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EXPERT REVIEW

Nanoparticles in the Brain: A Potential Therapeutic System Targeted to an Early Defect Observed in Many Neurodegenerative Diseases

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ABSTRACT Currently, there are no effective treatments or cures for many neurodegenerative diseases affecting an aging baby-boomer generation. The ongoing problem with many of the current therapeutic treatments is that most are aimed at dissolving or dissociating aggregates and preventing cell death, common neuropathology often seen towards the end stage of disease. Often such treatments have secondary effects that are more devastating than the disease itself. Thus, effective therapeutics must be focused on directly targeting early events such that global deleterious effects of drugs are minimized while beneficial therapeutic effects are maximized. Recent work indicates that in many neurodegenerative diseases long distance axonal transport is perturbed, leading to axonal blockages. Axonal blockages are observed before pathological or behavioral phenotypes are seen indicating that this pathway is perturbed early in disease. Thus, developing novel therapeutic treatments to an early defect is critical in curing disease. Here I review neurodegenerative disease and current treatment strategies, and discuss a novel nanotechnology based approach that is aimed at targeting an early pathway, with the rationale that restoring an early problem will prevent deleterious downstream effects. To accomplish this, knowledge exchange between biologists, chemists, and engineers will be required to manufacture effective novel biomaterials for medical use.

KEY WORDS anterograde transport · axonal transport · dynein · kinesin · neurodegenerative disease · neurons/axons · organically modified silica particles · retrograde transport · synthetic vesicle · therapeutic vesicle

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ABBREVIATIONS

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
APP	Amyloid precursor protein
Aβ	Amyloid beta
HD	Huntington's disease
HTT	Huntingtin
KHC	Kinesin heavy chain
KLC	Kinesin light chain
MT	Microtubules
ORMOSIL	Organically modified silica
PD	Parkinson's disease

INTRACELLULAR TRANSPORT WITHIN AXONS IS CRITICAL FOR VIABILITY

Our nervous system can be thought of as requiring a transport system composed of highways that move essential cargoes from a central location (cell bodies in the spinal cord/brain) to places of action (nerve terminals/synapses) very much like a busy freeway system. Neurons are highly specialized cells with elaborate, long cytoplasmic processes termed dendrites and axons. Axons are unique in that essentially all material in the axon is synthesized within the cell body and delivered through the lengthy axon to sites of function/need by processes collectively known as axonal transport. Molecular motors (kinesin and dynein) are proteins that power cargo transport. Motor proteins use ATP hydrolysis to move vital cargoes on MT-tracks. Within axons, organelles, vesicles, cytoskeletal proteins, signaling molecules, and other supplies synthesized in the cell body must be transported by kinesin motors in the anterograde direction to nerve terminals/synapses, while signaling molecules and other components that need to be returned to the cell body from synapses are transported in the retrograde direction by dynein.

Classically, axonal transport can be divided into two, fast and slow transport, based on the speeds of cargo movement. Fast

axonal transport moves cargoes such as synaptic vesicles at speeds of 1 μm/s while slow axonal transport moves components of the cytoskeleton at speeds of 1 mm/day (reviewed in 1). The kinesin-1 motor complex, responsible for anterograde transport contains two heavy chains (KHC) and two light chains (KLC) (Fig. 1) (2). KHC is the motor that binds directly to MTs, while KLC interacts with intracellular cargo vesicles. The cytoplasmic dynein motor is a large and complex molecule. It is composed of two heavy chains (DHC), several intermediate (DIC), light intermediate (DLIC), and light chains (DLC) (3). DHC functions as the motor that directly binds MTs, and DIC, DLIC, and DLC are thought to mediate associations between the motor and its cargos (Fig. 1). Dynactin binds dynein and is a large complex composed of eleven different subunits (Fig. 1). It binds to dynein and to MTs through the p150-glued subunit and also mediates interactions with cargo. Thus, molecular motors and long distance transport is crucial for many neuronal and cellular processes. Defects in this pathway could lead to initiation, progression, and finally the chronic development of dysfunction.

AXONAL TRANSPORT DEFECTS IN NEURODEGENERATIVE DISEASE

Recent work has demonstrated that kinesin and dynein not only function as motors, but also have critical roles in long-distance events (Table I). For example, mutations in the kinesin super family motor protein KIF1Bβ result in Charcot-Marie-Tooth disease Type2A, a condition characterized by progressive dysfunction of peripheral neurons (14). A missense mutation in the neuronal kinesin heavy chain gene KIF5A causes Hereditary Spastic Paraplegia, which is a condition that arises due to axonal degeneration of motor and sensory neurons (6,33,34). Inhibition of axonal transport by excess dynamitin, a component of the dynactin complex, was sufficient to cause motor neuron degeneration observed in amyotrophic lateral sclerosis (ALS) (22). While transgenic ALS mice showed abnormalities in MT-based transport, with decreased rates of slow axonal transport and degeneration of motor neurons (35), the dynamitin transgenic mouse showed late-onset progressive motor neuron degeneration. A mutation in the human p150Glued subunit of the dynactin gene caused human motor neuron disease (32), and missense mutations in cytoplasmic dynein heavy chain gene caused selective impairment of retrograde transport, cell death, Lewy body-like inclusions, and progressive motor neuron degeneration (21). Taken together these results demonstrate that disruption of axonal transport is an important determinant in the initiation, and the progression of neuronal dysfunction. Work has also shown that axonal transport defects are an early precursor in many neurodegenerative diseases (reviewed in 1,36–38 (10,11,39 (AD), 27,40 and reviewed in 41 (HD), 21,22,32,35 and 42 (ALS))).

Axonal Transport and Alzheimer's Disease

Several reports suggest that amlyoid precursor protein (APP), the protein involved in Alzheimer's disease (AD), is transported down the axon by kinesin-1 (10–12,43). To date, two closely related models have been proposed for APP-dependent axonal

