PATHOLOGICAL ALPHA-SYNUCLEIN PREPARATIONS INDUCE COGNITIVE IMPAIRMENT AND NEURODEGENERATION

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α-synuclein pathology is clearly linked to Parkinson’s disease (PD) and related dementia, which happens early in the disease process. Drug discovery for PD needs translational in vitro and in vivo models that are recapitulating natural disease onset. Here, we present translational models, induced by minute amount of highly reproducible α-synuclein oligomers (αSO) or fibrils (αSF) for drug screening and discovery.

Characterization of α-Synuclein Aggregates

Recombinant endotoxin-free αS monomers (>97% purity), detected by Coomassie-stained (left), are treated to generate oligomers (αSO) or fibrils (αSF) in a highly reproducible manner. αSO are stable in SDS-PAGE and can be detected by monomer-directed antibodies (right).

Antibodies Prevent αSO-Induced Neurodegeneration

Primary mouse striatal neurons (DIV 10) were incubated for 72 h with vehicle or αSO and different dilutions of αS-synuclein antibodies. Cell viability was evaluated by the MTT assay.

αS Induce Cognitive Impairment in Wild-Type Nontransgenic Mice

A single intrastriatal inoculation of αSO or αSF (4 µg) into wild-type mice induced cognitive deficits in the novel object recognition test at different time points. αSO-induced defects was observed within 15 days and stay the same for up to three months. However, αSF-induced cognitive dysfunction was not observed before 3 months. * p<0.05 vs. vehicle

αS-Induced Neurodegeneration in Dopaminergic Primary Neurons

Primary rodent striatal neurons (DIV 10) were incubated for 72 h with 1 µM of αS monomers (αSM), αSO, non-solicited αSF or sonicated αSF fibrils (αSFs).

Primary rodent astrocytes were incubated with αSO or αSF (10 µM) and astrocyte-conditioned media (CM) were harvested at 1, 3, 6 and 24 h post treatment. CM were analyzed by ELISA to quantify pro-inflammatory cytokines levels IL1β, TNFα & IL6 (N=3, n=2).

αS Induce Dopaminergic Degeneration, Neuro-Inflammation and Synaptic Loss

Iba-1 staining showed microglia activation in striatum of αSO-administered mice. ELISA analysis of mouse striatal lysate showed a significant reduction in dopamine active transporter (DAT) and tyrosine hydroxylase (TH) content in αSO-inoculated mice. Hippocampal lysate showed increased pro-inflammatory cytokine production (IL1β) in mice inoculated αSO, and decreased levels of synaptic markers (PSD-95).

αS Induce Neurodegeneration in Human HIP iPSC-Derived Neurons

Human hippocampal iPSC cells were differentiated for five weeks and treated with 0.3 µM αSO, BDNF, or epigallocatechin gallate (EGCG) for 72 h. Neuronal survival was determined by neuron specific enolase (NSE) ELISA.

A single intrastriatal injection of αSF (4 µg) into wild-type nontransgenic mice led to the cell-to-cell transmission of pathologic αS and Parkinson’s-like Lewy pathology in anatomically interconnected regions. Brain staining for phosphorylated Ser129 showed spreading of αS aggregates within 15 days post αSF intrastriatal administration.