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Novel treatment strategies targeting alpha-synuclein in multiple system atrophy as a model of synucleinopathy

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Abstract

Neurodegenerative disorders with alpha-synuclein (α -syn) accumulation (synucleinopathies) include Parkinson's disease, Parkinson's disease dementia, dementia with Lewy bodies and multiple system atrophy (MSA). Due to the involvement of toxic α -syn aggregates in the molecular origin of these disorders, developing effective therapies targeting α -syn is a priority as a disease-modifying alternative to current symptomatic treatments. Importantly, the clinical and pathological attributes of MSA make this disorder an excellent candidate as a synucleinopathy model for accelerated drug development. Recent therapeutic strategies targeting α -syn in *in vivo* and *in vitro* models of MSA, as well as in clinical trials, have been focused on the pathological mechanisms of α -syn synthesis, aggregation, clearance, and/or cell-to-cell propagation of its neurotoxic conformers. Here we summarize the most relevant approaches in this direction, with emphasis on their potential as general synucleinopathy modifiers, and enumerate research areas for potential improvement in MSA drug discovery.

Keywords

synucleinopathy; multiple system atrophy; alpha-synuclein; therapies; clinical trials

Introduction

In the US alone over 1.5 million people [1] are affected by Parkinson's disease (PD), PD dementia, dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). In all these neurodegenerative disorders the synaptic protein α -synuclein (α -syn) accumulates in the CNS and peripheral organs, and for that reason the term “synucleinopathies” is often used to refer to them [2, 3]. Despite the fact that synucleinopathies are nowadays the second leading cause of dementia and parkinsonism in the elder population, no significant strides have been made yet towards the development of effective disease-modifying therapies that significantly stop or delay the progression of the neurodegenerative pathology.

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In synucleinopathies, α -syn aggregates can be observed within neurons in the form of Lewy bodies (LBs) or neuronal cytoplasmic inclusions (NCIs), and within glial cells as glial cytoplasmic inclusions (GCIs) [4-10]. Interestingly, α -syn may also be present in the amyloid plaques of Alzheimer's disease, where it co-aggregates with amyloid beta [11, 12]. Moreover, co-aggregation of α -syn with other proteins such as tau or TDP-43 has also been reported in synucleinopathies [13, 14]. These observations suggest a common neuropathological mechanism to most neurodegenerative disorders involving the abnormal aggregation and accumulation of a few toxic protein conformers. It is very likely that in the causes and consequences of this pathological protein aggregation, and in the ability of these toxic conformers to propagate within the CNS, lays the foundation of most age-dependent neurodegenerative disorders. Most importantly, targeting these mechanisms is a promising therapeutic alternative for the development of disease-modifying alternatives for those ailments.

MSA as a synucleinopathy model for the development of therapies targeting α -synuclein

While in recent years considerable effort has been devoted at understanding the pathogenesis of PD, less is known about MSA, which is a rapidly progressive and fatal neurodegenerative disease characterized by parkinsonism, dysautonomia [15, 16] and α -syn accumulation within oligodendroglial (GCIs) and neuronal cells (NCIs) [17, 18]. This accumulation is accompanied by neuroinflammation [19, 20], demyelination [21, 22] and neurodegeneration [23, 24]. Parkinsonian features reflecting striato-nigral neurodegeneration predominate in 80% of MSA patients in the United States (MSA-P subtype), while the major motor feature in 20% of patients is cerebellar ataxia due to olivo-pontocerebellar atrophy (MSA-C subtype) [25]. Clinically, the rapid progression, the lack of response to L-DOPA [26], and pathologically the extensive accumulation of α -syn within oligodendrocytes differentiates MSA from other synucleinopathies [27].

However, the mechanisms through which α -syn accumulates within oligodendroglial cells in MSA are not completely understood. One possibility is that α -syn is produced by oligodendroglial cells, which in turn over-express or fail to intrinsically clear α -syn; the other is that α -syn propagates from neurons to oligodendrocytes due to neurons over-expressing and/or displaying defects in the physiological mechanisms of α -syn clearance. While some studies suggest that low levels of α -syn mRNA might be detected in oligodendroglial cells [28], others have not been able to confirm these findings [29-31]. However, given the high levels and widespread distribution of α -syn aggregates in MSA, it is possible that both propagation and oligodendroglial α -syn expression are occurring simultaneously. Supporting the possibility of propagation, several studies have shown that α -syn aggregates can transmit from neuron to neuron [32], neuron to astroglial [33] and oligodendroglial cells [34], and oligodendroglial to astroglial cells [20], leading to neuronal dysfunction [35, 36], apoptosis [32] and neuroinflammation [20, 33]. Furthermore, recent studies have shown that injection of homogenates from MSA brains propagate α -syn pathology in a prion-like fashion in the murine brain [37, 38]. Despite the considerable clinical and pathological overlap between PD and MSA-P in early disease stages, MSA

