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Altered Microtubule Dynamics in Neurodegenerative Disease: Therapeutic Potential of Microtubule-Stabilizing Drugs

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Abstract

Many neurodegenerative diseases are characterized by deficiencies in neuronal axonal transport, a process in which cellular cargo is shuttled with the aid of molecule motors from the cell body to axonal termini and back along microtubules (MTs). Proper axonal transport is critical to the normal functioning of neurons, and impairments in this process could contribute to the neuronal damage and death that is characteristic of neurodegenerative disease. Although the causes of axonal transport abnormalities may vary among the various neurodegenerative conditions, in many cases it appears that the transport deficiencies result from a diminution of axonal MT stability. Here we review the evidence of MT abnormalities in a number of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and traumatic brain injury, and highlight the potential benefit of MT-stabilizing agents in improving axonal transport and nerve function in these diseases. Moreover, we discuss the challenges associated with the utilization of MT-stabilizing drugs as therapeutic candidates for neurodegenerative conditions.

Keywords

Alzheimer; Amyotrophic Lateral Sclerosis; Axons; Frontotemporal Lobar Degeneration; Microtubules; Neurodegeneration; Parkinson; Transport; Traumatic Brain Injury

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Introduction

As discussed throughout this special issue, a common feature of many neurodegenerative disorders is an apparent disruption of normal axonal transport that likely contributes to the neuronal dysfunction observed in these diseases. Axonal transport is critical to the health and viability of neurons, as maintenance and functioning of axonal projections, which can reach many feet in length in certain motor neurons, requires the delivery of a variety of cellular cargo to and from the axon terminus (Roy et al., 2005). The process of axonal transport depends on the integrity of the microtubule (MT) architecture within the neuron, as MTs serve as the conduits upon which cellular constituents are shuttled in both the anterograde (towards the axonal terminus) and retrograde (towards the neuronal soma) directions. The movement of materials along the MTs is facilitated by molecular motors, with kinesins responsible for anterograde transport (Hirokawa and Noda, 2008) and dyneins (Ishikawa, 2012) for retrograde transport. Thus, the faulty axonal transport observed in neurodegenerative disease could result from alterations in MT structure and/or the molecular motors required for axonal transport (Ballatore et al., 2012; Brunden et al., 2014).

As there are reviews in this issue dedicated to the molecular motors involved in axonal transport, this topic will not be further discussed in detail herein. Instead, we focus on the potential contribution of MT deficits in neurodegenerative disease. In this regard, it is important to recognize the unusual features of MTs that allow them to play a critical role in fundamental cell biological processes, such as mitosis and the distribution of intracellular organelles, as well as the more specialized function they serve in axonal transport. MTs are polymeric structures comprised of repeated assemblies of α- and β-tubulin heterodimers that form linear protofilaments which align to form MTs, with 13 protofilaments found within typical MTs (Desai and Mitchison, 1997; Wade, 2007). There are a large number of α- and β-tubulin isoforms and the composition within MTs can vary depending on cell type and developmental stage, although the differences in structure and/or function conferred by these various isoforms is not fully understood (Wade, 2007). MTs have directionality that results from the orientation of the assembled α- and β-tubulin heterodimers, such that the (-) end of a MT has an exposed α-tubulin subunit whereas the (+) end has an exposed β-tubulin (Figure 1a). In many cells, the (-) end of a MT is associated with a microtubule organizing center (MTOC), although neurons appear not to have a classical MTOC and MTs are discontinuous in neuronal processes (Ori-McKenney et al., 2012; Stiess et al., 2010). A key property of MTs is their dynamic instability, in which elongation can occur at the (+) end through the addition of α/β heterodimers in which each of the subunits contain a bound GTP molecule (Desai and Mitchison, 1997). This GTP is hydrolyzed to GDP after addition to a MT, and prolonged exposure of a terminal GDP-α/β heterodimer at the (+) end can lead to MT depolymerization. Such MT dissociation appears to be less common at the (-) end due to the association of the MT with a MTOC, although as noted, it appears that a classical MTOC may not exist in neurons. Thus, periods of MT growth can be followed by bouts of MT shortening and this “dynamic instability” is critical to certain cellular processes, such as the eventual collapse of MTs after the segregation of chromosomes along MTs during cell division. MTs within neurons appear to have a lower degree of dynamic instability than in most other cell types, presumably because of the specialized role that MTs play in axonal

transport. MT dynamics in neurons appear to be governed, at least in part, through interactions with MT-associated proteins (MAPs) (Wade, 2007),.

