α-SYNUCLEINOPATHY-INDUCED REGIONAL BRAIN ATROPHY ASSOCIATED WITH OLFATORY DEFICITS AND SLEEP DISTURBANCES

Elodia Briot1, Simon P. Zehringer1, Alex P. Elgin2, Kevin L. Lut3, Barry J. Bedell1, Elodie Brison1, V.M. Zhang2, O’Brien P.1, K.C. Hutson2, J. Carroll3, B. Zhang2, and J. Fleming1

1Biospective Inc., Montreal, QC, Canada; 2University of Pennsylvania, Philadelphia, PA, USA; 3Reasearch Institute of the McGill University Health Centre, Montreal, QC, Canada

Introduction

Parkinson disease (PD) is currently diagnosed based on motor impairment and neuropsychiatric disturbances, although non-motor deficits, such as olfactory impairment, typically precede the classic motor symptoms by several years. This early stage of PD represents an ideal window for therapeutic intervention to prevent development of motor symptoms. PD is associated with progressive loss of neurons, as well as the presence of abnormal aggregates of misfolded α-synuclein. This misfolded α-synuclein is a primary target for new, disease-modifying therapeutic agents. The overarching objective of this work was to develop an in vitro mouse model of α-synucleinopathy to characterize early stages of the disease process, and to test the reproducibility of using state-of-the-art, multi-modality imaging techniques in order to provide well-validated tools to accelerate the development of disease-modifying treatments for PD.

Methods

ADβ42/Tg Model of α-Synucleinopathy

- Induced in 8‐10‐week-old male M3T91/tg mice
- Performed human α-synuclein fibrils (PFFs) (Luk, 2012; were injected into the left Anterior Olfactory Nucleus (AON)
- Injections of Phosphatase-Radiolabeled Saline (PSG) were used as a negative control

Behavioral Testing

Olfactory Dysfunction Testing

- Animals were tested for olfactory deficits at 8, 16, and 24 weeks post-surgery (w/wt)
- Burned Pallet Test
- Latency to find the pellet (5 min maximum) was measured on four consecutive days (Pelling, 2008)

Sleep Dysfunction Testing

- Animals were tested for sleep dysfunction at 8, 11, and 16 weeks post-surgery
- The PhazIPaw Mouse Behavior Tracking System was used to record continuous and real-time sleep/wake behavior over a 72 hour period
- Access to food and water was ensured and monitored closely

Anatomical MRI & Analysis of Volumetric and Cortical Thickness Data

- Mouse brain tissue was fixed and embedded in paraffin
- Tissue sections were collected over ~120 levels covering the entire brain
- Stained for pathologist phosphoSer129 α-synuclein (p-Syn) and NOS for neuronal cell body
- Digitalized using a microSlide2 digital whole slide scanner (Carl Zeiss, Canada)
- Analysis of the stained sections was performed using Biovisio’s PERMITS™ software

2D and 3D Quantitative Immunohistochemistry (qIHC)

- Mouse brain tissue was fixed and embedded in paraffin
- Tissue sections were collected over ~120 levels covering the entire brain
- Stained for pathologist phosphoSer129 α-synuclein (p-Syn) and NOS for neuronal cell body
- Digitalized using a microSlide2 digital whole slide scanner (Carl Zeiss, Canada)
- Analysis of the stained sections was performed using Biovisio’s PERMITS™ software

References


Conclusions

We have developed an inducible mouse model of α-synucleinopathy that demonstrates behavioral deficits (olfactory dysfunction and sleep disturbances). We have reproduced a representative pattern of pathology spreading through the olfactory network with a significant decrease in regional neuroanatomical volume and cortical thickness over a 16 week period. Our approach allows for a comprehensive understanding of the disease development utilizing in vivo MRI as an imaging biomarker. This rapid, robust inducible model can be used for preclinical studies to accelerate the development of disease-modifying treatments for PD and other neurodegenerative diseases.

Acknowledgements

This work was funded by Biospective Inc. and the Quebec Consortium for Drug Discovery (CQDM).

