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Polymer brain-nanotherapeutics for multipronged inhibition of microglial α-synuclein aggregation, activation, and neurotoxicity

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ABSTRACT

Neuroinflammation, a common neuropathologic feature of neurodegenerative disorders including Parkinson disease (PD), is frequently exacerbated by microglial activation. The extracellular protein α-synuclein (ASYN), whose aggregation is characteristic of PD, remains a key therapeutic target, but the control of synuclein trafficking and aggregation within microglia has been challenging. First, we established that microglial internalization of monomeric ASYN was mediated by scavenger receptors (SR), CD36 and SRA1, and was rapidly accompanied by the formation of ASYN oligomers. Next, we designed a nanotechnology approach to regulate SR-mediated intracellular ASYN trafficking within microglia. We synthesized mucic acid-derivatized sugar-based amphiphilic molecules (AM) with optimal stereochemistry, rigidity, and charge for enhanced dual binding affinity to SRs and fabricated serum-stable nanoparticles via flash nanoprecipitation comprising hydrophobe cores and amphiphile shells. Treatment of microglia with AM nanoparticles decreased monomeric ASYN internalization and intracellular ASYN oligomer formation. We then engineered composite deactivating NPs with dual character, namely shell-based SR-binding amphiphiles, and core-based antioxidant poly (ferrulic acid), to investigate concerted inhibition of oxidative activation. In ASYN-challenged microglia treated with NPs, we observed decreased ASYN-mediated acute microglial activation and diminished microglial neurotoxicity caused by exposure to aggregated ASYN. When the composite NPs were administered *in vivo* within the substantia nigra of fibrillar ASYN-challenged wild type mice, there was marked attenuation of activated microglia. Overall, SR-targeting AM nanotechnology represents a novel paradigm in alleviating microglial activation in the context of synucleinopathies like PD and other neurodegenerative diseases.

1. Introduction

Aggregated and increased levels of extracellular α-synuclein (ASYN) are characteristic of Parkinson's disease (PD). As the primary cell type to rapidly internalize and degrade ASYN, microglia are a natural target for regulating ASYN trafficking and associated neuroinflammation [1]. While microglia play a role in the physiological clearing of ASYN, disruptions to the lysosomal clearance pathways caused by overabundance of ASYN [2] or by common mutations associated with idiopathic and familial PD [3] result in dramatic increases in extracellularly released ASYN [4]. Elevated levels of extracellular ASYN could in turn contribute to the interneuronal spreading of pathological ASYN species, particularly since neuron-to-glia transmitted ASYN has been observed to form pathogenic aggregates [5]. A considerable body of work also indicates that intercellularly transmitted ASYN from neuron-to-glia triggers microglial activation [5,6]. The result of chronic and excessive ASYN exposure and microglial activation is the secretion

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of neurotoxic substances including reactive oxygen species [7]. Sustained microglial activation has a disproportionate influence on dopaminergic (DA) neuronal loss, given the relative abundance (4.5 fold microglia relative to neurons) [8] of microglia within the substantia nigra (SN), a prominent site of DA neuronal cell death, and is exacerbated by the inherently low antioxidant capacity of DA neurons. As DA neurons die, further ASYN monomers and aggregates are released into the extracellular space, propagating a cycle of microgliosis and neurotoxicity. Preventing initial ASYN-mediated microglial activation and ASYN aggregation during PD pathogenesis are two key precursor events that could halt the progressive loss of DA neurons.

A number of factors that are known to enhance the intracellular ASYN aggregation, including low local pH [9], high calcium concentrations [10], restricted space and high macromolecule concentrations [11], are all characteristic of the vesicular space and account for why ASYN within intracellular vesicles has been observed to be especially prone to aggregation [12]. In order to reduce intracellular ASYN aggregation, it would then be desirable to identify and disrupt receptor-mediated internalization by microglia. Multiple membrane receptors, including toll-like receptors [13], scavenger receptors [6c,14], and integrin MAC1 [15] have been implicated in microglial interactions with pathological proteins and activation in neurodegenerative disease. While TLR4 [16], TLR2 [13b], and MAC1 [17] have been implicated as receptors that interact with ASYN, scavenger receptors have remained relatively unexplored. SRA1 and CD36 have been noted to mediate microglial interaction and activation by amyloid β [14b,18], which forms similar fibrillar structures as ASYN [19]. We hypothesized that scavenger receptors are a key target for regulating ASYN trafficking and reducing intracellular ASYN aggregation.