A predominant MAP that is thought to affect MT structure in neurons is the protein tau (Drechsel et al., 1992; Gustke et al., 1994). In the human CNS, there are six isoforms of tau (Figure 1b) that result from differential splicing of three exons (Ballatore et al., 2007; Brunden et al., 2014). One of these alternatively spliced exons (exon 10) encodes a MT-binding repeat domain that bears sequence homology to additional MT-binding repeats within the tau molecule, such that human tau contains either 3 or 4 MT-binding repeats (referred to as 3- or 4-R tau). These MT-binding domains, in conjunction with additional tau sequences, promote MT binding. Tau appears to interact predominantly with the more labile distal regions of MTs (Black et al., 1996; Kempf et al., 1996), and there is evidence that fluorescently-tagged tau may have a highly reversible interaction with MTs (Janning et al., 2014), although there is some disagreement about the relative “dwell” time of tau on MTs (Konzack et al., 2007). Tau is believed to be a MT-stabilizing protein (Amos, 2004), as it can facilitate MT assembly in vitro and mutations in tau associated with inherited forms of frontotemporal lobar degeneration can reduce this MT-assembly activity (Hasegawa et al., 1998). However, there is also evidence to suggest tau may prevent spontaneous MT collapse through its binding to more labile regions of MTs (Black et al., 1996; Kempf et al., 1996), as well as reduce MT severing by proteins such as katanin and spastin (Qiang et al., 2006; Sudo and Baas, 2011; Yu et al., 2008) and perhaps fidgetin (Leo et al., 2015). Although a variety of in vitro and neuronal culture studies indicate that tau can affect MT structure, it should also be noted that mice in which tau has been constitutively knocked out do not show dramatic phenotypic changes, although some CNS deficits have been reported (reviewed in (Ke et al., 2012)), and the neurons from these mice may not have compromised axonal transport (Yuan et al., 2008). This might suggest that tau is not required for normal MT structure and function, although the interpretation of results from constitutive knockout mice can be confounded by compensatory changes and there is evidence of other MAPs being increased in tau knockout mice (Dawson et al., 2001; Harada et al., 1994). In addition to its presumed role in regulating MT dynamics, tau may also govern the association of MT motors (Dixit et al., 2008; Vershinin et al., 2007), and thus although there is not universal agreement, tau is believed to play a critical role among in proper MT function in neurons.

MT dysfunction in Alzheimer’s disease and related tauopathies: the potential therapeutic utility of MT-stabilizing agents

A number of neurodegenerative diseases referred to as tauopathies are characterized by the deposition of insoluble inclusions comprised of fibrils of hyperphosphorylated tau protein (Kidd, 1963; Lee et al., 1991; Lee et al., 2001). These aggregates of tau are typically found in the neuronal soma and dendrites, where they are referred to as neurofibrillary tangles (NFTs) and neuropil threads, respectively. The major disorders among the tauopathies are Alzheimer’s disease (AD) and frontotemporal lobar degenerative (FTLD) conditions associated with tau pathology (FTLD-tau), including Pick’s disease, progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD). Whereas the non-AD tauopathies have tau inclusions as their primary pathological feature, the AD brain also has

extracellular Aβ peptide-containing senile plaques (Ball et al., 1997). In addition, most AD cases also have α-synuclein and TDP-43 inclusions (Irwin et al., 2013; Nelson et al., 2016), so AD is most commonly a mixed proteinopathy, and cerebrovascular pathology is also common in AD (Toledo et al., 2013). Although the Aβ pathology observed in AD has historically been the major focus of pharmaceutical efforts for the treatment of AD, based largely on familial mutations that clearly implicate the Aβ peptide in AD (George-Hyslop and Petit, 2005), the repeated clinical failures of Aβ-directed drugs has led to a growing interest in tau-directed therapeutic strategies (Khanna et al., 2016). As noted above, tau is believed to play a critical role in MT structure and function, and the hyperphosphorylation and deposition of tau into inclusions in the various tauopathies is believed to lead to the neuronal dysfunction and death observed in these diseases. This viewpoint is supported by the high degree of correlation of tau pathological burden and dementia in AD (Arriagada et al., 1992; Gomez-Isla et al., 1997; Wilcock and Esiri, 1982), and the seminal findings that mutations in tau can result in inherited forms of FTLD (Hong et al., 1998; Hutton et al., 1998). Hyperphosphorylation of tau reduces the binding affinity to MTs (Alonso et al., 1997; Alonso et al., 1994; Dayanandan et al., 1999; Hong et al., 1998) and increased phosphorylation may also increase the propensity of tau to form insoluble fibrils (Barghorn et al., 2000; Nacharaju et al., 1999). Thus, it is believed that tau-mediated neuronal toxicity is due to the formation of misfolded tau oligomeric or fibrillar species, and/or an alteration of MT structure and axonal transport that results from the MT disengagement of tau and its subsequent deposition into inclusions.

