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REVIEW ARTICLE

TARGETING α-SYNUCLEIN AGGREGATION FOR PARKINSON'S DISEASE TREATMENT

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ABSTRACT

Parkinson's disease (PD) is a debilitating neurodegenerative disorder for which a cure has yet to be found. A pathological hallmark of PD is intracellular inclusions called Lewy bodies (LBs), which contain insoluble fibrillar aggregates of the protein α-synuclein (AS), along with lipids and other proteins in lower concentrations. There is substantial evidence that the progression of PD, and other LB disorders, is linked to the rate at which aberrant aggregates of AS accumulate in the brain, although LBs themselves may not be the pathological culprits. Several strategies are thus being pursued for targeting and modifying the aggregation of AS in the search for future therapies and to enhance our understanding of the causes of PD. This review presents the current evidence for the role of AS in PD and the factors responsible for protein aggregation, and highlights some of the potential therapeutic approaches. One promising approach is the use of small molecules to prevent or otherwise modify the formation of cytotoxic intermediates that form on-pathway to the fibrillar aggregates. Examples of effective inhibitors and methods for their evaluation are given and the prospects for their eventual clinical application are assessed.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder affecting over 4 million people worldwide. PD is characterized by deterioration of the substantia nigra and loss of dopaminergic neurons, leading to impaired motor and cognitive function. Symptoms usually first appear in patients over 50 and the risk of developing the condition increases with age, although 5% of cases are diagnosed under the age of 40. The etiology is unknown, but genetic factors are responsible in a minority of cases and oxidative stress has been strongly implicated. There is no cure for PD at present, but improvements in symptoms can be achieved with drugs or surgical intervention (1). PD is the second most common neurodegenerative disorder after Alzheimer's disease (AD) and is an important health concern for Western countries.

Current PD drugs replenish, emulate or increase the bioavailability of the neurotransmitter dopamine. The major drug treatment is levodopa, the natural precursor for dopamine. It acts to replace the dopamine deficiency caused by the loss of dopamine-producing cells. PD patients can become desensitized to levodopa after long-term use, however, and dyskinesia is a notable side effect. Other treatments for PD, often coadministered to overcome levodopa-induced dyskinesia, include dopamine agonists, which act by directly stimulating remaining dopamine receptors (2), glutamate antagonists (3) and cholinesterase inhibitors, also used in isolation to treat other dementias (4). Extensive research is being conducted in an effort to gain a better understanding of the neurodegenerative processes associated with these diseases and to identify and evaluate potential new treatments.

PD AND PROTEIN AGGREGATION

PD is the most prevalent of a group of neurodegenerative disorders defined collectively as synucleinopathies in recognition of a common pathological marker, the 140-residue protein α-synuclein (AS), concentrated at the presynaptic termini of the central nervous system (CNS) (5). In PD brains, insoluble aggregates of AS are localized within intracellular inclusions called Lewy bodies (LBs), chiefly within the substantia nigra. LBs are 10-μm diameter circular assemblies with a dense core surrounded by a peripheral halo, consisting of fibrillar AS and a mixture of other proteins and lipids (6). Positive identification of LBs is made post mortem, but early clinical diagnosis of PD is difficult owing to the similarities in the symptoms of PD and dementia with Lewy bodies (DLB), which has led some to argue that both are manifestations of the same disease process at different stages of progression. It is unclear whether the inclusions contribute to neurodegeneration or are the end product of a pathological process involving the accumulation of AS.

The physiological function of AS is unknown, but the protein associates with presynaptic vesicles (7) and may play a role in regulating vesicle formation and plasticity (8, 9). Monomeric AS is intrinsically disordered in aqueous solution (10), but it can misfold and aggregate into amyloid-like filamentous assemblies (11), characterized by a cross-β architecture consisting of arrays of β-strands aligned perpendicular to the fibril axis. LBs and their precursors, Lewy neurites, are stained strongly by antibodies specific for AS, indicating that AS constitutes the major filamentous protein (12). A central hydrophobic region (residues 61-95) of AS, identified as the non-amyloid component (NAC) of AD plaques (13), is believed to be responsible for aggregation. Various residues within the NAC region have been suggested as the key initiating sequences, including residues 66-74 (14), 68-78 (15) and 71-82 (16). Studies of AS aggregation in vitro indicate that self-assembly proceeds via one or more partially folded intermediates, or "protofibrils", which can propagate in various ways to form either the insoluble filaments or fibrils associated with LBs, or precipitate as amorphous aggregates (17). Dopamine-related catecholamines have been shown to undergo oxidative ligation to monomeric AS and prolong the lifetime of the protofibrils formed after aggregation (18). This observation may explain the dopaminergic selectivity of AS-associated neurotoxicity in PD.