progresses more rapidly and is fatal, making it a good candidate for accelerated drug development. Importantly, the use of MSA as a model in the development of disease-modifying therapeutic strategies targeting common pathophysiological mechanisms for all synucleinopathies (such as α -syn aggregation, prion-like propagation and autophagy deficits) might reduce the number of negative clinical trials and redirect resources towards earlier development stages [39, 40].

Novel therapies targeting alpha-synuclein in MSA and related disorders

Despite sharing other neuropathological features, the accumulation of α -syn is the pathological characteristic that defines synucleinopathies as a group of disorders, and it is probably mechanistically involved in the origin of the disease. It follows that reducing the α -syn pathology would have important disease-modifying consequences for synucleinopathy patients, especially at early stages. Importantly, in MSA, α -syn accumulation is prominent in both neurons and glial cells (astrocytes and oligodendrocytes). While astroglial α -syn accumulation is normally observed in synucleinopathies, oligodendroglial α -syn accumulation is specific to MSA [41]. Although in this review we will focus on α -syn-targeted, disease-modifying approaches for MSA, other review articles have described α -syn-targeted approaches for other synucleinopathies [42-44] or other novel therapeutic approaches for MSA [45].

The molecular events leading to the neuropathological accumulation of α -syn may include increased expression and synthesis of the protein, increased aggregation, defective protein clearance, and cell-to-cell prion-like propagation (Figure 1). Although dysfunction of these molecular mechanisms has been observed in the brain of synucleinopathy patients, it is still unclear which one of these processes is the driving force at the origin of the disease. While it is widely accepted that most over-production of α -syn occurs in neurons, expression of the *SNCA* mRNA in oligodendrocytes has also been detected [28]. Abnormal aggregation and accumulation of α -syn due to defect in protein clearance mechanisms has been observed in primary oligodendrocytes [46]. Finally, intercellular propagation is believed to be responsible for the accumulation of α -syn in oligodendrocytes and other glial cells, and neuron-to-neuron α -syn propagation is probably concomitant although more difficult to quantify. Moreover, increased α -syn aggregation may lead to defective protein clearance and autophagy deficits [47] and, therefore, to increased release of α -syn to the extracellular environment and consequent spreading. The prion-like characteristics of α -syn aggregates may also lead to abnormal α -syn conformational changes and trigger its accumulation in acceptor cells [37, 38, 48, 49]. Therefore, it follows that α -syn-centric therapies should target one or more of these dysregulated mechanisms in order to effectively reduce α -syn pathology.

1. Therapies targeting α -syn expression

An increased dosage of the α -syn gene (*SNCA*) can cause PD, as observed in patients with *SNCA* triplication [50]. Moreover, elevated expression levels of *SNCA* are found in affected regions of the PD brain, supporting the hypothesis that an increase in α -syn expression is associated with the development of sporadic PD [51]. This increased expression of α -syn observed in synucleinopathies could be therapeutically targeted by the delivery of antisense,

small interfering RNA (siRNA) or short hairpin RNA (shRNA) constructs directed against the *SNCA* mRNA. In this sense, in animal and *in vitro* models of PD with *SNCA* overexpression, gene therapy with antisense constructs has been reported to have disease-modifying effects [52, 53].

It can be inferred that these therapies might be also applicable to the treatment of other synucleinopathies such as MSA. Although a genetic link between *SNCA* and MSA has not been found (e.g. *SNCA* multiplications do not increase the risk of suffering from MSA [54]), and some studies have reported that total *SNCA* mRNA levels are not significantly elevated in MSA brains [28], single nucleotide polymorphisms at the *SNCA* locus are significantly associated with an increased risk for MSA [55]. Moreover, the translational repression that could be achieved by the use of antisense sequences in MSA models may prevent the excessive synthesis of this protein. However, further research is still needed in this direction.