The hypothesis that tau loss-of-function, with resulting MT dysfunction, contributes to the neurodegeneration of tauopathies is supported by the observation of decreased MT numbers in AD brain (Cash et al., 2003) and reduced levels of acetylated α-tubulin (Hempen and Brion, 1996), which is a marker of stable MTs (Fukushima et al., 2009). Moreover, there is evidence of MT abnormalities in transgenic (Tg) mouse models with NFT-like tau pathology, including decreased MT density (Brunden et al., 2010; Zhang et al., 2012; Zhang et al., 2005), reduced axonal transport (Zhang et al., 2012; Zhang et al., 2005) and increased MT hyperdynamicity (Barten et al., 2012). These observations have led to an interest in assessing the potential of MT-stabilizing agents for the treatment of AD and other tauopathies. MT-stabilizing drugs have been used for decades in the treatment of cancer, as these molecules alter the mitotic spindle during the proliferation of cancer cells (and other dividing cells) (Zhao et al., 2016). Although it would be undesirable to affect dividing cells in patients with neurodegenerative diseases, the hope is that low doses of certain brain-penetrant MT-stabilizing drugs might compensate for MT abnormalities in the diseased brain without substantially affecting dividing cells in the periphery. The first report to test this hypothesis utilized a formulated version of the cancer drug, paclitaxel, in a tau Tg mouse model in which tau pathology is largely confined to brain stem and spinal cord (Zhang et al., 2005). The paclitaxel-treated Tg mice had significantly increased MT numbers and axonal transport in spinal axons, and these proof-of-principle data revealed that a MT-stabilizing drug could provide benefit in a tauopathy model. However, as paclitaxel does not cross the blood-brain barrier (BBB) (Brunden et al., 2011), there was still uncertainty whether MT-stabilizing agents would provide benefit in mouse models with brain tau pathology and associated cognitive deficits, as observed in AD and other tauopathies.

Several tau Tg mouse models develop tau pathology within the brain, with associated neuron loss and impaired learning and memory. However, the valid evaluation of MT-stabilizing agents in such models necessitates that test compounds have good brain exposure, and many of the most commonly employed MT-stabilizing drugs do not readily enter the brain, including most taxanes (Brunden et al., 2011). This led our laboratories to examine a number of known MT-stabilizing natural products or related congeners for their ability to enter the brain, and the epothilone family of compounds was generally found to be fully brain-penetrant (Brunden et al., 2011). An elucidation of the pharmacokinetic (PK) and pharmacodynamic (PD) properties of various epothilones revealed that epothilone D (epoD) had many desirable features, including excellent brain exposures and notably, the ability to increase the level of acetylated-tubulin in the brains of wild-type mice for prolonged periods after a single administration (Brunden et al., 2011). This extended biological activity appears to result from the retention of epoD in the brain, with a half-life that far exceeds that in blood (Brunden et al., 2010). Although prolonged brain exposures would typically be undesirable for a drug, this property might prove beneficial in the case of a MT-stabilizing agent for the treatment of tauopathies, as peripheral exposures would be minimized due to a lower required dosing frequency. This should result in decreased peripheral side effects, such as the inhibition of blood cell proliferation that occurs in cancer patients receiving drugs of this type. In fact, the initial studies with epoD revealed that doses that were equivalent to ~1/30th of those employed in cancer clinical trials were sufficient to elicit lasting MT stabilization in the brain.

In subsequent studies, epoD was evaluated in both preventative (Brunden et al., 2010) and interventional (Zhang et al., 2012) dosing schemes in the PS19 tau Tg mouse model, in which NFT-like tau inclusions develop as the mice age. The PS19 mice show a modest deficit in MT density and have reduced axonal transport relative to non-Tg littermates. In both studies with PS19 mice, doses of epoD that were much lower than those used for cancer treatment were well tolerated, resulting in improved MT density and decreased axonal dystrophy that presumably resulted from defective axonal transport. Moreover, in both the preventative and interventional studies, epoD treatment reduced the cognitive deficits normally observed in the PS19 mice. Of significance, when epoD was administered to mice with existing tau pathology, the drug improved axonal transport and prevented the neuron loss that develops in the mice with age. Somewhat surprisingly, epoD was also found to reduce the burden of tau pathology in the Tg mice (Zhang et al., 2012). As the intent of epoD treatment was to compensate for MT abnormalities that might result from tau loss-of-function, the attenuation of tau pathology was unexpected, and would seem to indicate that there is a linkage between axonal transport and the development of tau inclusions. Such a mechanistic link has been suggested previously, as disruption of anterograde transport through knockout of kinesin results in an exacerbation of tau pathology (Falzone et al., 2010; Falzone et al., 2009).

