Non-Invasive, Targeted Overexpression of AAV2.SIRT3-myc using MR-guided Focused Ultrasound: A Disease-Modification Strategy in Parkinson’s Disease

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Introduction

Upon diagnosis of Parkinson’s disease (PD), individuals are relatively young, and able to lead independent lives. In the advanced stages, symptoms of PD become disabling, and often palliative care is required. If a therapeutic was available that could halt or reverse progression of PD, the quality of life of individuals with PD would be significantly improved. The aim of the current study was to develop a disease-modifying therapy to slow the progression of PD pathology, that can be safely administered to individuals with PD.

Post-mortem and genetic studies have shown that mitochondrial dysfunction is central to PD pathology. Mitochondrial abnormalities, such as increased oxidative stress, mutations in mtDNA, decreased function of the electron transport chain, and reduced ATP output have all been linked with PD pathology. Sirtuin 3 (SIRT3), is the major protein deacetylase in the mitochondria, linked with cytoprotection and longevity-enhancing effects, with over 700 substrates[9]. In non-neuronal cells, such as liver, muscle and heart, SIRT3 has been shown to reduce oxidative stress, increase ATP, and generally maintain mitochondrial health[8].

Hypothesis

1. In a rat model of PD, viral-mediated overexpression of SIRT3 will prevent motor dysfunction by preventing PD-like pathology such as dopaminergic neuronal loss. This occurs through the stabilisation of mitochondrial bioenergetics.
2. MR-guided focused ultrasound (MRg-FUS) induced blood brain barrier opening (BBBO) can be used to non-invasively and selectively target AAV-SIRT3 to brain regions affected in PD.

Materials and Methods

Mutant (A53T) α-synuclein rat model of PD: In rats, adeno-associated virus (AAV2/2) expressing A53T α-synuclein or control (EV) was intra-retinally injected bilaterally to induce a motor Parkinsonian PD model. Following AAV-α-synuclein infusion, this model shows motor impairment after 8 weeks, and apolipoprotein E overexpression after 6 weeks. Stereotaxic infusion of AAV2/2:SIRT3: Rats received intra-nigral or intra-striatal injections of AAV2/2:SIRT3 (100 μl, AAV2/2:SIRT3 at 1 × 1012 vector genomes (vg) /μl, AAV2/2: EV at 1 × 1012 vg/μl) via a 33 gauge needle respecting the stereotaxic atlas of rats. Anticipated Substrate: On completion of the AAV2/2:SIRT3 injection, rats were observed for 14 days for any signs of toxicity. Survival: 60 rats (30 AAV2/2:SIRT3 and 30 AAV2/2: EV) were used.

Dopaminergic neuronal loss: 6 weeks following AAV2/2:SIRT3, brains were removed and immunostaining (67) performed per mouse in the SNc, using antibodies against tyrosine hydroxylase (TH) and NeuN, followed by quantification stereology.

Results

In a rat model of PD, AAV.SIRT3 reverses motor dysfunction

Figure 2: In the virally-mediated mutant α-synuclein overexpressing rat model of PD, SIRT3 overexpression in the SNc reversed motor impairment. Data are expressed as mean ± standard deviation in number of forelimb placements ± SEM. Abbreviations: Control = Empty Vector + Empty Vector, PD = A53T α-synuclein. ANOVA with Tukey post-hoc. n = 10–12 per group. *p < 0.05

In a rat model of PD, AAV.SIRT3 prevents loss of dopaminergic neurons

Figure 3: In the virally-mediated mutant α-synuclein overexpressing rat model of PD, nigral SIRT3 overexpression prevented dopamine (DA) cell loss. Data are expressed as mean ± standard deviation ± SEM. Abbreviations: Control = Empty Vector + Empty Vector, PD = A53T α-synuclein. ANOVA with Tukey post-hoc. n = 10–12 per group. **p < 0.001, ***p < 0.0001

MR-g-FUS induced BBB opens the BBB to target brain regions affected in PD

Figure 5: Following infusion of AAV2/2:SIRT3-myc into the SNc, brains were removed, and the mitochondrial function stabilized. SIRT3-myc overexpression resulted in deacytlation of mitochondrial proteins as shown by SDS-PAGE and Western blot followed by quantification of optical density (OD). Data are expressed as mean ± standard error at 4°C. (A) SIRT3 (B) Tom20 (C) Sirt3 (D) Tom70. (Abbreviations: EV = Empty Vector, SIRT3 = AAV.SIRT3-myc). Mann-Whitney U test. n = 4–5 per group. *p < 0.05

Discussion

In the AAV mutant α-synuclein rat model of PD, intra-retinal injection of SIRT3 using AAV has disease-modifying effects. Over-expression of SIRT3 reversed motor deficits, and prevented dopamine cell loss, showing proof of concept that SIRT3 may be a useful therapy in the clinic[2,3].

Transduction of SIRT3-myc using AAV results in mitochondrial targeting of SIRT3 and deacytlation of mitochondrial proteins, demonstrating target engagement in vivo (Fig. 4.5).

Ectopic SIRT3-myc improves mitochondrial health by decreasing the mitochondrial respiration rate of dopaminergic neurons, which decreases oxidative stress. These may underlie the disease modifying effects of SIRT3-myc (Fig. 6).

MRg-FUS induced BBB to deliver AAV is safe in humans, consistent with the lower tropism and liver toxicity that can be achieved with AAV2 compared to other AAVs[4].

Using MR-g-FUS induced BBB, we have demonstrated the selectivity and non-invasive targeting of AAV2.SIRT3-myc into brain regions affected in PD (Fig. 7).

Further pre-clinical studies are required to conclusively determine the efficacy of MRg-FUS induced BBB to deliver AAV2.SIRT3-myc as a disease-modification strategy for individuals with PD.

Conclusion

MRg-FUS induced BBB provides a non-invasive method for the delivery of AAV2.SIRT3-myc, a disease-modifying agent which has neuroprotective properties in parkinsonian rats.

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References


Image 1: Schematic for the MR-g-FUS delivery of AAV2/SIRT3-myc into the rat brain. Created with BioRender.com

Image 2: Ectopically expressed SIRT3 engages the mitochondrial target

Figure 4: Following infusion of AAV2/SIRT3-myc into the SNc, SIRT3 overexpression is observed in the mitochondria of neurons, shown with immunofluorescence labelling. This shows that ectopically expressed SIRT3 can translocate from the nucleus to the mitochondria (white arrows). Antibodies: TOM20 (mitochondria), myc (ectopic SIRT3-myc), and TH (dopaminergic neurons).

Figure 6: In an in vitro differentiated dopaminergic neurons, SIRT3 overexpression decreases oxygen consumption rate in the mitochondria

Figure 7: Following infusion of AAV2/SIRT3-myc conjugated to microbubbles, MR imaging with gadolinium contrast agent shows the FUS targeted areas as bright spots (red arrows), indicating the BBB was opened and contrast agent was able to concentrate in the SNc and striatum.

Figure 9: Notable protein targets of SIRT3. Created with BioRender.com