α-SYNUCLEIN AS A DRUGGABLE TARGET FOR PD

Studies in humans and animal models have uncovered substantial evidence that AS is a contributing factor in PD and LB diseases. A link between AS aggregation and the onset of parkinsonian symptoms has been found in *Drosophila melanogaster* overexpressing human AS, which exhibit phenotypic inclusions resembling LBs in the brain, with associated loss of dopaminergic neurons and motor processes (19). The progression of PD and other LB disorders appears to be linked to processes that increase the rate at which aberrant aggregates of AS are formed. These processes include the elevation of protein concentration via either upregulation of AS expression or a decreased rate of AS degradation. Families have been identified with duplication and triplication of the AS gene (*SNCA*), causing high expression and accumulation of AS in cell bodies of neurons and early onset of PD (20, 21). Three missense mutations within the amino terminal of the AS gene have been discovered in patients with rare early-onset autosomal dominant forms of PD. The first (A53T) was discovered in Italian and Greek families (22), followed by A30P in a German family (23) and E46K in a Spanish family (24). Individuals with these mutant versions of AS show LB formation and other neuropathological characteristics associated with PD. In vitro studies indicate that A53T and E46K AS oligomerize and fibrillize more rapidly than wild-type protein, whereas A30P AS shows an increase in oligomerization, but not fibrillization (25-27). In addition, altered forms of AS, such as truncations (28), or chemical modifications by oxidative reactions (29) have been identified and studied as possible causes of increased AS concentration resulting in aggregation and inclusion formation.

Several transgenic mouse models have been produced expressing wild-type and mutant variants of AS (recently reviewed in 30, 31). In general, higher levels of AS expression result in greater dopaminergic and behavioral deficits, implying that a threshold level of AS has to be reached before disease symptoms occur, although region-specific expression patterns in the brain may also be important. Indeed, discrepancies in the results of various studies may be accounted for by the different strains of animals and promoters used for expression, resulting in varying protein levels and distribution throughout the brain (32-39). The first reported work on transgenic mice overexpressing wild-type AS showed development of intraneuronal inclusions in similar areas of the brain affected by synucleinopathies (40). These inclusions contained AS, although not in the fibrillar form characteristic of LBs, but dopamine levels were reduced and motor performance hindered, consistent with disease progression. Double-transgenic mice coexpressing AS and β-amyloid showed pathology resembling DLB and LB variants of AD (41). Mice overexpressing wild-type, A53T and A30P AS show accumulation of protein in neuronal cell bodies and neurites throughout the brain, with varying neuronal abnormalities and motor deficits similar to PD (34).

It is not known which species of AS are pathogenic, but AS and AS peptide fragments increase in neurotoxicity upon aggregation (42) and monomeric AS is not toxic to cells. Work by the Lansbury group found that AS forms annular protofibrils structurally resembling bacterial pore-forming toxins, indicating a possible pore-forming action contributing to the toxicity of these protofibrils (43-45). The permeabilization of membrane vesicles could cause leakage of dopamine into the cytoplasm within brain regions affected by disease. Mice overexpressing AS have phenotypic motor deficits and a loss of dopamine-producing cells, but no fibrillar deposits, supporting the argument that protofibrils are the toxic species (35). Regardless of the precise mechanism, there is substantial evidence that pathological aggregation of proteins, including AS, involves the formation of a key, partially folded intermediate that promotes specific protein–protein contacts (17, 46).

THERAPEUTIC APPROACHES

A potential treatment for PD that is attracting much interest is modification of the AS aggregation pathway, either by targeting AS aggregation directly or by manipulating the external factors that promote aggregation (see 47). The direct modulation of AS aggregation or the dissolution of fibrils by small molecules is perhaps the most popular strategy in this context, and is reviewed more comprehensively in the following section. Other more speculative approaches are outlined below.

