ABSTRACT – HOPE 2020 (600 words max)

Evaluation of dopaminergic neuronal loss and synaptic changes in an α-synuclein overexpressing model of PD

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Introduction

Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder, pathologically characterized by a loss of dopaminergic neurons in the substantia nigra (SN). The resulting dopamine deficiency in the striatum underlies some of the observed motor symptoms such as rigidity and tremor. A histopathological hallmark of PD is the presence of Lewy Bodies in the brain; aggregates containing, amongst others, misfolded α-synuclein protein[1]. In the present study, we aim to evaluate neuronal loss and synaptic compensation mechanisms in a rodent PD model that unilaterally over-expresses mutant (A53T) human α-synuclein, a missense mutation found in familiar cases of PD[2].

Materials & Methods

Two cohorts of rats (n_{total}=16) were unilaterally injected in the SN with AAV2/6 viral vector coding for mutated (A53T) human alpha-synuclein. Animals were evaluated at early (n=8;6-8 weeks post-injection (wpi)) or late time-point (n=8;10-12wpi). At both time-points, all animals underwent *in vivo* behaviour and PET imaging, and *post mortem* immunohistological and qPCR analysis.

PET imaging was performed using 6-[18F]fluoro-L-m-tyrosine [3](FMT) - a substrate for AADC, and [18F]-LBT999 [4](LBT) - a radioligand for dopamine transporter (DAT). AADC enzymatic rate (Ki) and DAT binding (BP_{ND}) were calculated using Patlak and Logan graphical methods respectively, employing the cerebellum as a reference region. For behavioural analysis, rats were subjected during 5 minutes to the cylinder test, in which contralateral and ipsilateral paw use was compared. After the *in vivo* studies, rats were sacrificed for histological studies using tyrosine hydroxylase immunohistochemistry, and biochemical analyses using qPCR. Paired student t-tests were used to compare the contra- and ipsilateral sides in imaging studies, while an ANOVA was used to compare contralateral paw use to a control group.

Results

The cylinder test revealed a clear motor deficit at both the early (-37%;n=7;p=0.025) and the late time-point (-35%;n=8;p=0.032). FMT-PET imaging did not reveal any significant differences in AADC enzymatic activity in the striatum at either time-point, nor did we observe a difference in AADC mRNA levels in the SN. In contrast, we observed a significant asymmetry in DAT binding in both the early (-

26%;n=6;p=0.027) and the late cohort (-42%;n=4;p=0.003). However we only demonstrated a significant left/right difference in DAT mRNA levels in the SN (-25%;n=5/6;p=0.01) and DAT protein levels in the striatum (-26%;n=8;p=0.005) at the late time-point.

Finally, we demonstrated significant dopaminergic cell loss in the SN with TH stereology in both the early (-14%;n=6;p=0.013) and late cohort (-28%;n=8;p=0.009), and a significant TH protein loss in the striatum at the late time-point (-33%;n=8;p=0.038).

Discussion/Conclusions

Based on behavioural data, and compared with previous studies on a complete lesion PD model, the AAV2/6-A53T model shows mild progressive neuronal loss between the early and late time-point. However, we were not able to confirm the dopaminergic neuronal loss using an *in vivo* PET marker of AADC enzymatic, neither were we able to demonstrate any evidence of AADC compensation mechanisms, as previously suggested in the literature[5]. Although our DAT binding at the early time-point was greater than neuronal loss measured with stereology, we were not able to demonstrate any compensation effect of DAT by *post-mortem* data. However, this compensation effects were evident at the late time-point, as previously suggested by Lee *et al.*[6]. Future research will have to unveil whether these compensation effects occur at the ipsilateral and/or contralateral synapse. Mild progressive PD models liked used in this study, unlike full lesion models, will allow to study compensatory mechanisms, and could give us new information about PD pathology.

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