The potential of epoD for the treatment of AD and related tauopathies was confirmed in further studies using two additional tau Tg mouse models, where again epoD was found to improve several efficacy outcomes, including a reduction of neuron loss and improved cognitive performance (Barten et al., 2012). Of note, these studies also confirmed that epoD reduced MT hyperdynamicity at doses that were appreciably lower than those used for the

treatment of cancer. The effects of epoD on MT dynamicity suggest that the drug may, as has been suggested for tau, act to prevent MT depolymerization at the distal ends of MTs. Subsequent to the publication of the studies evaluating epoD in Tg mouse models of tauopathy, the drug (referred to as BMS-241027) entered clinical testing in AD patients in a 9-week Phase 1b study in which safety and initial efficacy measures were evaluated (ClinicalTrials.gov identifier: NCT01492374). Although the study results have not been formally published, a prior Phase 1 safety study indicated that BMS-241027 was well tolerated at the doses tested (Malamut, 2013), which were roughly equivalent to those used in the aforementioned mouse studies when adjusted for species. There have been no further reports on the clinical development of BMS-241027. More recently, TPI-287, an abeotaxane MT-stabilizing drug, has entered clinical evaluation in AD patients (ClinicalTrials.gov identifier: NCT01966666), as well as in those with PSP and CBD (ClinicalTrials.gov identifier: CT02133846). Although there is limited published information on TPI-287, which makes it difficult to ascertain how the relative PK/PD profile of this compound compares to epoD, this compound is reported to be brain-penetrant (Fitzgerald et al., 2012). As with the Phase 1b trial of BMS-241027, those with TPI-287 are planned for 9-weeks duration, and it is uncertain whether these short trial durations will be sufficient to observe a meaningful change in disease biomarkers or cognitive status.

More recently, our laboratories have evaluated another natural product MT-stabilizing agent, dictyostatin, in an interventional study in 6-month old PS19 tau Tg mice. Like epoD, dictyostatin shows excellent BBB penetration and prolonged brain retention, and elicits a lasting increase in brain acetylated tubulin in wild-type mice (Brunden et al., 2013). When administered once-weekly at a low dose of 0.1 mg/kg, dictyostatin showed improvement in CNS/brain measures that were quite similar to those noted for epoD (Makani, 2016). In particular, dictyostatin treatment led to increased MT density and reduced axonal dystrophy. Moreover, dictyostatin reduced the further deposition of tau pathology in the PS19 mice, with a strong trend towards improved hippocampal neuron survival. However, the PS19 mice showed clear evidence of intolerance to dictyostatin, with gastrointestinal (GI) complications that led to the death of some mice, particularly at higher doses. Although further study would be required to fully understand the nature of this GI side effect, we speculate that intestinal epithelial cells may be affected by dictyostatin, as has been reported in some cancer patients receiving taxane MT-stabilizing agents (Daniels et al., 2008). Interestingly, the dictyostatin-treated mice did not have reduced neutrophils or other blood cells, which is one of the major side effects observed in patients receiving MT-stabilizing drugs for cancer treatment (Bedard et al., 2010; Cortes and Baselga, 2007). Thus, it is unclear whether humans would show the same dose-limiting GI side effects observed in PS19 mice, but additional safety evaluations would clearly be required before considering dictyostatin for human use.

In addition to these brain-penetrant MT-stabilizing natural products and derivatives thereof, selected non-naturally occurring triazolopyrimidine and related heterocyclic MT-stabilizing molecules have been identified that are brain-penetrant and increase markers of stable MTs in the brain after administration to mice (Kovalevich et al., 2016; Lou et al., 2014). Moreover, these compounds show oral bioavailability. Finally, several studies have been conducted with an octapeptide referred to as NAP (sequence is NAPVSIPQ) that is derived

from the activity-dependent neuroprotective protein (Gozes and Ivashko-Pachima, 2015). NAP is reported to have MT-stabilizing activity, as well as several other biological effects (Gozes and Ivashko-Pachima, 2015). NAP has been tested after intranasal administration to Tg mice that form both Aβ plaque and tau pathology, and the peptide was reported to lower both Aβ and hyperphosphorylated tau levels (Matsuoka et al., 2007). A similar effect on phospho-tau was observed in another tau Tg mouse model (Matsuoka et al., 2008). The NAP peptide (also referred to as davunetide) progressed to clinical testing in FTLD-Tau patients, where it was again dosed via the intranasal route. Unfortunately, davunetide did not lead to clinical improvement in a Phase 2/3 trial in PSP patients (Boxer et al., 2014). The reason for the clinical failure is unknown, but it should be noted that drug levels were not assessed in the cerebrospinal fluid of patients within this trial, and given the intranasal route of delivery it may be that insufficient brain drug levels were achieved.

Finally, it should be noted that whereas the clear benefit provided by brain-penetrant MT-stabilizing agents in tau Tg mouse models of tauopathy is consistent with a tau loss-of-function hypothesis, it is possible that the MT and axonal transport deficits observed in these mice result instead from effects arising from the pathological tau species that form in these mice. A tau gain-of-function effect on MTs would be compatible with the more general observation of MT abnormalities in multiple neurodegenerative diseases, as discussed below. In addition, as previously noted, studies in constitutive tau knockout mice suggest that a loss of tau is not detrimental to neuronal function or axonal transport (Yuan et al., 2008), although there is some disagreement about whether tau knockout is truly benign (Ke et al., 2012) or whether compensatory changes confound the interpretation of results from such studies. Although there is clearly interest in better understanding the mechanism(s) that lead to the alterations of MT structure and function observed in mouse models of tauopathy, it is gratifying that MT-stabilizing drugs have reproducibly provided significant CNS improvements in these mice, irrespective of the underlying cause(s) of MT abnormalities.