Inhibition of post-translational modification of AS

AS contains several sites for phosphorylation, nitration, oxidation and ubiquitination (48). Post-translational modifications affect AS aggregation and degradation, and targeting these modification pathways could provide a method of lowering the concentration of aberrant pathological aggregates. AS is phosphorylated extensively in LBs and in vitro studies have shown enhanced fibril formation upon phosphorylation at S129 (49), suggesting prima facie that manipulation of AS phosphorylation might present therapeutic opportunities. The role of phosphorylation has been called into question by a more recent structural study, however, which found that phosphorylation of S129 inhibited the formation of AS oligomers and fibrils, while increasing the flexibility and destabilizing the long-range tertiary interactions of the monomers (50). The effect of phosphorylation in vivo, and whether modification occurs before or after inclusion formation, is thus unclear and further inves-

tigation will be necessary to evaluate this therapeutic avenue. Nitration or oxidation of tyrosine residues promotes the formation of stable oligomers and polymers of AS (29). AS within LBs is nitrated at tyrosines 39, 125, 133 and 136, although it is not known whether this modification occurs before or after accumulation of the protein aggregates (51). AS has been found to be ubiquitinated within inclusions at lysine 6, 10 and 12 (52, 53), suggesting that a default occurs in the degradation pathway by the ubiquitin–proteasome system, which could be targeted by drugs.

Promotion of chaperone activity

Molecular chaperones function under cellular stress conditions to control protein folding. Chaperones are candidates for targeting neurodegenerative disease because of their ability to lower the concentration of aberrant aggregates and prevent the accumulation of misfolded proteins. One chaperone, HSP104p, known to resolubilize aggregated denatured proteins, protects against AS fibril formation in vitro (54). Another chaperone, HSP70, protected cells from AS-induced toxicity in a cell culture model cotransfected with both AS and the chaperone (55). In the same work, detergent-insoluble AS aggregates isolated from AS transgenic mice were reduced in AS mice cross-bred with HSP70-overexpressing mice. In addition, the directed expression of HSP70 in *Drosophila* protected against dopaminergic neuron loss, one of the primary defining features of PD (56). Taken together, these results suggest that enhancement of chaperone activity could in principle be used to reduce AS aggregation and toxicity associated with cell loss in PD. The correlation between chaperone function and AS aggregation and toxicity is not completely resolved, however. For example, HSP70 mutated within the ATPase domain maintains the ability to enhance AS degradation and prevent aggregate formation, but is unable to abolish AS toxicity (57). Moreover, targeting or upregulating chaperones may create complications arising from the disruption of their essential cellular functions.

Promotion of inclusions

There is some evidence that intracellular inclusions associated with PD and Huntington's disease may in fact protect against pathological damage (58). Promoting the formation of such inclusions could lower the concentration of toxic intermediates. Compounds have been identified that increase the formation of inclusion bodies in CHO cells while lowering the pathological effects of synuclein expression (59, 60). The consequences for tissue damage of increasing the volume of inclusions are not known, however, and the clinical impact of such an approach is far from clear. For example, a recent study demonstrated that enhancing polyglutamine aggregation in live cells increases oxidative stress and, in turn, cell death, suggesting that promotion of amyloid inclusions may not be beneficial (61).

Downregulating AS expression

Hippocampal infusion of murine-specific siRNA has been shown to downregulate AS production (62). This approach is currently useful as a research tool for understanding the role of AS in disease, but could eventually provide the basis for a therapeutic strategy.

SMALL-MOLECULE INTERVENTION IN AS AGGREGATION

The design and discovery of agents that arrest the onset or propagation of protein aggregation could provide drug candidates for a range of protein misfolding diseases, including AD and PD (63). A wide variety of compounds prevent the formation of toxic AS protofibrils and sarkosyl-insoluble filaments and stabilize nontoxic monomers, dimers or soluble oligomers over a defined time period (Fig. 1). Two principal mechanisms have been proposed for the inhibition of fibril formation by small molecules. In one model, the inhibitor-stabilized oligomers are SDS-resistant intermediates formed on-pathway to protofibrils and filaments (64). In an alternative model, inhibitors bind to natively unfolded AS monomers and promote unstructured, nontoxic aggregates that assemble off-pathway from the toxic species (65). The precise mechanism(s) of inhibition is a matter of intense debate, which has been fueled by the difficulty in distinguishing unambiguously the on- and off-pathway forms. The assembly pathway of inhibitor-stabilized AS aggregates was recently studied using epitope-specific antibodies (64). Some antibodies were sensitive to the conformational changes associated with AS aggregation and showed different immunoreactivity to monomers and fibrils. Inhibitor-stabilized oligomers and dimers showed intermediate reactivity between that of monomers and fibrils, from which it was inferred that the stabilized species were on-pathway to the fibrillar endpoint of aggregation.

