IN VITRO AND IN VIVO MODELING OF MUTATED A53T ALPHA-SYNUCLEIN PATHOLOGY

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BACKGROUND

Aggregation of alpha-Synuclein (alpha-Syn) plays a central role in Parkinson's Disease (PD). Mutations in the alpha-Syn gene have been identified in rare forms of familial PD and are reported to accelerate its oligomerization and aggregation. In subjects affected by the alpha-Syn A53T mutation, the age of onset is earlier compared to sporadic PD. The development of new PD drugs halting the production of alpha-Syn aggregates and the resulting neurodegeneration is thus the main focus of PD research. To be able to test these new drugs, appropriate *in vitro* and *in vivo* models are needed.

We therefore evaluated toxicity and seeding properties of different recombinant human alpha-Syn peptides and fibrils in primary cortical neurons.

Additionally, human A53T alpha-Syn transgenic mice with murine Thy-1 promoter on a C57BL/6J background and age-matched non-transgenic littermates are currently characterized for general health and motor deficits. Animals of both sex and different age groups are evaluated.

MATERIALS and METHODS

Pre-formed fibrils and oligomers from Stressmarq (Active Human Recombinant Alpha Synuclein Pre-formed Fibrils (Type 2); Active Human Recombinant A53T Mutant Alpha Synuclein Protein Preformed Fibrils (Type 1); Active Human Recombinant Alpha Synuclein Protein Monomer (Type 2); dopamine stabilized oligomers) were used.

For assessment of toxicity, mouse primary cortical neurons were cultivated until DIV8 and treated once for 24 h with 100 µg/ml of the different alpha-Synuclein fibrils. Viability and toxicity were measured with MTT and LDH assay, respectively.

For testing the seeding properties of the fibrils, they were added to the mouse primary cortical neuron cultures on DIV1 at 5 µg/ml and re-treatment was carried out on DIV7. On DIV14, cells were fixed and Immunocytochemically assessed for human (data not shown) and murine alpha-Synuclein. Aggregation was also measured with ThioS staining.

A53T alpha-Syn transgenic mice are currently characterized, and results are not yet available.

SUMMARY and CONCLUSION

Our *in vitro* results show that monomeric human alpha-Syn has no impact on cell viability, while pre-formed wild type and A53T human alpha-Syn fibrils, but especially oligomers, have toxic effects on primary cortical neurons. Only A53T alpha-Syn fibrils of the tested isotypes showed seeding properties. It can be concluded that primary cortical neurons treated with preformed A53T alpha-Syn fibrils are a valuable *in vitro* model to analyze alpha-Syn aggregation and toxicity.

RESULTS

Alpha-Synuclein Toxicity and Seeding in primary neurons

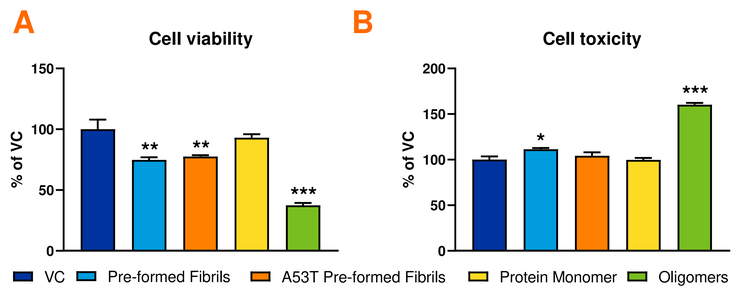


Figure 1: Evaluation of alpha-Syn toxicity on primary cortical neurons tested with MTT and LDH assay. Cells were treated for 24 h with different pre-formed alpha-Syn species (Stressmarq) and thereafter cell viability was assessed with (A) MTT and toxicity was measured with (B) LDH assay. Data are displayed as % of vehicle control (VC). Data are presented as bar graphs and standard deviation. For statistical analysis, One-way ANOVA followed by Bonferroni's *post-hoc* test (vs VC) was used. **p<0.01, ***p<0.001.

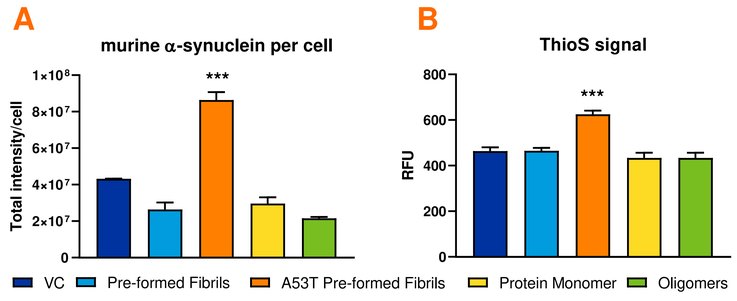
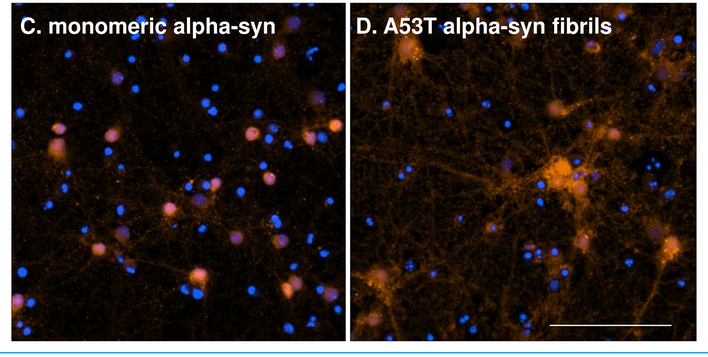


Figure 2: Evaluation of seeding properties of different alpha-Syn species. Neurons treated with alpha-syn species for 14 days and (A) quantification of immunocytochemically analyses for murine alpha-syn to visualize the induction of aggregates of the endogenous alpha-Syn by the human fibrils as well as by (B) ThioS staining. Data are presented as bar graphs and standard deviation. For statistical analysis, One-way ANOVA with Bonferroni's *post hoc* test (vs vehicle control: VC) was used. ** p<0.01, *** p<0.001.
(C,D) Representative images: Neurons were treated with (C) monomeric and (D) A53T preformed fibrils and after incubation immunocytochemically stained for murine alpha-syn. Nuclear stain DAPI = blue; murine alpha-syn = red; scale bar 100 µM.



For more information about the model please visit:
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