Serotonergic Signalling Activation as a Therapeutic Approach for Neurodegenerative Diseases

Hypothesis
Our hypothesis states that modulation of the serotonergic signalling prevents protein aggregation and pro-toxicity likely through an enhancement and/or adaptation of the cellular protein control mechanisms.

Aims
- Determining the effects of Citalopram treatment on protein aggregation, neuropathology, and behaviour of a mouse model of Tauopathies.
- Determining the effects of serotonin drugs treatment on protein aggregation, neuropathology, and behaviour of a mouse model of Parkinson’s Disease.

Previous Results

In recent studies in our laboratory, it was demonstrated that modulation of serotonergic signalling by citalopram (CIT) and other selective serotonin reuptake inhibitors (SSRIs) suppressed PolyQ aggregation and neurotoxicity. SSRIs are known to block serotonin reuptake by the serotonin transporter SERT, and to desensitize the 5-HT1A autoreceptor, meaning that there will be an increase of serotonin availability at the synaptic cleft, leading to the activation of post-synaptic receptors.

Does Citalopram Treatment supress Tau Pathology?

1. Model: Transgenic mice expressing the aggregation-prone P301L-Tau protein. These mice express 2N4R human Tau under a CAMKII promoter.

2. Experimental Timeline

Citalopram at 8mg/kg will be administrated in the drinking water.

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- Motor, Anxiety, Amnesia & Memory
- Spatial Learning, Anxiety, Amnesia, Stress & Memory

Tools: Biochemical & Neuropathology Evaluation

Does CIT Treatment supress α-synuclein Pathology?

1. Pre-Formed Fibrils Formation


2. Experimental Timeline

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- Sudden death
- Motor Impairment, Coordination, Sensitivity, Agility, Spatial Learning, Memory, & Stress
- Gastrointestinal, Biochemical & Neuropathology Evaluation

3. Stereotaxic Surgery

- The localization and amount of fibrils used, can affect the number of neurons that will develop inclusions.
- Direct intracerebral unilateral injection into the right striatum of three-month-old Wild Type C57Bl/6 mice.
- Injection rate: 0.2uL/min (2.5uL total per site).
- Concentration: 2mg/mL (total injected: 5ug).
- Coordinates:
  - From Bregma: +0.2mm.
  - From Midline: +2.0mm.
  - Beneath Dura Mater: +2.6mm.

4. Electron Microscopy Observation

- Stereotaxic Surgery

Figure 1 – (A) This graph represents the results of the Motor Swiming Test performed when the mice were six months old. As expected, we see no significant difference, since Tauopathies do not show movement disorders. (B) This graph represents the result of the Sucrose Preference Test. Also, performed when the mice were six months old. We see a significant effect in the treated mice, since they have less preference for drinking sucrose, than the vehicle mice. (C) In this graph we can see the results of the Nesting Test, performed at six months of age, in which we can not see a significant difference.

P301L Behavioural Analysis

Preformed Fibrils Quality Control

1. Thioflavin T Assay

- Two protocols: an one hour-measurement protocol and a kinetics protocol.
- Kinetics Protocol
  - 7 replicates from same monomers aliquots;
  - Fibrillation conditions were mimicked (except shaking);
  - Over-time measurements: every half hour during 7 days.

- One Hour-Measurement Protocol
  - Fluorescence measurement after incubation for one hour at room temperature;

Figure 2 – Variability can be observed between replicates.

Figure 3 – These results show the formation of α-synuclein fibrils. The values obtained were correspondent to the ones expected, since the fibrils fluorescence is, approximately, 10x bigger than the monomers fluorescence.

2. Sedimentation Assay

- Supernatant (S) and Pellet (P) of both the Monomers and the Pre-Formed Fibrils;
- Standard SDS-PAGE Protocol was performed.

Figure 4 – These results show the presence of α-synuclein fibrils. The presence of band in the supernatant of the fibrils can be explained by the fact that the 7-day incubation might be insufficient to complete the process of fibrillation of all monomers.

5. Electron Microscopy Observation

- Stereotaxic Surgery

Figure 5 – (A) Visualization of α-syn monomers and α-syn fibrils via transmission electron microscopy. Representative micrographs of α-syn monomers and fibrils: mouse α-syn monomers, full length α-syn PFFs and mouse α-syn PFFs after sonication; (B) distribution of sonicated mouse α-syn PFF lengths.