Evidence of MT abnormalities in other neurodegenerative conditions and potential beneficial effects of MT-stabilizing agents

A. Traumatic brain injury

There is growing recognition that traumatic brain injury (TBI) can lead to lasting effects in the brain that can manifest many years after the initial injury (Young et al., 2016). Furthermore, there is increasing evidence that repetitive brain injuries resulting from contact sports or other causes can lead to a syndrome known as chronic traumatic encephalopathy (CTE), which is characterized by neuronal loss and behavioral/cognitive abnormalities (Saulle and Greenwald, 2012). A common feature of brain trauma appears to be diffuse axonal injury, in which there is mechanical breakage of axonal MTs and a disruption of axonal transport (Johnson et al., 2013; Tang-Schomer et al., 2012; Tang-Schomer et al., 2010). Given the previously noted relationship between deficits in axonal transport and the development of tau pathology (Falzone et al., 2010), it is interesting that a feature of repetitive TBI/CTE is the presence of distinct patterns of tau pathology that first appears in cortical sulci and then may spread to other brain regions (Johnson et al., 2013; McKee et al., 2016). Indeed, TBI can often result in AD and other neurodegenerative conditions (Gardner

et al., 2014). Given the parallels in MT deficits and axonal dysfunction in AD and TBI/CTE, it would seem MT-stabilizing agents might provide benefit to the latter neurodegenerative conditions. There is increasing evidence that this may be true, as treatment with paclitaxel reduced axonal degeneration in an axon stretch model that was developed to mimic the mechanical injury of TBI (Tang-Schomer et al., 2010). In addition, epoD was demonstrated to improve post-injury axonal sprouting in an in vitro model of nerve trauma (Brizuela et al., 2015). More recently, paclitaxel was examined in a mouse model of TBI. As paclitaxel does not readily cross the BBB, the drug was applied directly to the injury site. The paclitaxel-treated group showed improvements in brain-related measures, such as reduced lesion volume and edema, as well as preserved axonal myelination (Cross et al., 2015). Moreover, improvements in motor function were observed in the TBI mice receiving paclitaxel. Thus, although research into the pathological features and biological consequences of acute and repetitive brain trauma is ongoing, there is growing evidence that MT dysfunction may be a hallmark of such injury and that MT-stabilizing agents may provide benefit.

B. Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative condition characterized by a significant loss of dopaminergic neurons in the substantia nigra pars compacta, with resulting motor impairments. The nigral neurons of PD patients accumulate intra-neuronal inclusions known as Lewy bodies that are comprised of α-synuclein (Braak et al., 2003; Lee and Trojanowski, 2006; Spillantini et al., 1997), a protein that normally appears to play a role in synaptic function (Burre, 2015). Although PD has historically been viewed as a disease that affects the nigral-striatal system, it is becoming clear that additional brain regions are also affected, including those involved in cognitive processes (Irwin et al., 2013). As in AD, there is evidence of MT abnormalities in the PD brain that likely contribute to the observed neurodegeneration, and given the extensive axonal arborization of nigral dopaminergic neurons (Matsuda et al., 2009), it is likely that aberrant axonal transport would be particularly detrimental to these cells. Some of the initial studies demonstrating a linkage between MTs and PD were in model systems that employ environmental toxins that have been suggested to increase the risk of PD (Shaw and Hoglinger, 2008). In particular, rotenone appears to affect MT polymerization (Brinkley et al., 1974; Marshall and Himes, 1978), and dopaminergic neurons may be particularly sensitive to this toxin (Ren et al., 2005). Similar observations have been made with additional agents employed to model certain aspects of PD, such as herbicides (Holy, 1998; Rosso et al., 2000) and MPTP (Cappelletti et al., 1999; Cappelletti et al., 2005; Cartelli et al., 2010). Notably, treating mice with MPTP leads to increased MT dynamicity, consistent with findings from culture systems (Fanara et al., 2012).