2. Therapies targeting α -syn aggregation

Aggregated forms of α -syn are more resistant to degradation than monomeric, non-toxic forms [56], which would explain the prominent α -syn accumulation observed in MSA brains despite the apparent lack of increased *SNCA* expression. Aggregation of α -syn on the form of dimers, oligomers, proto-fibrils and fibrils is believed to be an early event on the α -syn pathology [57]. Oligomers and proto-fibrils are reportedly the toxic species [58, 59], while fibrillar aggregates have been traditionally associated with reduced toxicity [41]. However, several reports have shown that fibrillar forms can also propagate through the brain and contribute to the neurodegenerative pathology [60-63]. In this sense, therapies targeting this molecular mechanism include anti-aggregation agents and small molecules that act as molecular chaperones. An example of those compounds is Anle138b, an α -syn aggregation inhibitor that strongly blocks oligomer accumulation, neuronal degeneration, and disease progression in animal models of PD [64]. Other small molecules designed to stabilize or reduce the formation of toxic α -syn aggregates are also being developed at the preclinical stage, such as the conformational stabilizer NPT200-11 (Neuropore Therapies).

Degenerative movement disorders such as MSA, PD, Huntington's disease and progressive supranuclear palsy, exhibit increased iron content in brain [65-67]. In the case of MSA, dysregulation of iron metabolism may be relevant to the origin of the pathology, as oligodendrocytes are the predominant iron-containing cells in the brain, and iron stimulates α -syn aggregation [68]. Based on those observations, the use of iron chelation agents has been explored as a potential treatment for MSA. A phase III clinical trial with epigallocatechin gallate (EGCG) as an α -syn anti-aggregation approach for MSA is currently ongoing (NCT02008721). EGCG, a polyphenolic flavonoid extracted from green tea leaves, inhibits the formation of toxic α -syn oligomers *in vitro* and is able to transform α -syn oligomers into non-toxic species [69]. There is also evidence for a neuroprotective effect of EGCG in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of PD [70]. EGCG acts as an antioxidant and iron chelation agent, and this may in part explain its anti-aggregation properties. For these reasons, EGCG has also been evaluated in multiple sclerosis and Huntington's disease [71, 72], as well as in many non-neurological conditions.

3. Therapies targeting α -syn degradation and clearance

Recent evidence supports the notion that failure of protein clearance mechanisms (e.g., autophagy) might play a role in the process of α -syn aggregation [36, 73], release [73] and subsequent accumulation of α -syn pathological species in neurons and oligodendroglial cells in MSA and other synucleinopathies (Figure 2). Moreover, microglial pro-inflammatory activation leading to a M1 phenotype may result in a reduction in the ability of microglia to effectively clear out propagating, prion-like α -syn species, resulting in increased accumulation within oligodendrocytes and other α -syn acceptor cells [74]. Therefore, pharmacological stimulation of α -syn clearance could result in both reduced accumulation and impaired cell-to-cell propagation (Figure 2). Restoring clearance mechanisms in the α -syn donor cells (neurons, oligodendrocytes) would reduce its release to the extracellular medium and therefore its cell-to-cell spreading; and restoring clearance in acceptor cells (oligodendrocytes) would reduce α -syn accumulation after its internalization. Treatments targeting α -syn clearance include stimulation of autophagy mechanisms (e.g. nilotinib [75]), modulation of the phagocytic activity of microglia, and the use of extracellular degrading enzymes. Interestingly, some anti-inflammatory approaches are able to reduce oligodendroglial α -syn accumulation, suggesting that regulating the activation of microglia might have the double effect of reducing inflammation and stimulating α -syn clearance. We have recently reported a reduction in α -syn accumulation by anti-inflammatory agents such as certain antidepressants [20, 76] and immunomodulatory drugs [77] in transgenic (tg) mouse models of synucleinopathy.

Some studies have suggested that myeloperoxidase (MPO) inhibition might represent a novel candidate treatment strategy against MSA-like neurodegeneration, acting through its anti-inflammatory and antioxidant properties. In the 3-nitropropionic acid (3-NP) model of MSA, early-start treatment with MPO inhibitors reduces motor impairment and rescues vulnerable neurons in striatum, substantia nigra pars compacta, and other brain areas. Interestingly, MPO inhibition has been associated with suppression of microglial activation and results in reduced intracellular aggregates of α -synuclein [78]. However, a more recent report has shown that delayed-start MPO inhibition fails to impact on motor impairments and neuronal loss in contrast to the disease-modifying efficacy of early-start therapy in the 3-NP mouse model of MSA [79]. Despite these conflicting results, a Phase II clinical trial to assess the safety and tolerability of the MPO inhibitor AZD3241 in MSA patients is currently ongoing (NCT02388295, AstraZeneca). A similar study recently completed in PD patients (NCT01603069) has shown promising results regarding safety and tolerability.

