The Use of Imaging to Follow Astrocyte Responses to Pro-Homeostatic Elovanoids

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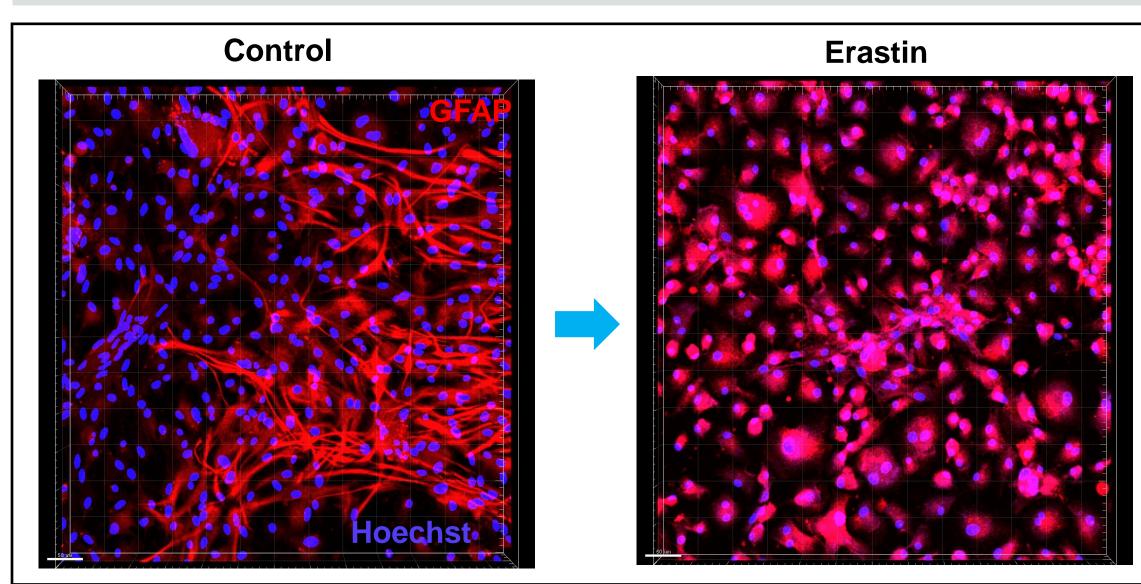
Introduction

The estimated number of persons diagnosed with Parkinson's disease (PD) in 2010 was 630,000, and this number is expected to double by 2040. The burden of PD will only grow over the next few decades as the size of the elderly population increases[1]. Currently, there is no cure for PD, and palliative therapies only provide temporary relief of symptoms as the neurodegeneration proceeds. Moreover, promising cellular replacement therapies are likely to fail unless the causes of the disease are uncovered so that the death of the transplanted cells can be prevented. Thus, it is essential to identify physiological abnormalities that lead to the death of dopaminergic (DA) neurons.

Astrocytes play an important role in the survival of the neurons, as they are the main support cell that controls the availability of neurotransmitters and ions required for the neuronal activity, provide neurons with neurotrophic factors, required for the neuronal activity, provide neurons with neurotrophic factors and nutrients and establish a two-way communication with the blood vessels in the brain[2]. Astrocytes also are the first line of defense of the neurons. When damage occurs, astrocytes become reactive and adopt a different phenotype[3]. We hypothesize that astrocytes promote survival by secreting bioactive lipids and thus the wellbeing of these cells may determine the fate of the dopaminergic neurons. Here we aimed to investigate the effects of Elovanoid 34 (ELV34) on the morphology of erastin-treated astrocytes from Hypothalamus, Sustantia Nigra (epicenter of the neuronal degeneration occurring in PD), Thalamus and Olfactory Bulb. Erastin is a small molecule that promotes Ferroptosis- a common type of cell death observed in PD. For that purpose we treated the astrocytes for 6 h with 10 μM erastin.