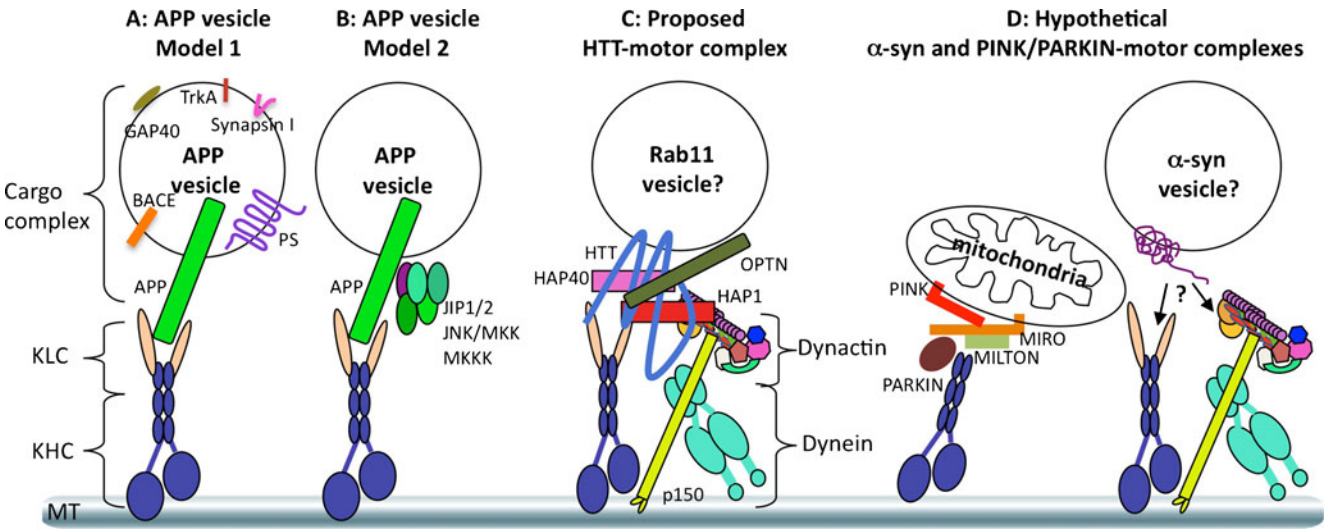

Fig. 1 Proposed motor-disease protein complexes. (**a**) Model 1 suggests that APP is contained within a vesicle that includes PS, BACE, GAP40, TrkA and synapsin 1, APP directly interacts with kinesin light chain (KLC) *via* it's C-terminus. (**b**) Model 2 suggests that the APP interaction with kinesin is indirect and is mediated *via* JNK and JIPs. (**c**) Proposed HTT vesicle. HTT may act as a scaffolding protein that links Rab 11 vesicles to motors *via* optineurin (OPTN). HAP1 can interact with both dynein and dynactin (p150) while HAP40 can interact with the Rab vesicle (KHC=kinesin heavy chain, MT=microtubule). (**d**) Hypothetical α-syn/PINK/PARKIN-motor complex. PINK and PARKIN interacts with MILTON/MIRO and KHC during mitochondrial motility. α-synuclein is thought to be present in vesicles and biochemical analysis has shown that it can bind to both kinesin-1 and dynein, although whether this binding is direct or indirect or occurs *in vivo* is unknown.

Table I Axonal Transport and its Relationship to Neurodegenerative Disease

Disease	Molecular motor involved	Proposed motor binding partner	Proposed cargo complex	Method of identification	*In vivo* evidence	References
Kinesin						
Neurofibromatosis	KHC	NF1, NF2	?	Biochemical	–	Hakimi *et al.*, 2002 (4)
Fragile X syndrome	KHC	mRNP complex	Purα, mStaufan, FMRP, Myosin Va	Biochemical	–	Ohashi *et al.*, 2002 (5)
Hereditary Spastic Paraplegia	KIF5B	?	?	Genetic	Loss of function	Reid *et al.*, 2002, Tessa *et al.*, 2008, Lo Giudice *et al.*, 2006 (6–8)
Alzheimer's disease	KLC	JIP1,2,3(SYD)	JNK, MPK, DLK, ApoER2	Biochemical 2-hybrid	Loss of function Gain of function	Verhey *et al.*, 2001 (9)
Alzheimer's disease	KLC	APP	PSI, GAP-43, BACE, TrkA, synapsin1	Biochemical, genetic	Loss and gain of function	Kamal *et al.*, 2000, 2001, Gunawardena *et al.*, 2001, Stokin *et al.* 2005 (10–13)
Charcot-Marie tooth disease type 2	KIF1Bα	PSD95, SAP97, S-SCAM	?	Biochemical	Loss of function	Mok *et al.*, 2002, Zhao *et al.*, 2001 (14,15)
Retinitis pigmentosa	KIF3A	?	Opsin, arrestin	Genetic	Loss of function	Marszalek *et al.*, 2000 (16)
Huntington's disease	KLC	HAP1	Rab 11	Biochemical	Loss of function/grain of function	McGuire *et al.* 2006, Power *et al.* 2012 (17,18)
Parkinson's disease	KHC/ KLC	Alpha-syn	?	Biochemical	–	Utton *et al.* 2005 (19)
Parkinson's disease	KHC	PINK, PARKIN	mitochondria	Genetic, biochemical	Loss of function/ gain of function	Wang *et al.* 2011 (20)
Dynein						
Motor neuron degeneration	Dnchc1	?	?	Genetic	Loss of function	Hafezparast *et al.*, 2003 (21)
Motor neuron disease	DIC	p150	p50, Arp1	Biochemical genetic	Loss and gain of function	LaMonte *et al.*, 2002 (22)
Retinitis pigmentosa, retinal cone dystrophy 1	Tctex-1	opsin	?	Biochemical 2-hybrid, genetic	–	Tai *et al.*, 1999 (23)
Lissencephaly	DIC	LIS1	CLIP170, NUDEL	Biochemical	–	Tai, *et al.*, 2002, Sasaki, *et al.*, 2000 (24,25)
Huntington's disease	DIC	Huntingtin	?	Biochemical	–	Caviston *et al.* 2007 (26)
Parkinson's disease	Dynein	Alpha-syn	?	Biochemical	–	Utton *et al.* 2005 (19)
Dynactin						
Huntington's disease	p150	HAP1,BDNF, HAP40	Huntingtin, Rab5, Rab 11	Biochemical 2-hybrid	–	Li *et al.*, 1998, Engelender *et al.* 1997, Gunawardena *et al.*, 2003, Gauthier *et al.* 2004, Pal *et al.* 2006, Power *et al.* 2012 (18,27–31)
Motor neuron disease	p150	?	?	Biochemical genetics	Loss of function	Puls *et al.*, 2003 (32)

transport (Fig. 1a, b). The first model proposes that axonal transport of APP occurs *via* direct interaction of the C-terminus of APP with the kinesin light chain subunits (KLC) of kinesin-1 (12,44), while the second model favors an indirect interaction between the C-terminus of APP and KLC *via* JNK (c-Jun NH2-terminal kinase)-interacting protein 1 (JIP1) (45). Regardless of whether APP-kinesin-1 interactions are direct or indirect, *in vivo* experiments in *Drosophila* and mice indicate that APP functionally interacts with kinesin-1 (10,11). Further, genetic data revealed an axonal transport phenotype in loss of function mutants of the APP-like gene in *Drosophila* (10). Overexpression of wild type human APP (hAPP) and hAPP with familial AD (FAD, mutations SWE, LOND) also showed axonal blockages but only when the C-terminus (which was proposed to interact

with kinesin-1) was present. Of importance is also the observation that loss of the APP C-terminus appears to interfere with its proper transport to presynaptic endings of axons in *Drosophila* (10). Consistent with these data, evidence suggests that a short peptide sequence (15aa) of the C-terminus of APP is sufficient for anterograde transport (46), indicating that the C-terminus is essential for the transport of APP. Reduction of kinesin-1 with excess APP showed a strong phenotypic enhancement in *Drosophila* (10) and in mouse (11), and expression of additional KLC rescued APP induced overexpression phenotypes in *Drosophila* (47). Interestingly, thus far only one single nucleotide polymorphism in the 5′ UTR of human KLC1 gene has been identified from AD patients (48). Taken together, these data indicate that normally APP has an important role in transport, and that excess APP poisons transport by titrating kinesin-1 away from other vesicles (Table I).

