1521-0103/389/S3/supplement: ASPET 2024 Annual Meeting Abstract
The Journal of Pharmacology and Experimental Therapeutics

dx.doi.org/10.1124/jpet.516.909300
J Pharmacol Exp Ther 389: June 2024

Neuroprotection in C. Elegans Parkinson's Disease Models: Discovering Therapeutic Insights via RNAi and Small Molecule High Throughput Screening

Alexis A. Surtel,[1] Emmanuel Akano,[1] Patricia K. Dranchak,[2] Carl T. Ash,[1] Emily E. Anderson,[1] Amy E. Moritz,[1] R. Benjamin Free,[1] Joseph P. Steiner,[3] James Inglese,[2] and David R. Sibley[1]

[1]Molecular Neuropharmacology Section, NINDS/NIH; [2]Assay Development and Screening Technology Laboratory, NCATS/NIH; and [3]Neurotherapeutics Development Unit, NINDS/NIH

Abstract ID 90930 **Poster Board 516**

Abstract Text Parkinson's disease (PD) is a prevalent neurodegenerative condition characterized by degeneration of dopaminergic neurons in the substantia nigra of the midbrain. This results in bradykinesia, tremors, rigidity, and other motor and non-motor deficits. The prevalence of PD is increasing, in part, due to the aging population. Despite this growing burden, the development of effective therapies to halt disease progression remains an unmet need. This challenge is due to gaps in our understanding of the molecular mechanisms underlying the disease and deficiencies of preclinical models in recapitulating all aspects of the pathobiology of PD. Consequently, deepening our understanding of pathobiology and discovering druggable targets remain a research priority. Herein, we describe assay development and optimization of a high throughput phenotypic screen in the roundworm *Caenorhabditis elegans* for discovery of novel druggable targets and genetic influencers of neurodegeneration and neuroprotection. We utilized two transgenic *C. elegans* strains expressing human PD-linked genes—one with mutant (A53T) alpha-synuclein (SNCA) and the other expressing mutant (G2019S) leucine-rich repeat kinase 2 (LRRK2). These strains exclusively express the PD-related transgenes and green fluorescent protein (GFP) in their dopaminergic neurons, facilitating the tracking of neurodegeneration through measurable changes in the GFP fluorescence. Using laser cytometry and high content imaging with worms growing in liquid culture within 384-well plates, we assessed the health of the dopamine neurons via GFP intensity, number of neurons, and area of green objects. We observed a 30-50% and 75-85% decrease in GFP intensity in the SNCA and LRRK2 worms, respectively, by day seven compared to wild-type controls lacking the PD genes but expressing GFP exclusively in the dopaminergic neurons. We crossed the worms to RNAi hypersensitive backgrounds carrying eri-1, rrf-3, and eri-1;lin-15B mutations to overcome the typical resistance to neuronal RNAi knockdown in *C. elegans*. We observed a robust RNAi knockdown in the control and mutant worms with a 50-75% knockdown on day seven with RNAi against GFP, ama-1, ceh-43, and unc-62 compared to empty vector control. RNAi specifically engineered against the LRRK2 transgene slows neurodegeneration in the LRRK2 mutant worms. Additionally, we have identified selective LRRK2 kinase inhibitors that confer neuroprotection in the LRRK2-carrying worms with promising properties to serve as controls for high throughput molecular library screening. Further optimization studies are ongoing with the goal of conducting a screen for genes influencing neuroprotection in these models. Additionally, we plan to conduct a small molecule library screen for novel neuroprotective compounds using these PD models. These lines of inquiry may identify new molecules or potential druggable targets for novel therapeutics for influencing neurodegeneration and PD progression.

Supported by the NINDS Intramural Research Program.