Methods

The cells were fixed, immunostained with GFAP antibody and imaged in a FluoView 1200 confocal microscopy. The Z-stacks obtained were converted to IMARIS files and processed using the IMARIS cell module. The volume of the cells under different treatments was plotted. The differences between treatments were tested for significance.
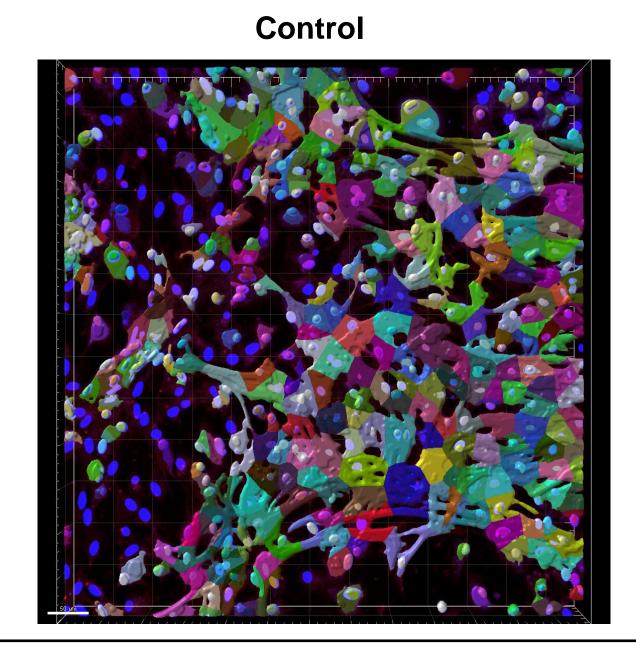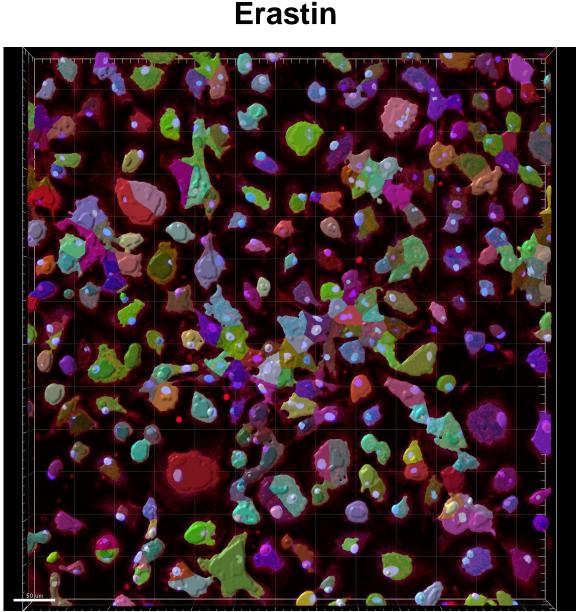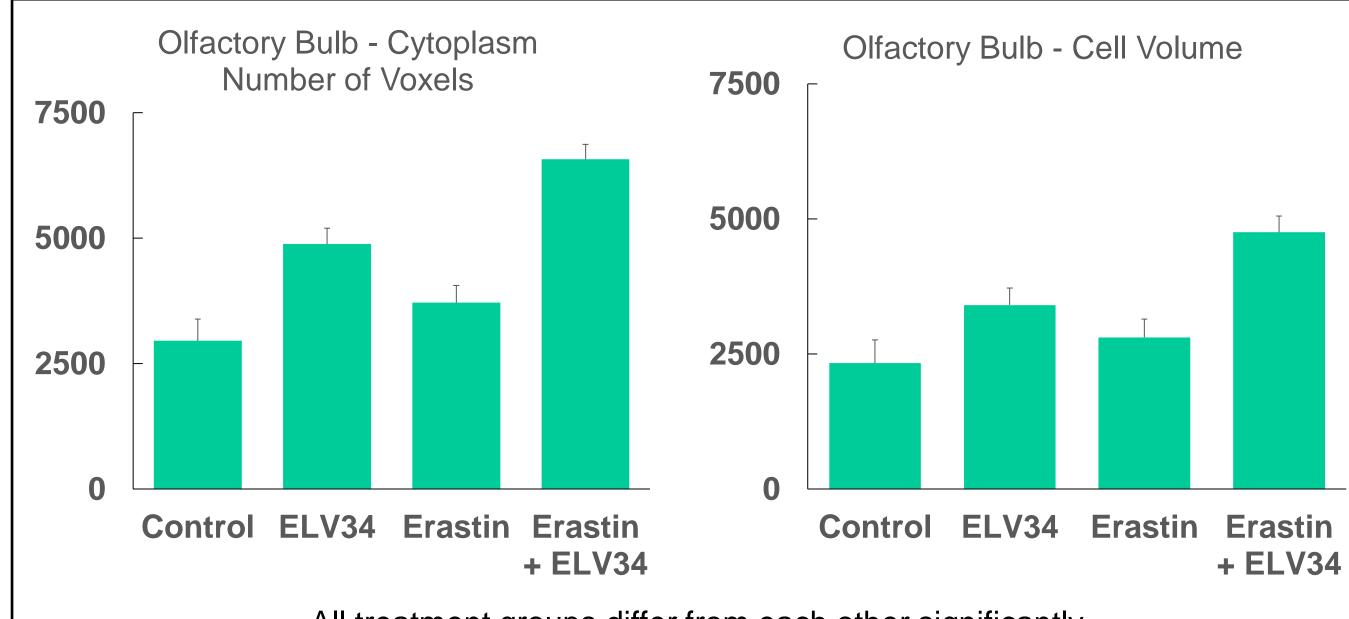
Astrocytes Morphology Change Induced by Erastin