Axonal pathology indicative of transport defects has also been reported in AD diseased states. Studies have shown that axonal swellings or blockages can develop in brain areas affected by AD prior to tau-related neuropil changes or before amyloid deposition (11). Such swellings have also been observed before the onset of amyloid deposition in a well-characterized mouse model of AD (49). These observations demonstrate that transport problems may correspond to the earliest axonal changes observed in AD having the potential to develop tau-related abnormalities, dystrophic neurites and senile plaques. Since the secretases (BACE and presenilin) responsible for the generation of pathogenic amyloid beta (Aβ) were shown to be present within APP vesicles transported by kinesin-1 (13), perhaps axonal swellings are sites of Aβ production. Thus, these swellings could perhaps be precursors for the induction of neuronal dysfunction or death pathways. Indeed in the fly, only lines expressing human APP showed cell death, suggesting that Aβ generation can be linked to neuronal death (10). Moreover, substantial accumulations of presenilin (PS), BACE, caspase 3, caspase mediated cleavage of APP, APP and Aβ were seen within sites of injury suggesting that injured or blocked regions may be locations where active APP proteolysis and Aβ formation occur (50). Taken together these observations indicate that the axonal transport pathway can trigger APP-mediated neuronal death observed in later stages of AD.

The Putative APP Vesicle

Several works indicated that APP transports a class of vesicles that contain PS, BACE, GAP43, TrkA, and Synapsin-1 (Fig. 1a) (13,51). Groups have also proposed that APP and JNK may be together in a complex (52,53). Perhaps the APP vesicle may not only include proteins involved in trafficking and cleavage, but may also include proteins that have a regulatory role. Indeed, JIPs, scaffolding proteins for kinases of the MAPK pathway were found to interact with KLC and APP (53), (Fig. 1b). Although not integral membrane proteins, JIPs are vesicle associated (37). JIPs are thought to bind to APP *via* its phosphotyrosine-binding domain and the YENPTY-containing site in the C-terminus of APP (45,54,55). Work also suggests that the APP-JIP complex can recruit KLC, suggesting that JIPs may facilitate the recruitment of kinesin-1 to APP containing vesicles (56). It is also thought that selective phosphorylation of APP in the cell body may recruit JIP-1, which may then facilitate the binding and/or activation of kinesin-1 (57). Together these observations support the proposal that APP is present within a sub-class of vesicles that is transported within axons.

Intriguingly, the other FAD protein, PS, has also been implicated in axonal transport. A deficiency in kinesin-1 mediated transport was reported in PS1−/− and PS1 knockin M146V mice (FAD mutant in PS), suggesting that PS may have an important role in transport (58). Anterograde transport of APP and Trk receptors was shown to be impaired in the sciatic nerves of transgenic mice expressing two independent FAD-linked PS1 variants, suggesting that defects in anterograde transport may underlie FAD-linked PS1-mediated neurodegeneration through a mechanism involving impairments in neurotrophic signaling and synaptic dysfunction (51). PS is present within the APP containing vesicle (13) and both APP and PS (including the holoprotein, PS CTF and NTF fragments) are transported from the entorhinal cortex to the hippocampus *via* axons of the perforant pathway (56,58,59).

PS is thought to influence kinesin-1-mediated transport *via* a GSK3β-mediated pathway (60). Levels of GSK3β activity was found to be increased in the absence of PS, and concomitant with increased GSK3β activity, levels of KLC phosphorylation were increased, while the amount of kinesin-1 bound, membrane-bound organelles were reduced (60,61). Perhaps expression of FAD-linked PS1 impairs anterograde transport, by increasing GSK3β activity, (51,62) causing enhanced phosphorylation of kinesin-1 and dissociation of kinesin from cargoes. Recently, GSK3β was also shown to have a role in regulating the transport of APP (63). Thus, like APP, PS could have a direct role in kinesin-1 mediated transport through an action on GSK3β. Collectively, these observations demonstrate that normally two AD disease proteins are present within a subclass of vesicles, and both have important roles in axonal transport.

Axonal Transport and Huntington's Disease

A similar mechanism can be proposed for pathogenesis observed in Huntington's Disease (HD) and other polyQ diseases (Table I). HD is characterized by the preferential loss of striatal neurons, and huntingtin (HTT) accumulations are found within axons of striatal projections in HD transgenic mice before behavioral defects are seen (40). Perhaps these striatal neuronal inclusions are correlated with the loss of striatal neurons and may result due to

perturbations in axonal transport. Indeed, wild type HTT was required for efficient vesicle trafficking of cortical BDNF, and mutant HTT interfered with anterograde transport of BDNF, contributing to BDNF depletion in the striatum (28,64). These observations propose that normally HTT is transported down the axon and may have an important role in this pathway. Indeed, while HTT knock out mice are embryonic lethal, reduction of *Drosophila* HTT (dHTT) caused axonal transport defects and degenerative eye phenotypes (27). Similarly, excess of HTT containing pathogenic polyQ repeats (in the context of HTT) also caused axonal transport defects together with nuclear and cytoplasmic aggregates, neuronal cell death, suggesting that disruption of HTT or excess of pathogenic HTT can cause pathology indicative of degeneration by directly poisoning the axonal transport pathway (27,28,65,66). Thus, HTT-mediated problems in intracellular transport can trigger events, which can ultimately contribute to neuronal dysfunction (1,67).