Although toxin models have been of value in eliciting the nigral neuron loss observed in PD, they do not typically recapitulate the α-synuclein pathology that is a hallmark feature of human PD. Thus, literature reports of possible linkages between MT alterations and α-synuclein expression lends additional credence to an element of MT dysfunction in PD. Among the first reports to demonstrate impaired MT function related to α-synuclein were studies showing that neurons or cell lines overexpressing α-synuclein showed MT dysfunction, including decreased MT networks, impaired MT-dependent trafficking, Golgi

fragmentation and neuritic degeneration (Lee et al., 2006). Subsequently, MT hyperdynamicity was observed in Tg mice expressing α-synuclein with the A53T mutation found in familial PD (Fanara et al., 2012). Additional studies provided further evidence of reduced MT stability in α-synuclein mouse models of PD, and the changes in MT structure and function observed in α-synuclein Tg mice may be linked to increases in tau phosphorylation and disengagement from MTs, as suggested in mice expressing either wild-type (Haggerty et al., 2011) or A53T mutant α-synuclein (Kaul et al., 2011; Wills et al., 2011). A possible involvement of hyperphosphorylated tau in the reduced MT stability observed in PD models is further supported by studies with LRRK2, a protein kinase that is mutated in familial cases of PD (Paisan-Ruiz et al., 2004; Zimprich et al., 2004). There is evidence that LRRK2 can directly phosphorylate tau that is bound to MTs, thereby reducing tau binding to MTs (Kawakami et al., 2012). Moreover, LRRK2 mutations enhance this tau phosphorylation (Kawakami et al., 2012), perhaps as a result of increased binding of mutant LRRK2 to MTs (Kett et al., 2012). Interestingly, overexpression of LRRK2 in A53T α-synuclein expressing Tg mice exacerbated the extent of α-synuclein pathology, and the acceleration of α-synuclein aggregation was attributed to impairments of MT dynamics (Lin et al., 2009). Thus, it appears that alterations in MT stability may lead to an enhancement of both α-synuclein and tau pathology, as noted previously.

Notably, a reduction of MT stability may also result from mutations in the Parkin protein that cause familial PD (Kitada et al., 1998; Oliveira et al., 2003). An analysis of iPSC neurons derived from fibroblasts of normal subjects and PD patients with Parkin mutations revealed a significant decrease in stable MTs in the latter neurons, with a consequent reduction in neurite length and complexity (Ren et al., 2015). Importantly, the neuritic morphology of the Parkin-deficient neurons could be restored by treatment of the cultures with the MT-stabilizing drug, paclitaxel (Ren et al., 2015). Although the mechanism of Parkin effect on MTs is not fully understood, it is interesting to note that Parkin appears to interact with HDAC6 (Jiang et al., 2008), which can deacetylate MTs and thereby possibly affect MT structure or transport. In fact, the literature indicating MT deficits in various rodent models of PD, as noted herein, would suggest that MT-stabilizing agents hold great promise in the treatment of PD. It is thus somewhat surprising that relatively few studies have examined the effects of MT-stabilizing compounds in PD disease models, although this may be a consequence of the lack of available brain-penetrant MT-stabilizing small molecules until the relatively recent publications on epoD, dictyostatin and other small molecules (Ballatore et al., 2012; Brunden et al., 2014). In this regard, epoD was shown to increase MT stabilization and attenuate nigrostriatal degeneration in a mouse MPTP model of PD (Cartelli et al., 2013). More recently, epoD was also utilized in a rat model of methamphetamine-induced striatal dopaminergic neuron loss. The methamphetamine-treated mice showed a reduction of stable MTs and a loss of dopaminergic markers within the striatum that were rescued by a low dose of epoD (Killinger and Moszczynska, 2016). Interestingly, a higher dose of epoD appeared to exacerbate the effects of methamphetamine, pointing to the importance of proper dosing with MT-stabilizing agents and the potential risks associated with excessive exposure to this class of drugs.

C. Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a disease of motor neurons that typically progresses at a rapid rate (Robberecht and Philips, 2013). Although the selective vulnerability of motor neurons in ALS is not fully understood, it is likely that deficits in axonal transport and/or MT structure would be particularly crippling to these cells as certain motor neurons have the longest axonal projections of any neurons in the body. A number of observations support the hypothesis that faulty axonal transport, and perhaps hyperdynamic MTs, play a key role in the motor neuron death observed in ALS. Mutations in the dynactin subunit, $p150^{glued}$, which plays a role in retrograde axonal transport through its association with dynein, cause motor neuron disease and perhaps ALS (Munch et al., 2005; Puls et al., 2003). Moreover, mutations in the superoxide-dismutase-1 (SOD1) gene, which are responsible for ~10% of inherited ALS cases, might also lead to compromised axonal transport. For example, several Tg mouse models with mutant SOD1 have been developed, and it appears that axonal transport deficits precede neurodegeneration in certain of these mice (Williamson and Cleveland, 1999; Zhang et al., 1997). A study in one SOD1 Tg mouse model confirmed that these mice had increased MT dynamicity, and treatment with the reported MT-stabilizing compound, noscapine, reduced MT turnover and normalized axonal transport, with a prolongation of lifespan (Fanara et al., 2007). More recently, MT hyperdynamicity was confirmed in two additional mutant SOD1 Tg mouse models of ALS using a novel methodology (Kleele et al., 2014), strengthening the evidence that SOD1 mutations lead to alterations in MT dynamics. The consequences of MT deficits in mutant SOD1 mice may extend beyond impairments of axonal transport, as a recent study reveals that two different SOD1 mutations lead to Golgi fragmentation that appears to result from an up-regulation in the production of the MT-severing proteins, stathmins 1 and 2 (Bellouze et al., 2016). The Golgi fragmentation observed in cells harboring the mutant SOD1 could be rescued by knockdown of stathmins, and notably, by treatment with paclitaxel (Bellouze et al., 2016).

