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Brain-penetrant microtubule-stabilizing compounds as potential therapeutic agents for tauopathies

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Abstract

Neurons within the brains of those with AD (Alzheimer's disease) and related neurodegenerative disorders, collectively termed 'tauopathies', contain fibrillar inclusions composed of hyperphosphorylated tau protein. Tau is normally enriched in axons, where it binds and stabilizes MTs (microtubules). Tau hyperphosphorylation and aggregation probably result in reduced MT binding that could affect axonal transport and neuronal function. A possible therapeutic strategy to overcome a loss of tau function in tauopathies is administration of MT-stabilizing agents, such as those used in the treatment of cancer. However, these drugs elicit severe side effects, and most existing MT-stabilizing compounds have poor BBB (blood–brain barrier) permeability, which renders them unsuitable for tauopathy treatment. We identified EpoD (epothilone D) as a brain-penetrant MT-stabilizing agent with preferred pharmacokinetic and pharmacodynamic properties. EpoD was evaluated for its ability to compensate for tau loss-of-function in an established Tg (transgenic) mouse model, using both preventative and interventional dosing paradigms. EpoD at doses much lower than previously used in human cancer patients caused improved axonal MT density and decreased axonal dystrophy in the tau Tg mice, leading to an alleviation of cognitive deficits. Moreover, EpoD reduced the extent of tau pathology in aged tau Tg mice. Importantly, no adverse side effects were observed in the EpoD-treated mice. These results suggest that EpoD might be a viable drug candidate for the treatment of AD and related tauopathies.

Keywords

Alzheimer's disease; axon; microtubule; tauopathy; therapeutic agent

Introduction

The neurons within the brains of patients with many neurodegenerative diseases, including AD (Alzheimer's disease), FTLD (frontotemporal lobar degeneration), PSP (progressive supranuclear palsy), Pick's disease and CBD (corticobasal degeneration), contain insoluble fibrillar aggregates of hyperphosphorylated tau protein [1,2]. The tau inclusions in these various 'tauopathies' reside either within the neuronal soma as NFTs (neurofibrillary tangles) or within neuritic processes as neuropil threads. As discussed in greater detail

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below, tau is normally a highly soluble protein that is enriched within axons, where it binds and stabilizes MTs (microtubules) [3–6]. The common occurrence of intracellular tau deposits in the various tauopathies suggests that misfolded tau causes neuronal dysfunction and damage. In fact, there is a strong correlation between NFT burden in the brain and cognitive deterioration in AD [7–9], and a familial form of FTLD is caused by mutations in the tau gene [10,11]. More recent genome-wide association studies reveal that SNPs (single nucleotide polymorphisms) within the tau gene are associated with an increased risk of Parkinson's disease [12,13], a condition where tau inclusions can be found in addition to the more common Lewy bodies [14,15]. Tau-mediated neurodegeneration probably results from a gain-of-function toxicity associated with one or more misfolded tau species [2,16], and/or a loss-of-function as a consequence of a decrease in tau interaction with MTs due to its hyperphosphorylation and sequestration in aggregates [17]. In the present paper, we examine the evidence supporting a tau loss-of-function hypothesis, and review recent data which suggest that a viable pharmacological approach might be compensation of impaired tau function with MT-stabilizing agents.

Tau functions

There are six isoforms of tau in adult humans that are generated through alternative splicing of three exons within the tau transcript [18–20]. The inclusion or exclusion of exon 10 results in tau with four (4-R tau) or three (3-R tau) MT-binding motifs, respectively, with the ratio of 4-R/3-R tau being ~1 in healthy humans [11]. The additional MT-binding domain of the 4-R tau isoforms results in greater avidity for MTs than is observed with 3-R tau species [21], and thus an imbalance of the normal 4-R/3-R tau ratio could affect MT dynamics. In AD, the insoluble tau found within NFTs consists of both 4-R and 3-R tau. Interestingly, in Pick's disease, the tau inclusions are composed nearly exclusively of 3-R tau, whereas the NFTs in CBD and PSP contain predominantly 4-R tau [22]. The reason for the differences of tau composition in inclusions from these various tauopathies is not understood, but may provide clues to unique aetiologies among these neurodegenerative diseases.

