyperphosphorylation. It was shown that treatment of primary rat cortical neuron cultures with cuprizone, a copper chelator, in combination with oxidant agents Fe2+ and H2O2, significantly increased GSK3β activity and pathologic tau hyperphosphorylation. By contrast, concomitant treatment of these cultures with LiCl significantly decreased GSK3β activity and reduced abnormal tau phosphorylation, identifying GSK3β as the kinase involved in tau phosphorylation following OS conditions in this model [191]. More recently, it was shown that, in neuronal PC12 cells cultured with 100 μM H2O2, treatment with low doses of GSK3β inhibitors protected the cells against H2O2-induced OS and apoptosis. By contrast, higher concentrations of GSK3β inhibitors induce opposite effects relative to apoptosis and tau phosphorylation, demonstrating the key role, albeit ill defined, of this kinase in the disease process. Taken together, these data suggest that GSK3β plays important roles in tau pathologies, and fine modulations of its activity may prevent apoptosis, as well as tau phosphorylation, induced by OS [192].

However, beside GSK3β, OS affects other signaling pathways and/or kinases mediating tau hyperphosphorylation. In particular, several tau kinases belong to the family of stress-activated protein kinases, which are activated in response to OS [193, 194]. In particular, it has also been shown that HNE directly activates two members of the stress-activated kinase family, JNK and p38, in NT2 neuronal cells [195].

Another possible link between OS and pathologic tau phosphorylation is peptidyl prolyl cis-trans isomerase 1 (PPIase) or Pin1. It has been shown that this enzyme is significantly downregulated and oxidized in AD hippocampus. Because Pin1 has been implicated in dephosphorylation of tau protein, it can be hypothesized that in vivo oxidative modifications of Pin1, as found in AD hippocampus, reduce Pin1 activity, leading to increased tau phosphorylation [196].

Insulin may also play a role in OS-induced tau phosphorylation. First, it has been established that OS conditions lead to decreased insulin secretion and sensitivity [197, 198]. Second, while insulin is highly sensitive to OS, it plays an important regulatory role in tau phosphorylation in neuronal cell cultures [199, 200]. In addition, insulin levels in mice lead to tau hyperphosphorylation in brain neurons [201, 202].

Thus, whereas accumulation of toxic hyperphosphorylated tau species has been shown to stimulate the production of ROS and thus OS conditions, these data strongly suggest that, in turn, OS directly promotes tau hyperphosphorylation. In this context, tau hyperphosphorylation and OS appear as two elements of a crucial “vicious circle” leading to a progressive coordinated increase in both ROS and abnormal tau and ultimately to cell death.

10. Antioxidants in Therapeutic Approaches

Following up the OS theories of aging and neurodegeneration, several antioxidant substances have been tested in different models of tauopathies, showing interesting therapeutic properties at least in these models. In mice overexpressing the smallest human tau isoform, which develop age-dependent filamentous tau inclusions accompanied by neuronal loss and behavioral alterations, dietary supplementation of vitamin E reduced mortality, decreased the number of tau-containing inclusions in the spinal cord, and improved behavioral
phenotypes [203]. In another study using a cell model of tauopathy induced by expression of a truncated tau fragment, treatment with the antioxidants vitamin C or vitamin E significantly decreased ROS production [204]. Curcumin, a naturally occurring substance found in turmeric (Curcuma longa), is another antioxidant displaying an interesting therapeutic potential. Treatment with curcumin decreased Aβ-induced tau hyperphosphorylation in PC12 cells [205] and okadaic acid-induced ROS production and tau hyperphosphorylation in mice [206]. In addition, in mice lacking superoxide dismutase, antioxidant treatment with catalytic antioxidant EUK189 decreases accumulation of OS markers [207]. Similarly, after chronic treatment with EUK-207 (a superoxide dismutase/catalase mimetic) from 4 to 9 months of age, that is, before the onset of symptoms, markedly reduced OS suggests that these events are two key components of a vicious circle that plays a crucial role in the pathologic relationship between intracellular ROS and tau hyperphosphorylation [209]. Lastly, treatment with vitamin E or overexpression of antioxidant enzyme thioredoxin peroxidase markedly ameliorated the neurodegenerative phenotype of tau R406W transgenic flies [179].

Although much evidence points to antioxidant substances as potential therapeutic agents for the treatment of neurodegenerative diseases of the AD and tauopathy types, translation of the results obtained in animal models into clinical therapeutic strategies has not yet led to significant advances. Hence a better understanding of the role of OS in these diseases is essential, and antioxidant strategies hold promise for slowing down or halting the progression of neurodegenerative tauopathies.

11. Conclusion

Both OS and tau hyperphosphorylation appear as key elements in the pathophysiology of tauopathies. However, the relationship between intracellular ROS and tau hyperphosphorylation remains unclear. Accumulation of hyperphosphorylated tau has been shown to cause OS, but ROS have also been shown to stimulate tau hyperphosphorylation. In this context, a better understanding of the role of OS in these pathologies may serve primarily to define novel markers of early stages of the disease and then to develop therapeutic strategies to attenuate, halt, or reverse disease progression. In addition, close interplay between tau hyperphosphorylation and OS suggests that these events are two key components of a vicious circle that plays a crucial role in the pathologic process in tau pathologies, including AD.

Conflicts of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References


43. C. H. Reynolds, C. J. Garwood, S. Wray et al., “Phosphorylation regulates tau interactions with Src homology 3 domains of phosphatidylinositol 3-kinase, phospholipase Cγ1, Grb2, and


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