Assessing a novel therapy for synucleinopathies using an alpha-synuclein pre-formed fibril mouse model

Sydney Weber Boutros1, Joanne Lee1,2, Jacob Raber1,3,4,5, Vivek K. Unni3,6,7

1Department of Behavioral Neuroscience, OHDS; 2Department of Neuroscience, University of Southern California; 3Department of Neurology, OHDS; 4Department of Radiation Medicine, OHDS; 5Division of Neuroscience, ONPRC; 6Jungers Center for Neurosciences Research, OHDS; 7Parkinson Center of Oregon, OHDS

Background

What we know about α-synuclein (αsyn):
- Small protein, exists everywhere in the body & abundantly throughout the brain
- Important in neurotransmitter vesicle cycling
- Phosphorylated form (p-αsyn) is the primary component of Lewy bodies (LBs), the pathological hallmark of synucleinopathies (Fig. 1; 2, 3)

What we know about synucleinopathies:
- Second most common form of neurodegeneration
- More common in men
- Includes Parkinson’s disease, dementia with Lewy bodies, & multiple systems atrophy
- LB pathology is progressive, spreading to more brain regions over time
- Parkinson’s disease presents with motor abnormalities (tremor, slow movement, rigidity, reduced balance and posture) and cognitive/emotional changes (decreased ability to concentrate, depression, anxiety)
- In vitro & in vivo research shows that αsyn pre-formed fibrils (PPFs) leads to the spread of LB inclusions, following the prion-like hypothesis (Fig. 2; 4, 5)
- Treatments focus on symptom management – there are no treatments that address neuropathology

What we know about antisense oligonucleotides (ASOs):
- ASOs can prevent the production of specific proteins by inhibiting translation of mRNA (Fig. 3; 6)
- Many neurodegenerative diseases are characterized by accumulation of certain misfolded proteins
- Thus, ASOs have the potential to slow or reverse disease progression by decreasing protein available to be converted into toxic aggregates

Methods & Materials

Mechanism of ASOs. 1) DNA is transcribed into RNA. 2) RNA carries the information from DNA to make proteins. 3) Targeted ASOs drop RNA from translating into proteins. 4) Native protein levels decrease

Figure 3: Mechanism of ASOs. 1) DNA is transcribed into RNA. 2) RNA carries the information from DNA to make proteins. 3) Targeted ASOs drop RNA from translating into proteins. 4) Native protein levels decrease

Acknowledgements

Thank you to the funding that supported this project, including the NSF Graduate Research Fellowship-GVP58004D9, the P30 ND41800, and the ARCS Foundation.

References


Results

ASO reduces αsyn & pathology

The αsyn-ASO successfully reduced amount of αsyn and αsyn protein in the hippocampus; it also appears to have mildly alleviated LB-like pathology

Assessing the therapeutic efficacy of a novel, αsyn-targeted ASO

Hypothesis

Treating PFF-injected mice with an α-synuclein specific antisense oligonucleotide will reduce pathology and rescue behavioral and cognitive decline

Materials & Methods

Animals

Mice injected with a αsyn pre-formed fibril (PFF) into mono (mono) or dihemispheres (PFF-Scramble).

ASO: 5ug αsyn-sequence oligonucleotide ("αsyn") or 5ug nonsense sequence oligonucleotide ("ASO") injected via an intracerebroventricular injection.

Behavioral & Cognitive Testing (Pre-ASO)

αsyn-ASO treatment was started 3 months following behavior assessment.

Behavioral & Cognitive Testing (Post-ASO)

34 male C57Bl/6J (wild type) received bilateral injections into the motor cortex of either 5ug of monomeric αsyn ("Mono") or 5ug αsyn pre-formed fibrils ("PFF").

3 months later, mice received a battery of behavioral tests to examine
- motor function (rotation, wire hang),
- anxiety-like behavior (open field), and
- cognitive performance (novel object, water maze, fear conditioning).

Following behavior, mice received 700ug of either a nonsense ASO ("Scramble") or an αsyn-specific ASO ("ASO"), delivered via an intracerebroventricular injection.

5 weeks later, mice received another battery of behavioral tests to assess the effects of the ASO on
- motor function (rotation, wire hang),
- anxiety-like behavior (open field), and
- cognitive performance (novel object, water maze).

Weekly body weights were recorded to track health. After behavioral assessment of ASO, the right hippocampus, cortex, and cerebellum were dissected and frozen, the left hemisphere was post-fixed; and intestine and plasma were collected.

Western Blots

Western Blots (protein levels)

Immunohistochemistry (pathology spread)

Gut Microbiome (diversity & inflammation)

Post Mortem Analyses

The αsyn-ASO successfully reduced amount of αsyn and αsyn protein in the hippocampus; it also appears to have mildly alleviated LB-like pathology

ASO-treated mice showed a decline in body weight and decrease in overnight food intake

Treatment with ASO caused a dramatic and continuous drop in body weight. ASO also reduced overnight food intake, suggesting that drop in the hypothalamus is important for regulation of typical body functions.

Summary

• The αsyn-targeted ASO successfully reduced αsyn and psyn.
• However, αsyn reduction caused severe weight loss, decreased food intake, and detriments in motor and cognitive function.
• This suggests αsyn has a vital function in the adult brain that is still unknown and needs to be further explored.