Control   Erastin

Imaris Cell Module Astrocyte Rendering

Control   Erastin

Elovanoid 34 Improves Olfactory Bulb Astrocyte Survival
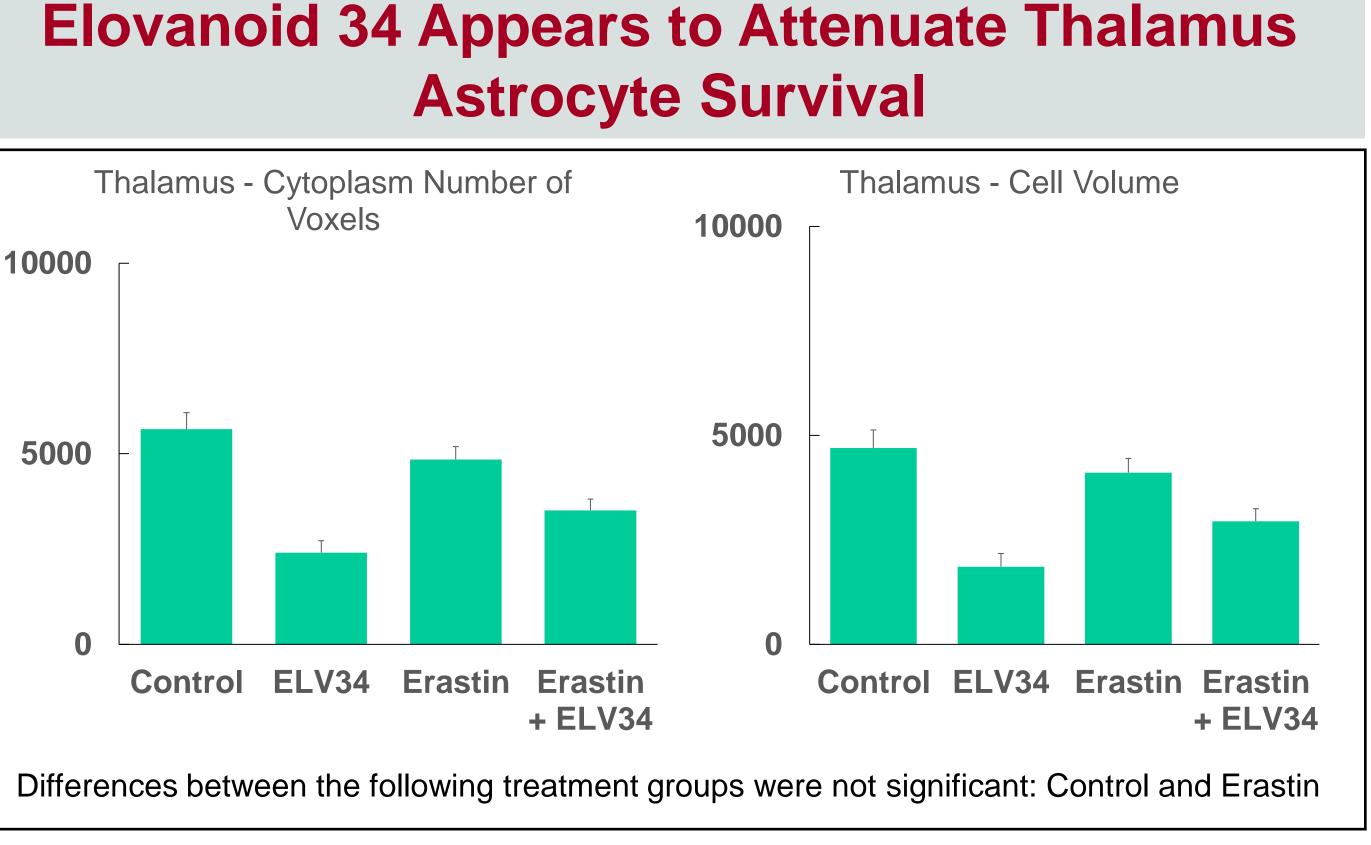
Olfactory Bulb - Cytoplasm Number of Voxels

Olfactory Bulb - Cell Volume

All treatment groups differ from each other significantly

Elovanoid 34 Appears to Attenuate Hypothalamus Astrocyte Survival

Hypothalamus - Cytoplasm Number of Voxels

Hypothalamus - Cell Volume

Differences between the following treatment groups were not significant: Control and Erastin + ELV34, Erastin and Erastin + ELV34

Elovanoid 34 Appears to Attenuate Thalamus Astrocyte Survival

Thalamus - Cytoplasm Number of Voxels

Thalamus - Cell Volume

Differences between the following treatment groups were not significant: Control and Erastin

Conclusions

Results Seen:
- ELV34 improves astrocyte survival in Olfactory Bulb
- ELV34 decreases astrocyte survival in Hypothalamus and Thalamus
- Change in Astrocyte morphology was observed between treatment groups (but not statistically tested)

Based only on the results of this project one may conclude that Elovanoid 34 has a positive impact on astrocyte survival in the Olfactory Bulb, but a negative impact in the Hypothalamus and Thalamus. As Elovanoid 34 has previously been shown to have effects that result in improved cell-survival[4567], these results may belie the reality. Because the difference between the Control and Erastin treatment groups was not shown to be significant in the Thalamus it is also questionable if ferroptotic conditions were properly achieved.

While working on this project, the following skills were learned and employed- cell culturing, immunostaining, use of confocal microscope, image analysis via Imaris software, and statistical analysis via Excel.

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