The Putative HTT Vesicle

Although HTT is enriched in the brain, HTT is widely expressed in all tissues and is associated with vesicles and microtubules (68), indicating that a HTT- vesicle could exist during transport within axons. HTT interacts with various proteins implicated in trafficking, including HTT-associated proteins (HAP) and HTT-interacting proteins (HIP). Strikingly, like HTT, HAP-1 itself is transported both anterogradely and retrogradely, associates with vesicles and microtubules and directly interacts with the dynactin subunit p150Glued (29,30,69). The Holzbaur lab demonstrated that HTT can specifically interact with DIC (26). The Li lab showed interactions between HAP1 and KLC (17). Functional interactions between HTT and kinesin or dynein motors were also shown in *Drosophila* (27). Thus, similar to APP, HTT could function as an adaptor protein that links a subset of vesicles (Fig. 1c). Indeed, HIP-14 has been implicated in the trafficking of cystein string protein (CSP) to synapses (70). Pal *et al.* found that HAP-40 makes a complex with HTT to mediate Rab5 dependent endosomal motility, essentially functioning as a switch that may regulate the movement dynamics of early endosomes from MTs to F-actin (31). Strikingly, recent *in vivo* studies showed that reduction of HTT perturbed the MT-based transport of Rab11 vesicles, but not Rab5 vesicles (18), and decreased Rab11 localization to membrane (71). Rab11 was shown to functionally interact with both kinesin-1 and dynein motors (18) and biochemically associates with optineurin a protein that was found to be involved in HTT associations with myosin (72). Perhaps HTT/HAP-1 or 40/HIP-14/optineurin could either together or differentially target a class of vesicles that contain CSP/Rab11 for MT-based transport. Thus, similar to APP, normally HTT is present within a subclass of vesicles and has an important role in axonal transport.

Axonal Transport in ALS and PD

There is also evidence for axonal transport disruptions in other neurodegenerative diseases (Table I). In mice expressing dynamitin, long distance transport was disrupted, and this was sufficient to cause motor neuron degeneration (22). Similarly, transgenic ALS mice showed abnormalities in MT-based transport, with motor neuron degeneration (35). Although biochemical interactions between kinesin and the ALS protein SOD1 have not been seen, associations between the dynein complex and SOD1 have been reported (73). *In vivo* assays showed axonal transport deficits in presymptomatic transgenic mice expressing human mutant SOD1 indicating that impairment of transport represents one of the earliest axonal pathologies observed in ALS (42).

Recent studies have also implicated impaired mitochondrial and axonal transport with Parkinson's disease (PD) (20,74). Two PD proteins PARKIN and PINK have been shown to form a complex with KHC for mitochondria transport within axons (20) (Fig. 1d). Alternations of axonal transport and decline in motor proteins were recently observed early in sporadic cases of PD before the death of dopaminergic neurons (74). Intriguingly, α-synuclein (α-syn) another PD protein travels within axons and is predicted to associate with vesicles through its amino-terminal lipid binding repeat region and FPD mutations of α-syn abolishes this activity (75,76) (Fig. 1d). Co-immunoprecipitation and GST pull down experiments have shown that α-syn can associate with complexes containing both kinesin-1 and dynein (19). Thus, although a putative vesicle or vesicles containing PD proteins are yet to be identified, together these studies propose a role for PD proteins in axonal transport and establish that defects in long distance transport are critical in the initiation or progression of degenerative disease, which can ultimately contribute to protein aggregation and death.

There is also evidence to suggest that proteins involved in other diseases can also form complexes with motors further strengthening the role of axonal transport in disease (reviewed in Table I). Since axonal transport defects (axonal blockages) are the earliest known pathological defects currently observed in degenerative diseases, developing and targeting therapeutic interventions to an early problem could eliminate or modulate downstream deleterious effects that propagate disease progression and cell death. In addition, such a therapeutic strategy will have the ability to potentially cure or treat disease at an early state, and also be applicable for use in many different degenerative diseases.

CURRENT TREATMENT STRATEGIES FOR NEURODEGENERATIVE DISEASE

Many neurodegenerative disorders share similarity in that disease protein accumulate and neurons degenerate. However the neuronal population or the type of neurons affected differs from disease to disease. In AD, degenerating cholinergic neuritis are seen with amyloid plaques containing the 4kd Aβ fragment and abnormal accumulations of hyperphosphorylated tau in neurofibillary tangles. In HD/polyQ diseases, mutant proteins are abnormally accumulated in nuclear and axonal inclusions together with degenerating striatal neurons. In PD, α-syn accumulations are seen in structures called Lewy bodies along with dopaminergic neuronal death. In ALS, phosphorylated neurofilaments and abnormal aggregations of ubiquitinated proteins are seen in structures called Bunina bodies and Lewy body-like inclusions together with motor neuron degeneration. In Creutzfeld-Jacob disease, prion proteins are aggregated with amyloid-like structures along with extensive neuronal death (reviewed in 1). Even though the type of aggregated disease protein and the region of the brain that is affected varies from disease to disease, common mechanistic pathways may link these diseases together. Thus a single therapeutic approach can be applicable across a broad spectrum of diseases. Indeed, currently the same treatments or drugs are used in many of these diseases.

Currently there are no cures for neurodegenerative diseases, all treatments are targeted towards reducing symptoms or prolonging disease progression, and only offer short-term relief. Most are also aimed at dissolving aggregates and protecting neurons. At present there are four major types of treatment strategies practiced; chemical compounds usually acting to inhibit aggregate formation, vaccines aimed at dissolving aggregates, and gene or stem cell therapies that supplement dead or dying neurons (Table II).

All of the therapies currently used for AD focus on modulating Aβ production. Normally, APP is cleaved by two secretases, beta (BACE) and gamma (PS) secretase to produce the small 4kd Aβ fragment. During disease however, there is increased cleavage leading to over production of Aβ. It is thought that soluble Aβ polymerizes to form oligomers, which fold to generate pleated sheet fibrils, which are insoluble producing senile plaques. Inhibitors of both beta secretase and gamma secretase, which interfere with APP cleavage can be used for therapeutics, but these have several problems. One issue is that the APP cleavage site that is used by beta secretase or the identity of the amino acids involved during this cleavage is not well defined, thus generating affective beta secretase inhibitors become problematic. The other issue is that since gamma secretase cleaves many other proteins than APP, toxicity could arise due to problems associated with cleavage of these other proteins. For example, gamma secretase inhibitors can lead to problems with Notch cleavage and Notch mediated signaling which is required for development and neuronal viability. Indeed, the drug Semagacestat, a gamma secretase inhibitor failed in Phase III clinical trials due to Notch toxicity problems (77). In addition, several nonsteroidal anti-inflammatory drugs (NSAIDS) were shown to selectively lower Aβ and to decrease risk of disease. However, R-flurbiprofen, derived from NSAIDS, which is thought to act as a putative gamma secretase modulator also failed in Phase III trails (78). The metal-protein-attenuating compound (MPAC) clioquinol has been shown to dissociate amyloid plaques in post mortem human brain by mechanisms that involve chelating Cu2+ and Zn2+ (79). Evidence from Phase II trials suggests that clioquinol could decrease cognitive decline in AD (80). However the effects of clioquinol long-term are unknown.