Although tau isoform composition can differ within NFTs from the different tauopathies, the tau within these inclusions is invariably hyperphosphorylated [23–25]. This increased phosphorylation generally decreases the binding of tau to MTs [26–28], and could contribute to a loss of tau function. In addition, tau hyperphosphorylation at some sites can enhance fibrillization [29,30], and thus could increase the rate of NFT and neuropil thread formation. As the primary function attributed to tau is MT stabilization within axons, a loss of tau function could result in axonal MT destabilization and a consequent impairment of the axonal transport that is critical for the proper trafficking of cellular materials. This has been demonstrated in a neuronal culture system, where the induction of tau hyperphosphorylation resulted in a diminution of stable MTs [27], whereas *in vitro* studies show that dephosphorylation of pathological tau isolated from AD brains restores the ability of tau to bind to MTs [31]. In addition to stabilizing MTs, tau may also modulate FAT (fast axonal transport) along MTs, as overexpression of tau in neuronal culture systems decreased the binding of motor proteins to MTs [32–34], thereby affecting FAT. It has been suggested that a distal axonal localization of MT-bound tau normally facilities kinesin disengagement, so that cargo undergoing anterograde transport is released at axon terminals. Thus the somato-dendritic relocalization of tau that is observed in tauopathies might cause an increase in tau bound to MTs in the proximal axon, such that kinesin cannot initially engage with MTs [33]. Although this possibility merits further investigation, it should be noted that the studies supporting a role of tau in regulating MT motor function have only been conducted *in vitro* under conditions of tau overexpression, and thus it remains to be determined whether tau affects kinesin-mediated FAT under the normal levels of expression that typically occur in human tauopathies.

In addition to interacting with MTs in axons, it has been suggested that tau has a potential dendritic role in which it assists in the postsynaptic targeting of the tyrosine kinase fyn [35]. This tau–fyn interaction could be disrupted by an N-terminal fragment of tau, resulting in reduced postsynaptic fyn localization. One consequence of this diminished fyn trafficking was decreased phosphorylation of the NR2b subunit of the NMDA (*N*-methyl-D-aspartate) receptor, with a resulting reduction of NR2b interaction with the PSD-95 (postsynaptic density 95) protein that led to diminished NR2b anchoring at the postsynaptic density [35]. Interestingly, a diminution of NR2b-PSD-95 binding appeared to prevent memory deficits and lethality associated with APP (amyloid precursor protein) overexpression and Aβ (amyloid β-peptide) production in Tg (transgenic) mice. This proposed dendritic function of tau thus provides a potential explanation for the observation that reducing endogenous tau levels led to an improvement of Aβ-induced memory deficits in an APP Tg mouse model [36].

Tau loss-of-function

The tau loss-of-function hypothesis is supported by studies which have examined MTs in AD brain. For example, there is a reduction in MT number and MT length in AD pyramidal neurons [37], and although this was not correlated with the presence of NFTs, the presence of tau pathology at sites distal to the perikarya in the axons and dendrites of affected pyramidal neurons could account for these MT abnormalities. Alternatively, the suggested absence of a direct relationship between NFT deposition and MT alterations in AD might suggest that it is predominantly tau hyperphosphorylation that affects MT engagement and stabilization, rather than tau sequestration into NFTs. This interpretation is consistent with the observation that an induction of tau hyperphosphorylation in NT2N cells, through the inhibition of protein phosphatases, led to an impairment of tau binding to MTs with resulting MT depolymerization and axonal degeneration [27]. However, another study revealed a decrease in stable MTs that correlated with the presence of NFTs within neurons in AD brain [38], and the accumulation of pathological tau in the AD brain is associated with a reciprocal depletion of normal tau when affected versus unaffected regions of the AD brain are compared [39]. Thus it is possible and perhaps likely that both tau hyperphosphorylation and sequestration within insoluble inclusions contribute to a loss of MT stabilization in AD brain.