α-SYNUCLEINOPATHY-INDUCED REGIONAL BRAIN ATROPHY ASSOCIATED WITH OLFATORY DEFICITS AND SLEEP DISTURBANCES

Elodia Briot1, Simon P. Zehringer1, Alex P. Elgin2, Kevin L. Lut3, Barry J. Bedell1, Elodie Brison1, V.M. Zhang2, O’Brien P.1, K.C. Hutson2, J. Carroll3, B. Zhang2, and J. Fleming1

1Biospective Inc., Montreal, QC, Canada; 2University of Pennsylvania, Philadelphia, PA, USA; 3Reasearch Institute of the McGill University Health Centre, Montreal, QC, Canada

Introduction

Parkinson disease (PD) is currently diagnosed based on motor impairment and neuropsychiatric disturbances, although non-motor deficits, such as olfactory impairment, typically precede the classic motor symptoms by several years. This early stage of PD represents an ideal window for therapeutic intervention to prevent development of motor symptoms. PD is associated with progressive loss of neurons, as well as the presence of abnormal aggregates of misfolded α-synuclein. This misfolded α-synuclein is a primary target for new, disease-modifying therapeutic agents. The overarching objective of this work was to develop an in vitro mouse model of α-synucleinopathy to characterize early stages of the disease process, and to test the reproducibility of using state-of-the-art, multi-modality imaging techniques in order to provide well-validated tools to accelerate the development of disease-modifying treatments for PD.

Methods

ADβ42/Tg Model of α-Synucleinopathy

- Induced in 8‐10‐week-old male M3T91/tg mice
- Performed human α-synuclein fibrils (PFFs) (Luk, 2012; were injected into the left Anterior Olfactory Nucleus (AON)
- Injections of Phosphatase-Radiolabeled Saline (PSG) were used as a negative control

Behavioral Testing

Olfactory Dysfunction Testing

- Animals were tested for olfactory deficits at 8, 16, and 24 weeks post-surgery (w/wt)
- Burned Pallet Test
- Latency to find the pellet (5 min maximum) was measured on four consecutive days (Pelling, 2008)

Sleep Dysfunction Testing

- Animals were tested for sleep dysfunction at 8, 11, and 16 weeks post-surgery
- The PhazIPaw Mouse Behavior Tracking System was used to record continuous and real-time sleep/wake behavior over a 72 hour period
- Access to food and water was ensured and monitored closely

Anatomical MRI & Analysis of Volumetric and Cortical Thickness Data

- Mouse brain tissue was fixed and embedded in paraffin
- Tissue sections were collected over ~120 levels covering the entire brain
- Stained for pathologist phosphoSer129 α-synuclein (p-Syn) and NOS for neuronal cell body
- Digitalized using a microSlide2 digital whole slide scanner (Carl Zeiss, Canada)
- Analysis of the stained sections was performed using Biovisio’s PERMITS™ software

2D and 3D Quantitative Immunohistochemistry (qIHC)

- Mouse brain tissue was fixed and embedded in paraffin
- Tissue sections were collected over ~120 levels covering the entire brain
- Stained for pathologist phosphoSer129 α-synuclein (p-Syn) and NOS for neuronal cell body
- Digitalized using a microSlide2 digital whole slide scanner (Carl Zeiss, Canada)
- Analysis of the stained sections was performed using Biovisio’s PERMITS™ software

References


Conclusions

We have developed an inducible mouse model of α-synucleinopathy that demonstrates behavioral deficits (olfactory dysfunction and sleep disturbances). We have reproduced a representative pattern of pathology spreading through the olfactory network with a significant decrease in regional neuroanatomical volume and cortical thickness over a 16 week period. Our approach allows for a comprehensive understanding of the disease development utilizing in vivo MRI as an imaging biomarker. This rapid, robust inducible model can be used for preclinical studies to accelerate the development of disease-modifying treatments for PD and other neurodegenerative diseases.

Acknowledgements

This work was funded by Biospective Inc. and the Quebec Consortium for Drug Discovery (CQDM).