Immunizations against the Aβ peptide could also be used as a potential treatment for AD (97). In 1999, Elan Pharmaceuticals developed the AN1792 vaccine which went into Phase II clinical trails but was abruptly discontinued as 18 of 298 (6%) patients given this treatment developed menigoencephalitis (inflammation of the brain). After stopping the treatment, 12 recovered to or close to baseline in weeks, but 6 remained with disabling cognitive or neurological problems. The inflammation of the brain was due to an inappropriate T-cell response caused by the adjuvant type, the antigen-enhancing agent used to promote antibody response. Currently, new studies are focused on minimizing T-cell responses by using alternative immunization protocols such as passive vaccines, which involve the production of monoclonal anti-Aβ antibodies. Although a passive vaccine, Bapineuzumab has been tested in clinical trials, recent reports indicate that it failed to help patients in a high-profile late stage phase III trial (77,81). Bapineuzumab is an injectable monoclonal antibody that works by attacking Aβ, thus preventing aggregate formation. Although considered a long shot to succeed, a similar drug, Solanezumab, developed by Eli Lilly & Co, has some hope as an AD vaccine (82). A study in Latin America is focued on testing an anti-Aβ antibody Crenezumab (developed by Genentech) on 100 FAD patients in Colombia. Similar to other passive vaccines, Crenezumab is designed to avoid immune responses such as swelling and microhemorrhages (83). A DNA vaccine is also being investigated against AD (84). This strategy does not contain Aβ itself, but instead uses a piece of APP. Researchers inject Aβ containing DNA coated beads into the skin of an animal ear. Once in the body, the DNA stimulates an immune response, including antibodies to Aβ (84). This vaccine however is yet to be tested in clinical trials. While still largely experimental, all of these treatment strategies are focused on dissolving amyloid plaques, which occur late in disease progression, to prevent disease symptoms, while none offer a cue for the disease. Furthermore, none of these treatments address early problems in AD.

Table II Current Treatment and Therapies for Neurodegenerative Disease

Disease	Treatment/Therapy	Mechanism	References
AD	Semagacestat	Gamma secretase inhibitor	Selkoe 2011 (77)
	R-flurbiprofen	Gamma secretase modulator	Golde *et al.* 2011 (78)
	Clioquinol	Dissociates amyloid plaques	Di Vaira *et al.* 2004, Ritchie *et al.* 2003 (79,80)
	AN1792 (Elan) vaccine	Dissociates amyloid plaques	Schenk *et al.* 2002
	Bapineuzumab (vaccine)	Dissociates amyloid plaques	Panza *et al.* 2012, Selkoe 2012 (77,81)
	Solanezumab (vaccine)	Dissociates amyloid plaques	Imbimbo *et al.* 2012 (82)
	Crenezumab (vaccine)	Dissociates amyloid plaques	Miller 2012 (83)
	DNA vaccine	Dissociates amyloid plaques	Bao-Xi *et al.* 2010 (84)
PD	Dopamine agonists (Bromocriptine, Cabergoline, Pramipexole Ropinirole)	Stimulates dopamine receptors	Brooks 2000 (85)
	Levadopa or L-dopa	Dopamine precursor	Cotzias *et al.* 1969 (86)
	Deep brain stimulation	Stimulates brain regions	Kringelbach *et al.* 2007 (87)
HD	Riluzole	Protects neurons	Landwehrmeyer *et al.* 2007 (88)
	Minocycline	Inhibits enzymes that promote neuronal suicide	Huntington Study Group, 2010 (89)
	Trehalose	Blocks aggregation of proteins	Katsuno *et al.* 2004 (90)
	Implantation of fetal neuronal tissue	Replaces dead neurons	Bachoud-Lévi *et al.* 2006 (91)
	Antidepressants, sedatives or antipsychotic drugs	Psychological problems	Alpay *et al.* 2006, Mason *et al.* 2009 (92,93)
ALS	Riluzole	Protects neurons	
	Vascular endothelial growth factor (VEGF), Insulin-like growth factor-1 (IGF-1)	Protects motor neurons	Henriques *et al.* 2010 (94)
	Synthetic SOD1 RNA	Against mutant SOD1	Miller, *et al.* 2005 (95)
	Grafted stem cells	Delivers vital growth factors to dying neurons	Glass *et al.* 2012 (96)

In PD more than half of the dopaminergic neurons are dead leading to loss of dopaminergic neurotransmission. Thus the simplest therapeutic strategy would be to increase dopamine production. However, dopamine cannot be administered directly since it does not pass the blood–brain barrier (98). Thus current pharmacological treatment strategies are focused on using dopamine agonists, compounds that directly stimulate postsynaptic striatal dopamine receptors such as Bromocriptine, Cabergoline, Pramipexole, and Ropinirole (85). Moreover, Levadopa or L-dopa, a precursor compound that the brain converts into dopamine has been extremely successful in the treatment of PD since the 1960s (86). L-dopa is an intermediate in the pathway of dopamine synthesis, which can pass through the blood–brain barrier. Since long-term use of most of these pharmacological treatments has been shown to cause decreased sensitivity, and there is constant difficulty in finding the right combination or dosage of drugs to circumvent individual symptoms, very often cocktails are used in treatments (99). Currently co-administration of agonists and L-Dopa offer the best relief for patients (100). Regardless of these approaches, over time current pharmacological treatments appear to decrease in their effect to bring relief. For these patients, the surgical treatment Deep Brain Stimulation (DBS) is available. Although the FDA approved DBS for PD in 2002 (87), due to the complexity of the surgery it is currently considered only for patients who no longer have an acceptable quality of life due to shortcomings of pharmacological therapy.

Current treatments for HD address only symptoms (101). Several drugs help with muscle contractions, side effects that limit the patients' mobility. Some drugs are used to delay or stop the destruction of neurons. For example, Riluzole a drug used against ALS, is also used in HD to protect neurons. Riluzole is currently in a European clinical trial involving 450 HD patients (88). Minocycline, an antibiotic in mice, inhibits the action of enzymes that set off neuronal suicide. However it failed a phase III clinical study (89). Trehalose, a natural sugar made by various desert plants, blocks aggregation of proteins (90). Antidepressants, sedatives or antipsychotic drugs are also used to help with psychological problems observed in HD patients (92,93). Implantation of neuronal tissue from aborted fetuses into the brains of HD patients has been done with some success, but some suffered hemorrhages (91). However this treatment has several technical and ethical issues since fetal tissue is difficult to obtain and prepare.