As individuals with SOD1 mutations comprise a small percentage of ALS patients, there would be value in demonstrating altered MT dynamics and/or deficient axonal transport in non-SOD1 ALS models. In this regard, recent studies suggest axonal transport deficits in TDP-43 Tg mouse models. As with SOD1, TDP-43 mutations can cause ALS, and TDP-43 is the primary protein found in ubiquitin-positive inclusions that are a hallmark pathology of ALS as well ~50% of patients with FTLD referred to as FTLD-TDP (Cohen et al., 2011; Neumann et al., 2006). When Tg mice expressing mutant TDP-43 were crossed with mice expressing a fluorescent protein that localized to mitochondria, defects in mitochondrial transport were observed before the onset of disease symptoms in the mice (Magrane et al., 2014). In addition to mutations in TDP-43 and SOD1, inherited forms of ALS can be caused by mutated FUS or repeat expansions in the C9orf72 gene (Peters et al., 2015). In a recent report (Baldwin et al., 2016), expression of mutant TDP-43, FUS and C9orf72, or knockout of the homologs of these genes, in *Drosophila* resulted in axonal transport deficits. Importantly, these axonal transport deficits manifested as motor deficits that generally worsened with age in most of the *Drosophila* models. Thus, in addition to the MT and axonal transport deficits observed in SOD1 mice, the studies of the other ALS gene mutations further implicate axonal transport deficiencies as a key feature of ALS animal models. However, there is relatively little understanding at this time of the molecular

mechanism(s) that cause impairment of axonal transport in the TDP-43, FUS and C9orf72 models, and it is unclear whether MT structure and/or dynamicity is affected, as has been suggested for models with SOD1 mutations.

Conclusions

As discussed herein, there is compelling evidence of axonal transport deficits in several neurodegenerative conditions, with at least some indication that the transport abnormalities are caused in part or in whole by reduced MT stability/increased MT dynamicity. Although the focus of this review has been on how MT alteration might affect axonal transport, it should also be noted that MT dysfunction could also affect the proper functioning of dendrites (e.g., see (Penazzi et al., 2016)). Currently, the largest body of literature supporting MT abnormalities is in tauopathies, including AD, and at least two brain-penetrant small molecule MT-stabilizing agents, as well as a peptide with reported MT-stabilizing activity, have shown evidence of providing significant benefit in Tg mouse models of tauopathy. However, there is an increasing number of studies revealing MT abnormalities in cell culture and animal models of several other neurodegenerative conditions, including TBI, PD and ALS, and in each of these there is evidence of benefit provided by MT-stabilizing agents.

Although the data reviewed here suggest that MT-stabilizing drugs hold promise for the potential treatment of several neurodegenerative diseases, there are challenges in the development of drug candidates of this type. As noted, MT-stabilizing drugs can inhibit cell division, and thus the ideal drug candidate for the treatment of CNS disorders would decrease MT dynamicity in the brain with minimal effect on proliferating cells in the periphery. The available data suggest that this balance is achievable, as evidenced by the studies with epoD where clear benefit was observed in multiple CNS endpoints without observed peripheral complications. However, the experience with dictyostatin in tau Tg mice, in which CNS benefit was observed, but with GI complications and mortality in some study mice, indicates that not all brain-penetrant MT-stabilizing agents are equal and that care will be required to ensure patient benefit is observed without significant side effects. In addition to the challenge of providing CNS benefit without peripheral complications, it is also recognized that the MT abnormalities observed in neurodegenerative diseases will likely not be found throughout the brain, but rather will be restricted to regions harboring pathology. Thus, it will be important to provide benefit with MT-stabilizing drugs in affected brain areas without eliciting MT dysfunction in other unaffected parts of the brain.

Another complication in moving MT-stabilizing drugs into clinical testing is the absence of a short-term marker of target engagement. As pharmaceutical companies have become more risk-averse in moving drug candidates into testing for neurodegenerative disorders such as AD, they are often requiring that a biomarker assay be in place that allows for the early detection of drug activity. This facilitates the determination of appropriate drug dosing, and provides confidence in early clinical testing that the drug is having the intended effect. For example, the activity of BACE1 inhibitors designed to lower the release of Aβ peptide from the amyloid precursor protein in AD can be monitored by measuring Aβ levels in biofluids, including cerebrospinal fluid. There is presently not a simple way to measure changes in MT stability or dynamicity, which complicates dose optimization studies in humans and

necessitates that alternative pharmacodynamic measures that are secondary to target engagement be utilized, such as changes in cerebrospinal fluid tau and/or p-tau levels. Such secondary endpoints are indirect and likely require that reductions in disease pathology occur before the biomarker change is observed, thus requiring longer clinical studies to garner evidence of compound activity.