In our laboratory we have analyzed the effects of the immunomodulatory drug lenalidomide in a tg mouse model of MSA that expresses human α -syn under the control of the oligodendroglial myelin basic protein promoter (MBP- α -syn) (Figure 3). Non-tg and MBP- α -syn tg mice were treated with either vehicle or lenalidomide at 100 mg/kg for 4 weeks, as previously described [77]. Neuropathologically, we observed a reduction in the number of α -syn positive cells in striatum after lenalidomide treatment (Figure 3A, 3B), together with a normalization of the pro-inflammatory microglial phenotype as measured by Iba1 staining in the same brain region (Figure 3A, 3C). The mechanisms that might lead to the reduction in the number of α -syn positive oligodendrocytes in lenalidomide-treated animals are still

under investigation; however, our preliminary results suggest that the pro-inflammatory activation of microglia in MBP- α -syn tg animals might impair microglial functioning, and the reduction in this microglial activation induced by lenalidomide could lead to an improvement in the phagocytic activity of these cells towards extracellular α -syn. We have previously observed a similar reduction in pro-inflammatory microglial activation by lenalidomide in a tg mouse model of PD [77]. Interestingly, administration of lenalidomide to non-tg animals may lead to a microgliosis phenotype in striatum that is not accompanied by other neuropathological or behavioral deficits (not shown), suggesting that the multi-target actions of immunomodulatory drugs and other anti-inflammatory compounds might account for their ability to modulate inflammation while simultaneously reducing α -syn accumulation.

4. Targeting α -syn cell-to-cell propagation

Recent studies have shown that injection of homogenates from MSA brains propagate α -syn pathology in a prion-like fashion in the murine brain [37, 38]. In MSA, neuronal cells (donors) release α -syn aggregates into the extracellular environment in clear vesicles [80] and exosomes [81] (Figure 2), and this extracellular α -syn may be taken up by other neurons, oligodendrocytes and astrocytes (acceptors) via endocytosis [82] (Figure 2). Now believed to be a central event at the origin of the neuropathology, cell-to-cell propagation of α -syn has recently become a prominent pharmacological target. The spreading of α -syn can be inhibited by compounds that recognize extracellular α -syn and stimulate its clearance, directly by degradation or indirectly through microglial mechanisms. These include the use of immunotherapy against α -syn (active and passive), and extracellular α -syn degrading enzymes such as neurosin [83-85], matrix metalloproteinases, and plasmin [86].

Active immunotherapy with small peptides mimicking abnormal α -syn conformations (AFFITOPEs) has yielded positive results in tg models of synucleinopathy [87, 88], leading to a Phase I clinical trial with the AFFITOPEs PD01A and PD03A in patients with early MSA (NCT02270489, Affiris AG). AFFITOPEs mimicking the C-terminus region of α -syn elicit an immune response specific to α -syn oligomers that leads to decreased accumulation of α -syn oligomers, reduced neurodegeneration, and improved motor and memory deficits in α -syn tg models of PD and DLB [88], and to reduced demyelination in a tg model of MSA [87]. Moreover, Phase I passive immunization approaches with the α -syn antibodies PRX002 (NCT02157714, Prothena Biosciences) and BIIB054 (NCT02459886, Biogen) are also currently ongoing. It has been shown that monoclonal antibodies recognizing an epitope in the C-terminus of α -syn are effective at clearing intracellular α -syn aggregates, inhibiting α -syn propagation, and preventing C-terminus cleavage of the protein in tg mouse models of PD [89-92]. Similar reports using antibodies against the N-terminus of α -syn show that passive immunotherapy is able to clear α -syn aggregates, reduce α -syn propagation and diminish motor deficits in diverse *in vitro* and *in vivo* models of PD [93, 94]. This evidence further supports the value of immunotherapy against α -syn as a disease-modifying option for MSA and related synucleinopathies. In this sense, recent efforts have been directed towards the development of therapeutic antibodies able to detect individual conformational species of α -syn [95-98]. These antibodies could be used to discriminate among protein

conformers [99], potentially including MSA-specific α -syn aggregates [62], for the differential treatment of synucleinopathies or for diagnostic purposes.

