of olfactory dysfunction, regional brain atrophy, and pathologic spreading in an inducible mouse model of α-synucleinopathy

Introduction

Parkinson’s disease (PD) is currently diagnosed based on motor impairment and neuropsychiatric disturbances, although non-motor deficits, such as olfactory impairment, typically precede the clinical 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 novel, disease-modifying therapies; yet, the overarching objective of this project was to develop an inducible mouse model of α-synucleinopathy that characterizes early pathologic changes associated with the olfactory system in mice using state-of-the-art, multi-modality imaging techniques in order to provide well-defined tools to accelerate the development of disease-modifying treatments for PD.

Methods

1. The mouse model of α-synucleinopathy was induced in 8-week-old, male Tg(129S1-Syn2tm1Saka) mice (Charles River, n=6), M38 (human A53T, A30P heterozygous (+/+) Tg mice), and M39 (human A53T, P30L heterozygous (+/+) Tg mice). Preformed mature or human α-synuclein fibres (PFs) (Luk, 2013) were injected into the olfactory bulb (Figure 3) or olfactory nerve (ANO) (Figure 4).

2. Animals were tested for olfactory deficits at 15 weeks post-surgery using the buried pellet test. Slightly after moderate food deprivation, the mice were put into a cage with a central pellet buried in the bedding. The amount of time to find the pellet (5 min maximum) was measured on four consecutive days (Ferring, 2008).

3. WT mice underwent baseline 3D anatomical MRI scans prior to inoculation at 7 weeks of age using a 7 T animal MRI system (Bruker, BioSpec 70/20). Mice were then randomized to PFF or PBS control groups, injected, and aged for 12 weeks. At the end of the post-inoculation period, WT and M38 and M39 mice underwent follow-up 3D MRI scans. All mice were re-analyzed using the fully-automated, production-level, NIGHTWING™ MRI processing platform (Figure 2).

5/ Injection of PFFs into the AON resulted in significant decreases in regional neuroanatomical volumes in WT and M38 Tg mice

Table 1. Comparison of the volumes of different ROIs at 17 weeks post-injection of PFFs or PBS into the AON in WT or M38 heterogeneous (-/-; n=3) mice. Actual Wt +/- SEM. L: Left injected and PFF (in parenthesis material). *p<0.05. **p<0.01.

We generated 3D quantitative IHC maps of the phosphoSer129 α-synuclein using Bioseptic’s PERMIt™ technology to visualize the pattern of spread. Representative coronal, sagittal and transverse sections stained with IHC show the region of inoculation surrounded by the spreading area (Figure 8). The qIHC parametric maps indicate the limited distribution of phosphoSer129 α-synuclein within the olfactory bulb, consistent with the immunochemistry data shown in Figure 9. IHC staining for neurofibrillary tangles, tangles (in Tg mice), or Lewy body pathology (in Tg mice) were not detected in any of the mice injected with PFFs or PBS (data not shown).

We have developed an inducible mouse model of α-synucleinopathy that demonstrates olfactory dysfunction, as well as a reproducible pattern of spread through the olfactory network with a significant decrease in regional neuroanatomical volumes. Future studies will focus on M38 (human AS1T) Tg mice as our preliminary studies revealed that this particular model has better potential for MRI studies and shows significant pathology. Our approach allows for a comprehensive understanding of the alterations underlying in vivo MRI-based imaging biomarkers. This rapid, robust, and reproducible model can be used for preclinical studies to accelerate the development of disease-modifying treatments for PD and other neurodegenerative disorders.

Acknowledgements

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