Currently, Riluzole is the only drug used in the treatment of ALS. It is aimed at symptomatic relief, prevention of complications, and maintenance of maximal function and optimal quality of life (102,103). It is thought to work by protecting motor neurons from overexposure to glutamate. Unlike dopamine, Riluzole can pass the blood–brain barrier. There are several other potential treatments for ALS that are under investigation. Administration of neurotropic factors such as the vascular endothelial growth factor (VEGF) and the insulin-like growth factor-1 (IGF-1) protects motor neurons (94). However, these cannot pass the blood–brain barrier so strategies such as direct injections must be developed to administer this therapy. Two clinical trails using viral delivery strategies are currently being done in Sweden. Since small RNA strands can interfere with the production of toxic proteins in neurons and glial cells, a synthetic RNA strategy is being designed against the ALS protein SOD1 (95). A mechanical pump to deliver the synthetic RNA directly to the cerebrospinal fluid of patients is also being tested on FALS patients. Similarly grafted stem cells can act as biological pumps for delivering vital growth factors to damaged motor neurons and this strategy is currently in Phase I trials (96).

Thus all current treatments for neurodegenerative diseases are engineered towards dissolving protein aggregates, maintaining optimal function of neurons and bringing systemic relief. Therefore, novel therapeutic interventions must be developed to target early defects, such as axonal accumulations so that transport pathways are restored for proper neuronal function, with the rationale of curing the disease and preventing cell death.

DEVELOPING SYNTHETIC VESICLE-LIKE STRUCTURES AS TREATMENTS FOR AXONAL TRANSPORT DEFECTS

Currently, there are no strategies focused on modifying axonal blockages. Since disease pathologies such as abnormal protein accumulations are seen in many human neurodegenerative diseases, which highlight interruptions in long distance transport, targeting this pathway for therapeutics is of benefit. Perhaps developing potential synthetic vesicle-like structures with modifier functionality against axonal accumulations should be considered. In this context, first, synthetic structures must be identified that can not only bind molecular motors but are small enough that they can easily enter axons and function as synaptic vesicles by interacting with endogenous motors to move on MTs to the site of defect. Second, a potential synthetic vesicle-like structure should be engineered to have the ability to carry a caged modifier to axonal transport defects such that the drug can be released at the site of the defect (Fig. 2).

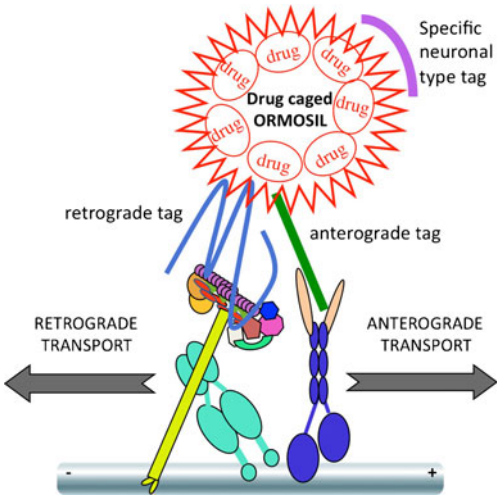

Fig. 2 A proposed therapeutic synthetic-vehicle targeted to defects in axonal transport. An ORMOSIL nanoparticle is conjugated with an anterograde tag and or a retrograde tag. The anterograde tag will bind to endogenous kinesin motors for anterograde transport, while the retrograde tag will bind to endogenous dynein motors for retrograde transport. The specific neuronal type tag will allow targeting to specific neuronal populations within the brain. Modifier drug(s) can be caged inside the porous ORMOSIL particle which can be released once the synthetic particle arrives at an axonal block. The size of the ORMOSIL particle will not change even through these conjugations are added to ORMOSIL.

Identifying Synthetic Structures

In vitro studies using purified motor proteins indicated that motors can bind to carboxylated beads and move along MTs (46,104). Charged carboxylated fluorescently labeled 100 to 500 nm beads have been shown to move down the axon (104). Western blot analysis showed that kinesin had a high affinity for these beads (105). The Bearer Lab (46) demonstrated that 15aa from the C-terminus of APP conjugated to beads was sufficient for directed anterograde transport. These conjugated beads traveled by fast axonal transport at average rates of 0.53 μ/s demonstrating that endogenous motors can be recruited to a synthetic material. Although these observations suggest that beads can utilize the endogenous motor machinery for movement, the beads that were tested were too large. Moreover, since axons are very narrow, steric hindrance due to large beads (106) or swollen mitochondria could potentially block transport (107).

Viruses can be used as carriers to build synthetic vesicle-like structures since many viruses normally use the axonal transport pathway for infection. For example, the Herpes Simplex Virus (HSV), a neurotropic virus that causes the cold sore, travels back and forth within neuronal processes at different stages in its life cycle. HSV, which is about 100–200 nm in diameter, does not encode its own motors and must recruit cellular motors from the host either directly *via* a viral protein that binds motors or indirectly by recruiting a motor receptor from the infected cell. Studies showed that HSV travels at

velocities four-times faster than synaptic vesicles and ten times faster than charged beads (108).

Although little is known about how impermeable, solid surfaced beads can be utilized to build therapeutic tools, experiments using viral-directed strategies for treatment have been performed and have shown some promise. Viral carriers have been successfully used in gene therapy treatments in animal models of disease and injury (109,110). However, prolonged exposure of these treatments in humans has caused excessive immune responses and insertional mutagenesis, activating oncogenes or silencing tumor-suppressor genes, leading to the development of cancer (111,112). Further complicating the long-term use of viral based therapies is the possibility that engineered viruses can revert back to their pathogenic form (113). Additionally, some clinical trials of viral based therapies have resulted in human deaths, thus halting further use of viral vectors (114,115).

Since the beads and viruses that have been tested thus far are much larger than endogenous synaptic vesicles (145), and since the viruses are also risky due to unforeseen immune responses, small sized nanoparticles could be bioengineered to develop safe and effective treatment approaches for humans. Moreover, because of their small size, nanoparticles have the potential to be conjugated with several linkers and modifier compounds for effective treatment strategies without dramatically affecting their over-all size (116,117).

Recent advances in the development of several forms of engineered nanostructures have shown potential to tremendously impact human health, especially by expanding new avenues in biomedical research aimed at developing novel types of therapeutic tools for disease. Currently, various nanoparticle-based formulations with numerous modifications exist. However, many are yet to be fully tested in biological organisms. The examination of these particles *in vivo* is key, since the same properties that make nanoparticles so attractive for use in biomedicine could prove deleterious when nanoparticles interact with living cells within a living organism. Although most studies to date have used nanoparticles as imaging tools (photoacoustic agents (118,119), QD labeling of MTs (120), no studies have yet used nanoparticles to develop vesicle-like structures with specific control or therapeutic functionality for neurodegenerative disease. Below I detail a nanoparticle based strategy and identify a novel nanoparticle that is ideally suited to develop a therapeutic synthetic-vesicle.