Organic molecules

Numerous small molecules interfere in the AS assembly process and reduce the cytotoxicity of aggregates. Masuda and coworkers carried out extensive in vitro testing of 79 chemically diverse compounds (66), identifying potent AS (aggregation) inhibitors, some of which are summarized in Table I. Of these, several polyphenols —neuroprotective compounds present in plants, fruits and vegetables— reduced the formation of sarkosyl-insoluble AS filaments with an IC_{50} of < 10 μM, which has been regarded by some to be a concentration threshold for effective inhibitors (66, 67). Several of the polyphenols (exifone, myricetin and dopamine chloride), as well as the phenothiazine lacmoid and the porphyrin hematin, promoted the formation of on-pathway AS dimers and SDS-resistant oligomers. The AS dimers and

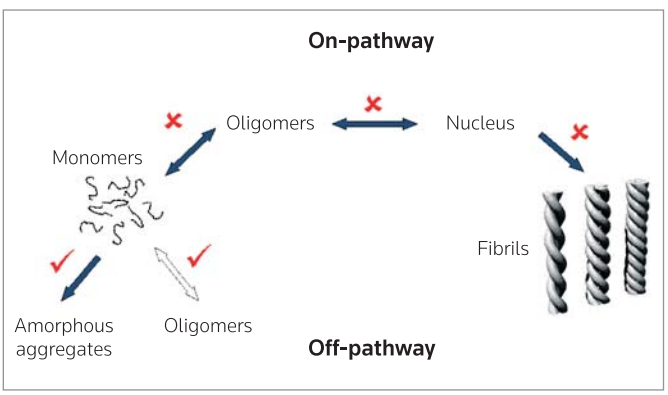

Figure 1. Scheme of the competing aggregation pathways for AS. Steps marked with a tick could be promoted by small molecules and peptides to stabilize nontoxic aggregates. Steps marked with a cross could be targeted by inhibitors preventing aggregation into fibrils or partially folded cytotoxic intermediates.

Table I. *Summary of small-molecule inhibitors of α-synuclein (AS) aggregation in vitro.*

Class of compound	Inhibitor	
	IC_{50} > 10 μM	IC_{50} < 10 μM
Polyphenols	Cyanidin (–)-Epicatechin-3-gallate Epigallocatechin Myricetin Quercertin 2,3,4-Trihydroxybenzophenone	Baicalein[a] Dopamine chloride Epigallocatechin gallate Exifone Hypericin Rosmarinic acid
Porphyrins	Hematin Phthalocyanine tetrasulfonate[b]	Ferric dehydroporphyrin IX
Phenothiazines	Lacmoid Perphenazine	
Polyene macrolides	Amphotericin B Filipin III	
Congo red and derivatives	Chlorazol black E FSB	Congo red BSB
Terpenoid	Asiastic acid	

Taken from Ref. 66, with additional sources as indicated. Compounds tested for protection against AS cytotoxicity are underlined. [a]Taken from Ref. 135; [b]taken from Ref. 68.

oligomers stabilized by dopamine chloride, exifone, lacmoid and hematin were isolated and found to be significantly less toxic to SH-SY5Y cells than were the AS protofibrils and filaments (66). In separate work, two forms of the compound phthalocyanine tetrasulfonate were evaluated for protection against AS cytotoxicity. The Cu(II) form of phthalocyanine enhanced AS aggregation and cytotoxic effects, whereas the apo-form inhibited AS aggregation and reduced the cytotoxicity of AS aggregates (68).

Several other compounds have been tested in vitro and in vivo for their ability to prevent AS fibril formation. Curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione), the major yellow pigment extracted from turmeric, is a brain-penetrating polyphenol that has shown neuroprotective effects in a mouse model of PD (69). Curcumin prevents the formation of β-amyloid oligomers and fibrils and reduces amyloid in vitro and in vivo (70), and has also been shown to inhibit AS fibril formation and stabilize soluble AS oligomers and dimers (71). Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen prevented the formation of AS fibrils and also destabilized preformed fibrils in vitro in a concentration-dependent manner (72). This observation is particularly interesting because NSAIDs have been shown to reduce the risk of PD in epidemiological studies; AS inhibition by these drugs might thus provide a specific protective effect in addition to the classical antineuroinflammatory indication. The antibiotic rifamycin is another compound that disaggregates preformed AS fibrils, stabilizing monomers and soluble oligomers, although the toxicological effects of the stabilized species were not reported (73). Finally, nicotine and related compounds have recently been shown to inhibit AS fibrillization and stabilize soluble oligomers, providing evidence that cigarette smoke contains neuroprotective chemicals responsible for the low incidence of PD in smokers (74, 75).