Similar reductions of MT density have been observed in Tg mouse models of tauopathy. For example, 12-month-old T44 tau Tg mice, which express the smallest human isoform of 3-R tau and accumulate insoluble, hyperphosphorylated tau inclusions primarily within the spinal cord and brainstem, have decreased MT density in spinal ventral root axons [40]. These same mice also demonstrate impaired FAT, which is consistent with a loss of MT function [41]. Similarly, PS19 Tg mice expressing 4-R tau with the P301S mutation found in inherited FTLD have reduced MT density in the optic nerve that coincides with the development of forebrain tau pathology [42], and recent data reveal that these mice also have deficient FAT [43]. Finally, it has been demonstrated that human 3-R tau expressed in *Drosophila* motor neurons is hyperphosphorylated and has reduced MT-binding. Interestingly, this hyperphosphorylated human tau also appears to sequester the endogenous *Drosophila* tau, further contributing to MT destabilization [44].

Compensating for tau loss-of-function

The idea that it might be possible to compensate for diminished tau binding to MTs with existing MT-stabilizing drugs, such as paclitaxel (Figure 1), was first hypothesized nearly two decades ago [17]. However, this concept was not tested until 2005, when the T44 3-R tau Tg mice described above, which develop tau inclusions in the spinal cord and brainstem

[40], were treated weekly with paclitaxel for a 3-month period [41]. The Tg mice dosed with paclitaxel showed improvement of MT density and FAT in ventral root axons, as well as improved motor performance, relative to Tg mice that received vehicle only. These data provided the initial proof-of-principle that it is possible to overcome a loss of tau function with a small molecule. However, paclitaxel does not readily cross the BBB (blood–brain barrier) and thus is not suitable for the treatment of human tauopathies, where tau pathology is found primarily in the higher learning centres of the telencephalon. Although our laboratory and others have attempted to develop taxanes with improved BBB permeability [45–47], these efforts have been largely unsuccessful. More recently, we have identified the epothilone class of MT-stabilizing agents as being generally brain-penetrant, and among this family of compounds, EpoD (epothilone D; Figure 1) was found to have preferred pharmacokinetic and pharmacodynamic properties [48]. This led to the evaluation of EpoD in the previously mentioned PS19 tau Tg mice, which develop tau pathology in the forebrain with age. Asymptomatic 3-month-old PS19 mice received weekly intraperitoneal administrations of 1 or 3 mg/kg EpoD until 6 months of age [42], a time point at which these mice normally begin to develop detectable tau pathology. Whereas vehicle-treated PS19 mice had reduced MT density and increased axonal dystrophy relative to non-Tg littermates, those PS19 mice that received either the high or low dose of EpoD showed a normalization of these endpoints. Moreover, spatial learning deficits that were observed in the vehicle-treated 6-month-old PS19 mice were largely corrected by EpoD treatment. Importantly, the beneficial effects of EpoD were achieved at doses that were 1/30 and 1/10 of the dose utilized in a Phase II clinical trial with cancer patients [49]. There was an absence of any observable side effects in mice that received these low doses of EpoD, including neutropenia and sensory nerve neuropathy, which are the primary side effects observed in patients treated with MT-stabilizing agents [50,51].