Characteristics of a Therapeutic Nano-Vesicle

An ideal therapeutic vesicle will have the ability to travel to the location of the defect and specifically treat the problem in a "target and treat" approach without affecting its environment. Such a "target and treat" approach needs to be developed to an early event in disease such as axonal blockages, in order to stop or prevent deleterious outcomes such as behavioral and pathological phenotypes which are observed at the end stages of the disease. In this context, recently, micro devices loaded with kinesin have been developed and used *in vitro* (121). Although *in vivo* studies have not yet been performed with these devices, such structures have the potential of being used as smart biosensors within an organism for remote sensing in human health. Similarly, synthetic nano-vesicles could be built and used, not as biosensors, but as vehicles that carry therapeutics to axonal blockages within an axon.

To achieve such a goal, knowledge of how biological machinery function *in vivo* must be adapted to engineer and assemble nano-chemical structures, which have functionality within an organism. These chemical structures must mimic biological structures and not cause adverse effects inside an organism. Such structures must also have the ability to travel to specific neuronal populations. In addition, within the axon these synthetic structures must be able to attach to endogenous motors for movement on MT tracks. Therefore, ideally, a therapeutic nano-vesicle-complex for potential treatment specifically directed or targeted to axonal blockages must include the kinesin/dynein motor to propel the vesicle-like particle *in vivo*. To accomplish this, the synthetic vesicle must have linker(s) that have the capability of binding to a specific motor(s) for directional travel on the MT track (Fig. 2). Although axonal transport defects are observed in many neurodegenerative diseases, the type of aggregated disease protein and the region of the brain that is affected vary from disease to disease. Thus, targeting or directing therapeutics to specific types of neurons located in specific regions of the brain is also crucial. To achieve this, synthetic vesicles must also carry tags that can direct them to a specific location or neuron. Further, these synthetic vesicles must also carry treatment compounds loaded within them so that they can correct the defect once they reach the site of the problem (Fig. 2). Such a therapeutic strategy can direct treatments to specific neuronal populations and to a specific defect so that beneficial effects are maximized while deleterious, secondary, global effects are minimized. Towards achieving this goal, a specific nanoparticle must be identified that facilitate the development of a synthetic vesicle that not only mimic biological vesicles but also has functionality *in vivo*. In this context, a novel class of nanoparticles called ORMOSIL is of interest (122).

Organically Modified Silica Nanoparticles as Synthetic Vesicles

There is tremendous interest in the use of new silica-based nanoparticles in the development of therapeutic applications for drug/gene delivery. Specifically, organically modified silica (ORMOSIL) particles are porous particles and have been found to be less toxic than quantum dots (QDs) (123,124) with superior delivery and cell uptake (116,125). These particles

have also shown robust gene transfer efficiency in brain cells and neuronal tissues (126,127,147).

These particles have a number of advantages over other particles in that they can be easily and reproducibly synthesized with ultra-low size (radius approx 10 nm) and narrow size distributions (7.5–12.5 nm radius). In addition, the design of silica-based particles has shown promising potential for use in controlled gene/drug delivery for actively targeted optical imaging and other biotechnological and biomedical applications (116,128). These inert, optically transparent materials can be conjugated with any desired fluorophore (visible/NIR), leading to the generation of robust, fluorescent particles (128,129). Furthermore, these porous particles have a tunable pore size, and can be conjugated with bioactive molecules such as enzymes, genetic material such as DNA/RNA, chemotherapeutic drugs, or tagged with a specific peptide for a specific biological function (130,131,146), thus allowing targeting to specific cell types, tissues, or specific neuronal populations. In addition, the chemistry of silica provides the opportunity for a variety of surface functionalities (hydroxyl/amino/thiol/carboxyl groups) (132,133), which can be used to incorporate therapeutic and biotargeting molecules or to release/activate/inactivate a caged compound. Efficient optical imaging of tumor cells *in vitro* has already been demonstrated using ORMOSIL conjugated with fluorophores and targeting ligands (134,135). These particles have also been tested for potential use in photodynamic therapy (PDT) and as a targeted optical probe for imaging of pancreatic cancer cells (117,125,136). In such studies ORMOSIL was rapidly taken up by tumor cells in culture and showed phototoxicity in treated cells after irradiation with light, demonstrating their potential in PET/SPECT imaging while preserving their therapeutic functionality (117). Thus ORMOSIL has the potential to be used for both diagnosis and PDT in cancer, using a "see-and-treat" approach (137). In addition, its unique characteristics can also be explored for treatment strategies for neurodegenerative disease.

ORMOSIL *In Vivo*, in the Brain, and in Neurons

For any nanoparticle to be used in an application to whole organisms and in treatments targeted to living neurons, the *in vivo* biocompatibility and safety of these particles must be evaluated given their expected and potential use as therapeutic agents in human health. Although the potential of these porous ORMOSIL particles are enormous, these particles have not been fully tested *in vivo* in whole organisms. Work in mice has begun to evaluate the systemic effects of ORMOSIL after intravenous injections, and work in *Drosophila* has recently evaluated the long-term effects of ORMOSIL during development of an organism by oral administration (122,138). In both these studies the *in vivo* applicability of these particles show great promise.

Bio distribution studies using mice intravenously injected with ORMOSIL showed a greater accumulation of particles in liver, spleen, and stomach than in the kidney, heart, and lungs (138). Almost 75% of injected ORMOSIL accumulated in the liver and spleen, whereas the lungs, kidney, and heart accounted for less than 5% of accumulations. These observations, together with clearance studies over a period of 15 days, indicate the excretion of these particles from the organism, with 20% of the particles being released out of the animal. Moreover, histological analysis of these tissues containing particles confirmed the absence of adverse effects such as cell death or abnormalities such as morphological defects (138). Physical and neurological evaluations on ORMOSIL injected mice for more than 1 month failed to show any behavioral or neurological problems compared to wild type none injected mice indicating that the presence of ORMOSIL is not a burden to the organism. Taken together, these observations indicated that ORMOSIL slowly clears out of the animal *via* excretion, without exerting toxicity and tissue damage. Thus slow excretion could be beneficial in a therapeutic setting for the release of the carrier particle after its treatment function.