Peptides

An attractive approach in the search for AS inhibitors is to design short peptides that hydrogen-bond to the target protein and block aggregation (76-78). The peptide sequences are selected so as to be complementary to a specific sequence —or self-recognition element (SRE)— within the target protein, but are modified to incorporate nonproteinogenic or other non-native amino acids. Although peptides are not good drugs for neurodegenerative disorders, they are synthetically facile and flexible templates that can be optimized to produce more drug-like small molecules.

Examples of peptide modifications that prevent the aggregation of amyloid proteins (including the AD β-amyloid polypeptide and the human islet amyloid polypeptide associated with type 2 diabetes) are β-sheet-breaking amino acid (e.g., proline) substitutions (79), *C*-terminal polyhydroxy (e.g., polyethylene glycol) chains (80), *N*-methylated amino acid substitutions (81), addition of *N*- and *C*-terminal-blocking or -disrupting groups (82), replacement of amide bonds with ester linkages and the introduction of α-disubstituted amino acids (83). Peptides designed to be complementary to the AS sequence have been tested for their ability to reduce fibril formation. An *N*-methylated variant of the AS sequence V^{77}AQKTV and a sarcosine-substituted *N*-methylated derivative of the AS sequence 68-78 have been shown to block the aggregation of AS and AS-derived peptides, and reduce cytotoxicity (77, 78). Peptides substituted with γ-aminobutyric acid (GABA) or β-alanine have also recently been shown to be effective AS aggregation inhibitors and to reduce cytotoxicity by 20% at 20 μM (83). More detailed investigation is necessary, however, to elucidate the nature of the nonfibrillar forms of AS that are stabilized by these peptides.

The AS homologue β-synuclein, which lacks the fibrillogenic NAC region and does not aggregate, appears to act as a natural inhibitor of AS aggregation in vivo (84, 85). The two synuclein proteins are predicted to be present in presynaptic termini of human brain in approximately equal concentrations (86). Transgenic mouse models have shown that the presence of β-synuclein is able to reverse disease-associated functional deficits induced by overexpression of AS and reduce the formation of LBs by up to 40% (87). In vitro aggregation studies show that inhibition of AS fibrillization by β-synuclein is

concentration-dependent. An equimolar concentration of β-synuclein delayed and reduced fibrillization of AS, resulting in increased amorphous aggregates, whereas a fourfold molar excess of β-synuclein completely prevented oligomerization of AS (88). Deletion of the *N*-terminal amino acids 1-15 from β-synuclein removes the potential to prevent AS aggregation, suggesting that these residues are important for the inhibition of AS. Small peptides derived from β-synuclein have shown antiaggregatory and neuroprotective properties (89).

Antibodies

Antibody fragments that bind to a target protein can be engineered to be expressed intracellularly as intrabodies that bind to their specific target within cells. Intrabodies can contain antibody fragments that specifically recognize different conformations of AS, and they may have therapeutic value in controlling the misfolding and aggregation of AS in vivo when expressed in dopaminergic neurons (reviewed in 90, 91). Single-chain antibody fragments have been engineered that can specifically target, bind to and stabilize monomeric AS, inhibiting disease-associated aggregation and cytotoxicity (92-94). Antibody fragments have also been produced that bind to and isolate different oligomeric forms of AS, specifically small (0.4 and 1 nm) particles produced early during AS aggregation (95) or larger (> 4 nm) species produced upon continued incubation in vitro (96).

A recent study tested the ability of intrabody binding to AS monomers and oligomers to reduce toxicity in a mammalian cell model (97). It was shown that cellular toxicity is abolished by the addition of a secretion signal to the oligomer-binding antibodies to facilitate clearance, further supporting the role of oligomeric aggregates in neurodegenerative disease. Immunization in a mouse model of PD with extracellular human AS antibodies can reduce accumulation of intracellular aggregates of AS and show protection against neurodegeneration (98). Antibody fragments could be applied in a similar way as therapeutic vaccines to specifically target and remove potentially cytotoxic AS species in PD.