In closing, deficits in axonal transport appears to be a common theme for most, if not all, neurodegenerative diseases, and there is evidence that the axonal transport impairments result, at least in part, from changes in MT dynamicity/stability. These observations have led to the testing of MT-stabilizing drugs in several neurodegenerative model systems, where benefit has generally been observed. Thus, there is incentive to develop MT-stabilizing drugs to treat the MT deficits observed in these various diseases, with the recognition of the challenges in achieving the favorable benefit-to-risk ratio required of a drug that will likely be administered on a chronic basis.

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Abbreviations

BBB	blood-brain barrier
CBD	cortical basal degeneration
CTE	chronic traumatic encephalopathy
EpoD	epothilone D
FTLD	frontotemporal lobar degeneration
MAP	microtubule-associated protein
MT	microtubules
MTOC	microtubule organizing center
PSP	progressive supranuclear palsy
SOD1	superoxide dismutase 1
TBI	traumatic brain injury

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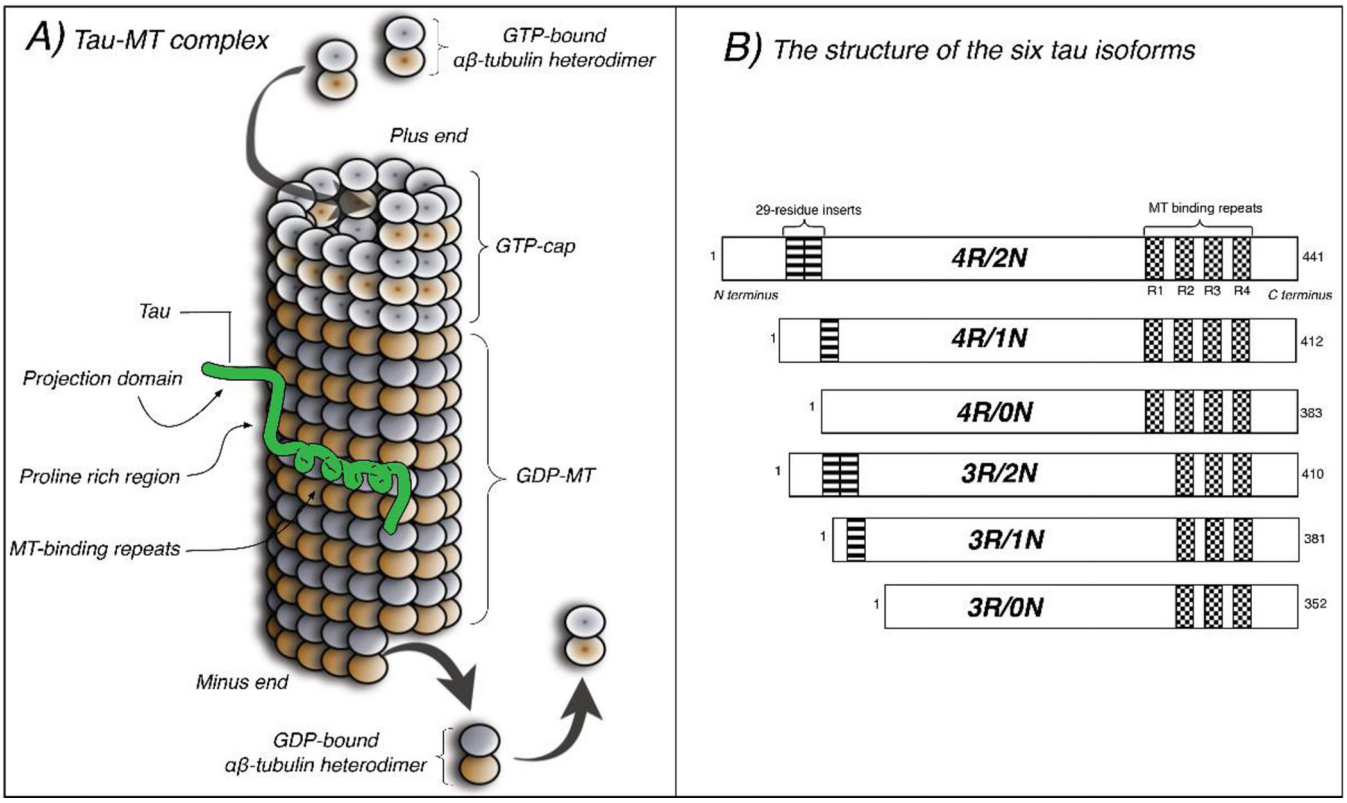

Figure 1.

A. Schematic representation of the complex between tau (drawn in green) and a MT, as well as **B.** the structure of the six human tau isoforms.