Astrocytic networks as a novel therapeutic target in Parkinson’s disease

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INTRODUCTION

Parkinson’s disease (PD) is a neurodegenerative condition with the highest rise in disability and is currently incurable. Dopamine replacement therapy ameliorates motor symptoms temporarily, but does not address non-motor symptoms (NMS) of PD, and often cause serious side effects; with up to 10 million people with PD worldwide and predicted economic burden of over $79 billion in the US alone by 2037, the unmet clinical need is large. Deeper understanding of GJ pathology is required to create new transformative therapies.

One cell type that received increasing attention in PD recently are astrocytes. Astrocytes express multiple familial PD-associated genes as much, or more than neurons, and develop alpha-synuclein (a-syn) immunoreactivity, which correlates with dopaminergic loss. Their causal involvement in PD symptom development was demonstrated in vitro and in vivo, where healthy astrocytes could rescue functions of neurons carrying PD mutations and reduce motor symptoms in rodent models, and diseased astrocytes could induce PD resembling dysfunction in human neurons.

A key aspect of astrocyte biology is their ability to form large networks transmitting calcium signals. Abnormal calcium signalling is also a hallmark of PD. We hypothesised that astrocyte networks are dysregulated in PD, and normalising calcium transmission in astrocyte networks could reduce a-syn aggregation and inflammation. Our first target is connexin43 (Cx43) which connects astrocytes via gap junctions (GJs) and promotes calcium transmission, and dysregulation of which is linked to inflammation activation through hemichannel (HC) opening.

OBJECTIVES

- To characterise Cx43 pathway disturbances in models of PD and in human PD post-mortem tissue;
- To establish causal relationship between the Cx43 disturbance and PD relevant cell dysfunction;
- To devise a clinically-relevant method of reversing Cx43 dysfunction in PD.

RESULTS

1. Inflammation or a-syn challenge lead to Cx43 Gl closure and Cx43 protein loss in astrocytes in vitro and in vivo. Functional Gl open/closed state was assessed using Cx43-permeable Lucifer Yellow (Luc) dye microinjection; Cx43 loss was visually confirmed in live imaging and immunoblotting techniques. Streak inflamation in vitro was induced by 100ng/mL LPS and 10mM interleukin 1β exposure or by 1mM LPS injection IP in vivo. A-syn challenge was triggered by a simple application of 0.5 μM a-syn pre-formed fibrils (PFFs) per 10,000 cells in vitro or by a peripheral injection into the tail with a 100 μg PFF per animal alongside a carrier protein RSV1 [1]. Error bars: SEM; statistics: ANOVA + Bonferroni correction / t-test. [2-8]

2. Cx43 Gl puncta and protein levels are reduced in PD human and are associated with non-motor symptoms such as depression. G43a gene encoding Cx43 was found in a stronger association with PD-linked gene regulatory networks (GRNs) in Parkinson's disease compared with published single cell dataset [2-8]. Cx43 Gl puncta and protein levels were reduced in the human cortex and striatum in PD vs age-matched controls; astrocytic process tree complexity was reduced in PD. Error bars: SEM; statistics: ANOVA with FDR control.

3. Cx43 knock-down (k/d) leads to functional cell abnormalities relevant to PD. Astrocytes with reduced Cx43 expression presented with diminished Gl coupling, calcium signalling abnormality similar to that seen with a-syn or inflammatory lesions, and reduced process tree. Mitochondrial respiration was affected in the midbrain astrocytes upon k/d. Phospho-a-syn aggregation was increased in cortical astrocyte-neuron co-cultures containing Cx43-deficient astrocytes. Cx43 shRNA was delivered via an AAV vector; control condition for Cx43 k/d was transduction with scrambled RNAi (siRNA). Error bars: SEM; statistics: ANOVA + Bonferroni correction / t-test. [2-8]

4. Opening Cx43-containing GJs and blocking HC is protective in models of PD. A pharmacological modulator of Cx43 (‘DrugB’) was employed. Treatment with ‘DrugB’ reduced the Gl block under a-syn challenge, normalised calcium signalling, and reduced a-syn aggregation and phosphorylation. Reduction in inflammatory cytokine and cytokine release was observed under conditions of a-syn challenge in vitro and in the C57 upon LPS-induced inflammation in vivo (1mg/kg LPS injection IP, 1.5 days total lesion; 10mg/kg ‘DrugB’ i.p.) upon ‘DrugB’ treatment. Error bars: SEM; statistics: ANOVA + Bonferroni correction / t-test. [2-8]

SUMMARY

- In vivo efficacy and non-GLP safety testing of our new pharmacological approach in 2 advanced models of PD (an-syn and LPS-induced);
- IND-enabling package generation: GLP toxicity, formulation optimisation for human use;
- Clinical trials; for Phase IIa – use of the pharmacological approach in early to mid-stage PD to assess disease-modifying properties of the treatment on motor and non-motor symptoms of PD.

FUTURE DIRECTIONS

- In vivo efficacy and non-GLP safety testing of our new pharmacological approach in 2 advanced models of PD (an-syn and LPS-induced);
- IND-enabling package generation: GLP toxicity, formulation optimisation for human use;
- Clinical trials; for Phase IIa – use of the pharmacological approach in early to mid-stage PD to assess disease-modifying properties of the treatment on motor and non-motor symptoms of PD.

BIBLIOGRAPHY


DISCLAIMER

The work is subject to a patent application. NH, SB, and PRS are directors and shareholders in Cellestial Health Ltd.