The ability of EpoD to safely attenuate axonal deficits and related cognitive impairments in PS19 mice that are developing tau pathology suggests that this compound might have potential as a primary or secondary prevention treatment for AD and related tauopathies. However, tauopathy patients will almost certainly have existing tau pathology in their brains by the time they present with clinical symptoms, and thus a more rigorous proof that EpoD has potential as an interventional therapeutic for patients with clinically manifest disease required demonstration of efficacy in a tauopathy model with pre-existing pathology. To this end, we recently completed an evaluation of EpoD in PS19 mice in which the animals received weekly dosing of 0.3 or 1 mg/kg of EpoD from 9 to 12 months of age [43], as 9-month-old PS19 mice have appreciable tau pathology. Notably, both doses of the MT-stabilizing compound reduced axonal dystrophy in these older Tg mice, with a resulting normalization of impaired FAT. Moreover, the EpoD-treated 12-month-old PS19 mice showed an improvement of both working and spatial memory performance, with the higher dose of the compound eliciting the greatest change. Somewhat unexpectedly, the extent of tau pathology in the brains of the aged PS19 mice was reduced by EpoD, as is evident from a reduction in forebrain phospho-tau and misfolded tau as detected by immunohistochemical staining. Moreover, the EpoD-treated PS19 mice had a lower amount of insoluble tau within the brain as determined by ELISA quantification. Importantly, EpoD treatment resulted in diminution of the hippocampal neuron and synapse loss that is normally observed in the aged PS19 mice as they accumulate increasing amounts of tau pathology [52]. Notably, no adverse effects of EpoD were observed in the interventional study with aged PS19 mice, which is highly significant for AD and related tauopathies since intervention therapy with EpoD or a related MT-stabilizing agent will probably require chronic administration of the drug for months to years. Thus these recent results provide encouragement that EpoD might be suitable for testing in human tauopathy patients.

Conclusions and future directions

The aforementioned studies which demonstrated that paclitaxel improved axonal and motor outcomes in a tau Tg mouse model with spinal cord pathology [41], and that the brain-penetrant MT-stabilizing agent EpoD was safe and efficacious in prevention [42] and intervention [43] studies in PS19 tau Tg mice with forebrain pathology, provide compelling evidence that this therapeutic approach holds promise for the treatment of tauopathies. In addition to these small-molecule MT-stabilizing agents, there is also evidence that the peptidic agent NAP, which has been suggested to act via stabilization of MTs, improved outcomes in AD-like Tg models [53,54]. The most parsimonious explanation for the beneficial effects of MT-stabilizing agents in these models is that they compensated for a deficiency of tau-mediated stabilization of MTs. However, it is also possible that the improvement of FAT and cognitive performance observed with the small-molecule compounds resulted, in part, from displacement of mislocalized tau from axonal MTs. There is evidence that paclitaxel can compete with tau binding to MTs [55,56], and EpoD and paclitaxel have a shared binding site on MTs [57]. Thus these agents could inhibit tau interaction with MTs, perhaps resulting in greater kinesin binding to proximal MTs in neurons with somato-dendritic tau, leading to improved FAT [33].

The hypothesis that a known MT-stabilizing agent such as EpoD might overcome a loss of tau function is based on the role that tau normally plays in facilitating MT stabilization. However, the observed reduction of tau pathology after EpoD treatment of aged PS19 tau Tg mice was unexpected, as it is not obvious that an improvement of MT stabilization should result in a decrease in insoluble and hyperphosphorylated tau. Although we do not have a mechanistic explanation for the EpoD-mediated reduction of misfolded tau, it has been demonstrated that tau pathology can be exacerbated when tau Tg mice are genetically crossed with mice that have altered FAT due to knockout of the kinesin light chain [58]. This observation would suggest that a deficiency in anterograde FAT leads to increased tau phosphorylation and misfolding [59]. Consequently, a vicious cycle may ensue, whereby the initial development of hyperphosphorylated and aggregated tau leads to diminished FAT, which then causes further development of tau pathology. This feed-forward mechanism could be ameliorated by an agent such as EpoD that normalizes FAT, which could then prevent development of tau pathology.