Research has been done previously to develop synthetic compounds that mimic the charge and hydrophobicity of scavenger receptor-binding ligands, including using sulfatide derivatives [20] and modified synthetic phospholipids [21]. Using amphiphilic macromolecules (AMs) as biomimetic synthetic ligands based on sugar-based backbone, aliphatic side chains, and a hydrophilic poly(ethylene glycol) (PEG) tail [22], new structure-activity relations indicated that optimal hydrophobicity, stereochemistry, and charge promote binding affinity to scavenger receptors SRA1 and CD36 [23]. Due to their amphiphilic nature, these molecules can be complexed around hydrophobic core solutes via kinetic flash nanoprecipitation techniques, forming nanoparticles (NP) with the potential for drug encapsulation and demonstrated resistance to AM release in serum-rich environments [24]. In this study, we advance microglial scavenger receptor-mediated internalization of ASYN as a therapeutic target in the pathway of ASYN aggregate formation and propagation.

The overall paradigm we investigated was the possible role of scavenger receptor-binding NPs in modulating the internalization of ASYN in microglia and the subsequent intracellular accumulation of aggregated forms of ASYN (Fig. 1). Our central hypothesis is that by targeting SR-expressing microglia, the AM NPs will counteract the microglial-mediated conversion of ASYN to higher order oligomers and fibrillar aggregates, whose transmission to neurons is a major risk factor in neuroinflammatory pathways. Given the acute role of microglia in elevating oxidative damage to neurons, we also engineered composite deactivating NPs (**CODE NPs**) via a concerted approach to introduce antioxidant polymers via NPs. In the process of microglial activation, intracellular reactive oxygen species (ROS) production is known to be a critical regulator [25]. While ASYN phagocytosis alone has been shown to result in microglial production of intracellular ROS [6b], internalized exogenous ASYN can result in ROS production through other means as well, including by disruptions in mitochondrial function [26]. While antioxidant therapies have shown promise in PD [27], their efficacy may be limited by factors including stability and ability to localize to relevant brain regions and cell types. Our research group has previously

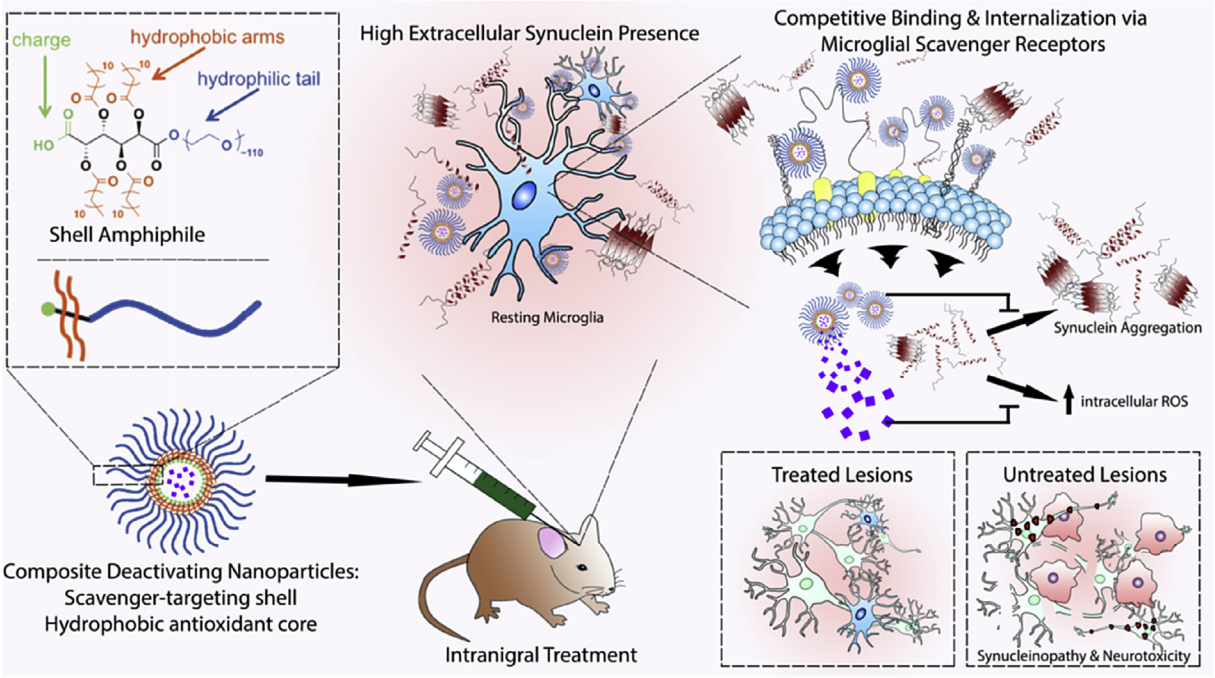

Fig. 1. Schematic of the new nanotechnology paradigm for scavenger receptor-mediated management of a-synuclein uptake and aggregation to counteract neurotoxicity in neurodegenerative diseases like PD. We envision nanoparticles comprised of synthetic scavenger receptor-binding amphiphilic molecules to reduce both intracellular ASYN aggregation and microglial activation by regulating microglial interactions with monomeric and aggregated ASYN and delivering anti-inflammatory agents along these pathways, ultimately decreasing microglial-mediated neurotoxicity and synucleinopathy propagation.