Lastly, we have recently observed that a modified, systemically delivered neurosin genetically modified for increased half-life and containing a brain-targeting sequence for delivery into the CNS, is able to reduce α -syn levels in oligodendrocytes and the spreading of α -syn to glial cells in a mouse model of MSA [85]. A similar lentiviral construct is also able to induce neuropathological and behavioral improvements in a tg mouse model of PD [84]. Taken together, these results suggest that the use of gene therapy with brain-targeted α -syn degrading enzymes may warrant further investigation as therapy for MSA and other disorders with α -syn propagation.

Final remarks

In this review we provide a brief overview of the current therapeutic approaches targeting α -syn for the treatment of MSA as a model synucleinopathy. Given the clinical progression and the pathophysiological characteristics of MSA, there is an urgent need for effective disease-modifying therapies for this disorder with potential application to similar neurodegenerative diseases with α -syn accumulation. This is even more relevant in the case of approaches aimed at blocking the cell-to-cell propagation of α -syn, as it is a pathological feature especially relevant in MSA compared to other synucleinopathies. However, it is also important to consider that MSA is a rapidly progressing neurodegenerative disorder often misdiagnosed as PD, therefore identifying early biomarkers and developing accurate diagnostic methods for MSA is a critical step for the success of therapeutic approaches targeting early pathological events such as α -syn accumulation. In this sense, the development of alternative animal models for MSA that fully reproduce all the neuropathological features of MSA is a research priority [100]. Early diagnostic approaches for MSA that have been recently explored include, among others: cerebrospinal fluid biomarkers such as microRNA levels [101, 102], neurofilament light chain levels [103], or the combination of α -syn and the ratio of phospho-tau to total tau [104]; differential pharmacodynamics of subacute levodopa [105]; identification of disease-specific α -syn conformations [62, 98, 99]; progressive thinning of the retinal nerve fiber layer and the macular ganglion cell complex [106]; etc. Moreover, combining the use of two or more biomarkers might be more successful than using single markers to increase sensitivity and specificity for the diagnosis of MSA [107]. Finally, elucidating the molecular mechanisms driving the oligodendroglial accumulation of α -syn in MSA would undoubtedly open the door for the identification of effective disease-modifying alternatives.

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Figure 1. Therapies targeting α -syn in MSA

The pathological behavior of α -syn in synucleinopathies has been associated with the dysregulation of its synthesis, aggregation, clearance and/or cell-to-cell propagation ability. The regulation of such mechanisms is interconnected, leading to a prion-like positive feedback loop and to the progression of the disease. Therefore, current disease-modifying therapeutic strategies targeting α -syn in MSA are primarily focused on reducing α -syn synthesis, aggregation and clearance, and/or blocking the spreading of toxic α -syn conformers.

Figure 2. Deficits in protein clearance mechanisms might increase α -syn propagation and accumulation in MSA

In MSA, it is believed that oligodendrocytes accumulate α -syn after a process of propagation from neurons and/or other oligodendroglial cells. The inhibition of clearance mechanisms such as autophagy might increase α -syn propagation by reducing its intracellular degradation in neurons and oligodendrocytes, and therefore potentiating its release to the extracellular medium. Moreover, clearance deficits may also lead to the intracellular accumulation of propagating α -syn within oligodendrocytes. Finally, impairments in microglial phagocytic activity hinder the physiological clearance of extracellular α -syn and further potentiate cell-to-cell propagation and glial pro-inflammatory activation.

Figure 3. Lenalidomide reduces α-syn accumulation and microgliosis in the striatum of MBP-α-syn tg mice

Mice (n=2-5 per group) were treated via gavage with lenalidomide 100 mg/kg or vehicle (0.5% methocellulose) for 4 weeks, and immunohistochemistry performed as previously described [77]. **A**, α-synuclein and Iba1 immunostaining of the striatum of MBP1-α-syn tg mice treated with vehicle or lenalidomide. Scale bar, 100 μm. **B**, Cell counts of α-syn positive cells in striatum. **C**, Optical density quantification of Iba1 staining in striatum. Statistical comparisons were performed using one-way ANOVA with Tukey's post-hoc test. *, p<0.05; **, p<0.01; ***, p<0.001. All experiments described were approved by the animal subjects committee at the University of California, San Diego (UCSD), and were performed according to NIH guidelines for animal use.