Although our results reveal that EpoD proved to be both safe and efficacious when administered to PS19 tau Tg mice, either in a preventative study design or in an interventional paradigm, the establishment of a reasonable projected therapeutic index (i.e., efficacy dose relative to the dose at which side effects are observed) will be paramount to the advancement of such compounds into clinical trials with tauopathy patients. As noted, MT-stabilizing drugs are known to elicit fairly severe side effects at the doses that are utilized for the treatment of cancer [49,51]. These dose-limiting side effects will probably be more noticeable in an aged population, such as AD patients. Moreover, whereas MT-stabilizing drugs are used for a fairly short period in an oncology setting, it is possible that tauopathy patients would require regular dosing for years. Thus it will be important to examine how well EpoD or comparable compounds are tolerated in humans upon repeated administration at doses similar to those that were efficacious in tau Tg mice (e.g. 1/30–1/100 of the doses used in cancer patients).

Finally, although EpoD appears to have a unique set of pharmacokinetic properties, including prolonged brain retention [42,48], that may make it a potential drug candidate for the treatment of tauopathies, it would be desirable to identify additional MT-stabilizing agents that have similar or improved features. To date, nearly all MT-stabilizing small molecules have been identified from natural products, and have relatively complex

structures that are difficult to synthesize. The identification of novel, brain-penetrant MT-stabilizing agents that are more amenable to synthetic chemistry would thus be of significant interest.

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Abbreviations used

Aβ	amyloid β-peptide
AD	Alzheimer's disease
APP	amyloid precursor protein
BBB	blood–brain barrier
CBD	corticobasal degeneration
EpoD	epothilone D
FAT	fast axonal transport
FTLD	frontotemporal lobar degeneration
MT	microtubule
NFT	neurofibrillary tangle
PSD-95	postsynaptic density 95
PSP	progressive supranuclear palsy
Tg	transgenic

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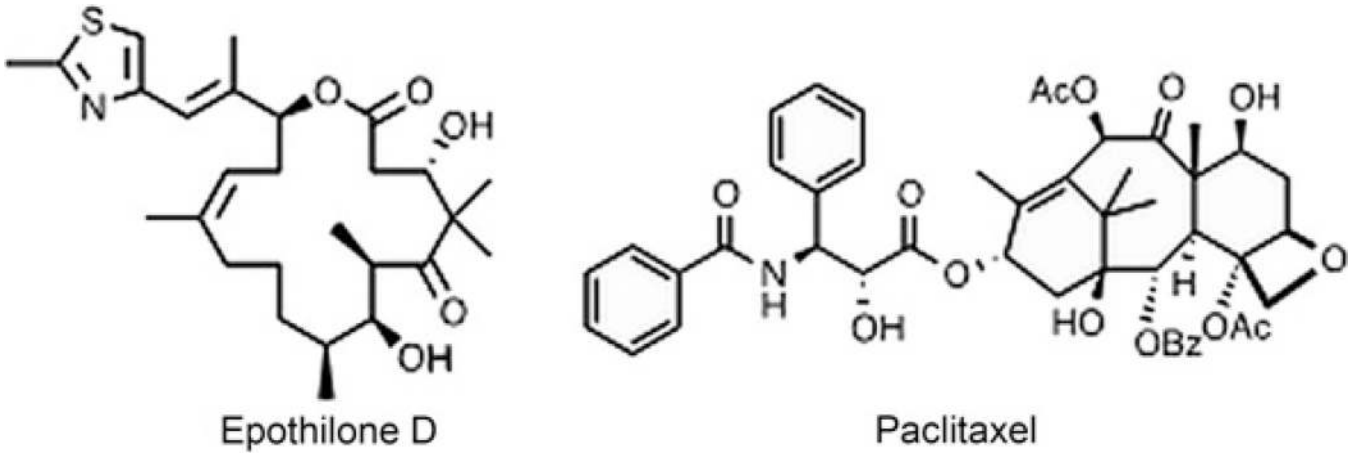

Figure 1.
Structures of EpoD and paclitaxel