Recent studies to evaluate ORMOSIL biocompatibility and distribution have been extended in *Drosophila*, since in the fruit fly, the entire developmental cycle of the organism can be investigated (122). Oral administration of ORMOSIL, at three different doses, from the time of the larval stage though puparation to eclosion of adults failed to show any toxic effects. As in mice, ORMOSIL was accumulated in the larval skin, malpigian tubes, and in the gut, indicating excretion of ORMOSIL. However, continued oral administration during development showed ORMOSIL in adult fly brains. No tissue damage was seen in larval tissues and no behavioral or neurological problems were observed in both ORMOSIL fed larvae or adult flies compared to wild type (122). While similar studies are needed in mammalian models, these observations suggest that ORMOSIL has great potential for systemic administration in routine clinical use in both diagnostics and therapeutics.

For any particle to be successful as a therapeutic tool for neuronal disease in humans, it must effectively and specifically incorporate into living neuronal tissues and must not cause any adverse effects to living neuronal cells or tissue. Recent studies have demonstrated that ORMOSIL particles satisfy these criteria. Direct, stereotaxic injection of ORMOSIL particles into mouse brains have been performed and showed no adverse toxicity effects in brain tissue (116,126,138). ORMOSIL was seen in neuron-shaped cells in the area of injection, the substantia nigra per compacta region of the brain, suggesting that these particles were intraneuronal. The efficiency of *in vivo* injections was found to be equal to

or greater than that obtained using HSV reported earlier (126,138). Furthermore, no marked toxic effects were observed in these ORMOSIL injected mice even after 2 months of injections. Further studies showed that targeting the FGF receptor 1 signaling pathway using ORMOSIL conjugated with DNA nanoplexes to efficiently transfect recombinant nuclear forms of FGFR1 and its FGF-2 ligand enhanced the latent potential of neuronal stem/progenitor cells to undergo neuronal differentiation, thus promoting neurogenesis in adult brains (127). Therefore, such studies demonstrate that ORMOSIL particles can be used for *in vivo* gene transfer methods directed to the brain or to specific neuronal populations with no adverse affects to normal neuronal differentiation and function. Furthermore, these *in vivo* injections indicated that ORMOSIL can be effectively targeted to neurons, with specificity of action dictated by the specific tag on the particle.

Recent work also demonstrated the potential of ORMOSIL in neurons, both within an organism and in neuronal cultures. In *Drosophila*, ORMOSIL readily incorporated into neurons after incubation (122). ORMOSIL was seen in the cell bodies and in neuronal projections and the presence of these particles did not affect neuronal growth. Immunohistochemical analysis suggests that ORMOSIL penetration into neurons was mostly *via* endocytosis (122). Within larval segmental neurons, ORMOSIL particles were seen as puncta. These particles did not cause adverse affects to the normal MT-based transport pathway and no axonal defects were observed. ORMOSIL incorporated larval brains were also devoid of neuronal death. Furthermore, these particles did not interfere with the normal transport of synaptic vesicles tagged with GFP as observed by *in vivo* imaging. However, ORMOSIL particles did not show movement within axons in the time frame tested, perhaps due to the fact that there was no direct contact between molecular motors and endocytosed ORMOSIL. Thus further study will be needed to engineer a "moving" particle that can directly interact with endogenous motors. In this regard, previous work has shown that direct injection of ORMOSIL into mouse brains successfully transfected DNA conjugated ORMOSIL into neuronal cells (127). ORMOSIL conjugated with various bioactive molecules such as transferrin, monoclonal antibodies against anti-claudin and anti-mesothelin has successfully targeted these particles directly to pancreatic cancer cells in cell culture (116). Furthermore, PEGylated or cell-penetrating peptide anchored ORMOSIL could be generated to directly target ORMOSIL to neurons so that endogenous motors readily have access to these particles in a neuron (139). PEGylation enables penetrating of both the cell membrane as well as the nuclear membrane (140,141). Moreover several other cell-penetrating peptides exist that can successfully enable translocation across biological membranes (142). Although further study is ongoing to build cell-penetrating forms of particles, these observations demonstrate that ORMOSIL is ideal for developing potential diagnostic and therapeutic tools since they can incorporate into neurons without inducing toxicity.

CONCLUSIONS AND FUTURE PERSPECTIVES

Currently, there are no effective treatments or cures for many of the neurodegenerative diseases affecting an aging baby-boomer generation. Although there is a great need to develop effective treatment strategies, the ongoing problem with many of the current therapeutic treatments is that they do not target specific areas or regions of the brain that are affected by disease, and most of these treatments are developed against pathologies that often occur later in disease. In addition, current treatments often have secondary effects that can be more devastating than the disease. Thus therapeutic interventions must be developed to directly target specific areas of the brain and specific populations of neurons early during disease. In this context, developing therapeutics targeted to the long distance axonal transport pathway is of importance, since several studies have indicated that perturbation of axonal transport is an early precursor in neuronal dysfunction.

Recently the use of engineering in biomedical research has impacted human welfare tremendously by opening new avenues in the development of novel types of therapeutic tools. In this regard significant research is focused on biomaterial engineering and the development of various nanoparticle-based formulations for potential medical use. Thus synthetic vesicle-like nanostructures can be designed and built to function as therapeutic vehicles for axonal transport defects. In this context, ORMOSIL nanoparticles have shown great promise. An ORMOSIL therapeutic vesicle will contain targeting tags that direct it to specific neurons and will associate with endogenous motor proteins using tags that bind to specific motors, so that it can be precisely moved to an axonal block. Once at the block, the caged modifier within the porous therapeutic vesicle will be released to dissolve axonal blockages and restore transport. Such a therapeutic system can target and treat a specific early defect in neurodegeneration. A recent study has shown that release of nanoparticle-loaded BDNF, a neurotrophic factor was able to rescue auditory neurons in the cochlea of guinea pigs with senorineural hearing loss (143). Antibody-conjugated PEGylated particles were able to modulate neuronal survival by targeting Aβ aggregates in a cell culture system (144). Similarly, a nanoparticle based therapeutic system specifically targeted to axonal defects could also modulate axonal blockages.

With the identity of effective compounds that modify axonal blockages or modulate early pathologies related to disease and dysfunction, future *in vivo* studies in *Drosophila*, mice, and human neurons should be focused on testing the functionality of therapeutic nano-vesicles or devices. Such rigorous investigations will expand our understanding of how nanoparticle-based therapeutic delivery systems can be effectively developed to target living neurons in a whole organism. Investigations must also be directed towards the application of such nanoparticle-based systems in humans. Whether intravenous injections, intraventricular brain injections, oral consumption, or nasal spray can be used to deliver such a therapeutic system to humans also needs to be explored. The advent of high-resolution *in vivo* brain imaging technology will enable studies to explore how such therapeutic vesicles/devices can be targeted to a specific brain region, to specific neurons affected by disease, and how effectively these devices can function to modify the defect.

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