INFLUENCE OF COFACTORS ON AS AGGREGATION

The rate of AS fibrillization is affected by various external factors that could be targeted by drugs in order to modulate aggregation indirectly. Aggregation in vitro is modified by polyelectrolytes found in intracellular inclusions, including nucleic acids (99-101) and, intriguingly, the extracellular glycosaminoglycans (GAGs) heparan sulfate and chondroitin sulfate (102, 103). Heparin and associated GAGs increase the rate of AS aggregation and fibril yield in vitro due to a direct interaction with AS fibrils. This implies that GAGs influence the formation and structural properties of AS fibrils in vivo. It has been suggested that leakage of GAGs into neurons could be a stimulating factor for the AS aggregation observed in LB disease (104). Targeting the interaction between heparan sulfate proteoglycan and the β-amyloid polypeptide has been considered as an anti-amyloid therapy for AD. This has been achieved by altering the structure of the sugar moiety of heparan sulfate (105-108) and with low-molecular-weight sulfated compounds that compete with glycan binding sites (109, 110). A similar approach could be applied to AS, although the intracellular location of the aggregates presents an extra challenge.

Post mortem studies have identified several manifestations of increased oxidative stress within neurodegeneratively diseased brains, including elevated iron content, impaired mitochondrial function and alterations to antioxidants (111). Two pathways relating AS and oxidative stress within disease have been suggested. One is the oxidation and nitration of tyrosine residues producing stable cross-linked AS polymers (29). The other is based on the observation that LB formation and AS aggregation are stimulated by cytochrome *c* (112) and metal ions, including iron (113) and copper (114, 115), in the presence of hydrogen peroxide. These conditions develop within cells under oxidative stress (116) and can be experimentally blocked using antioxidants. Chelation therapy using iron chelators is a further approach that can be targeted to reduce cellular oxidative stress in PD. Chelators act by scavenging excess redox-active metals present to form a nontoxic metal complex, which can be excreted. A second function of iron chelators could be to prevent iron and other metal ions binding to AS and promoting aggregation to form toxic products (117).

METHODS FOR SCREENING INHIBITORS

Primary testing of AS inhibitors in vitro requires a range of techniques to monitor the effects on aggregation and toxicity. The density and morphology of the insoluble aggregates deposited at the endpoint of aggregation, analogous to the species localized in LBs, is determined principally by electron microscopy (118) and atomic force microscopy (119), while the size of the aggregating species is followed by size exclusion chromatography (66) and dynamic light scattering (77). Attendant amyloid characteristics are routinely detected by the diagnostic fluorescent dyes thioflavin S/T (120) and Congo red (121). Less routinely, NMR methods have recently been used to design an *N*-methylated peptide inhibitor of AS (77). Importantly there is no firm relationship between the size and morphology of AS aggregates and their pathology. Cytotoxicity evaluation is thus a critical component of initial testing, by determining the viability of cells treated with exogenous AS aggregates (122) or cells overexpressing AS (123).

The development of animal models has provided a gateway to pharmacological evaluation of AS inhibitors. Immunization in a PD mouse model transgenic for human AS has been shown to reduce accumulation of disease-associated aggregates (98), consistent with in vitro studies (124), and also to reduce the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity (125). The environmental toxin MPTP is known to induce parkinsonism clinically indistinguishable from idiopathic PD (126, 127). Transgenic mice expressing β-synuclein do not show any neurological abnormalities, and when crossed with AS transgenic mice, β-synuclein protects against AS aggregate accumulation and motor alterations observed in these mice (85).

PROSPECTS

In summary, a variety of methods are available for modifying the aggregation, cytotoxicity and pathogenicity of AS in vitro and in vivo, and have provided useful research tools with which to elucidate the causes and progression of PD. The feasibility of targeting AS aggregation for PD has yet to be demonstrated clinically, and future success will depend in part on whether AS turns out to be a key factor in

the disease, or whether it plays only a minor supporting role. Directing compounds such as AS inhibitors to their site of action into living cells and across the blood–brain barrier is also a challenge, although some progress has been made to this end (128). Using a polyarginine peptide delivery system, cell-permeable AS inhibitor peptides based on residues 68-72 of AS (GAVVT) were able to inhibit the DNA damage induced by Fe(II) and Cu(II) in neuronal cells transfected with AS (A53T) (129). AS inhibitor peptides without this delivery system did not reverse DNA damage. Nanoparticles and nanodevices are also ideal candidates for targeting neurodegenerative therapies owing to their small and controllable size (1-100 nm) and the ability to modify their functionality (130, 131). Drug encapsulation in nanoparticles can enable passage across the blood–brain barrier (132, 133) and can be designed to target specific proteins or aggregates such as the AD β-amyloid polypeptides (134) and, in principle, AS.

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DISCLOSURE

The authors state no potential conflicts of interest.

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