Characterisation of two genetic rat models of Parkinson Disease through two presynaptic PET tracers; [18F]-LBT999 and 6-[18F]fluoro-L-m-tyrosine

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Résumé

Introduction
Parkinson's disease (PD) is the second most prevalent age-related neurodegenerative disorder, characterized by a tremor at rest, rigidity, and slowness, pathologically caused by a loss of the dopaminergic neurons in the substantia nigra (SN), resulting in a dopamine (DA) deficiency in the striatum [1]. Although it is over 200 years ago now that James Parkinson described this disease, to date there is still no treatment available that halts or slows dopaminergic neuron degeneration [2]. A necessity in finding such therapy is a pathologically relevant model to test new treatments. We developed a genetic rodent PD model with progressive, but significant DA loss through overexpressing WT or mutant (A53T) human alpha-synuclein protein in the SN [3], similar to the human pathology[1]. Our aims are to accelerate dopaminergic neuron loss in these rat models and evaluate DA pathology by two different presynaptic PET tracers and correlated this with behaviour and histological results.
Materials & Methods
A total of sixteen rats were unilaterally injected in the SN with a viral vector (AAV2/6-PGK; 1.00E+11 vgc) overexpressing WT human alpha-synuclein (WT-α-syn; n=8, 573±40gr) or mutated alpha-synuclein protein (A53T-α-syn; n=8, 589±39gr) and were studied at 10-12wpi. PET imaging was performed using a ligand substrate for AADC, 6-[18F]fluoro-L-m-tyrosine ("FMT", 60min acquisition, 36.4-46.5MBq; pre-treatment by IP injection of 10mg/kg benserazide 30' before imaging [4]), or a ligand after DA transporter (DAT), [18F]-LBT999 [5] ("LBT", 90min acquisition, 54.4-63.0MBq). For behaviour, rats were subjected for 5 minutes to the cylinder test, in which contralateral- and ipsilateral paw use was compared. After the *in vivo* studies rats were sacrificed for stereological counting studies in the SN using tyrosine hydroxylase immunohistochemistry.

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From LBT and FMT PET scans, quantitative uptake images (BPnd and Ki) were calculated using Logan and Patlak graphical methods, respectively, with the cerebellum as a reference, Ki images were subsequently smoothed. The striatum contralateral to the injection site served as internal control. Results are expressed as the mean ± SD, and compared using paired Student t-test for contra- and ipsilateral sides in imaging studies, while a one-way ANOVA and Scheffe F post-hoc test was used to compare contralateral paw use to a control group.

Results

Injection of benserazide was not effective in 37% of the animals, thus Ki (constant of accumulation) could not reasonably be estimated. Additionally, Ki parametric images showed lower contrast to background than binding potential (BPnd) images. At 12wpi we did not observed lower Ki in the ipsilateral caudate putamen compared to the contralateral side of quantifiable scans for the WT-α-syn (n=3, p=0.255) nor A53T-α-syn rats (n=2, p=0.336). However, LBT data showed a decreased BPnd in the ipsilateral caudate putamen of A53T-α-syn animals (n=4, p=0.003), while a near significant difference was observed in the WT-α-syn group (n=3, p=0.051). These results are in concordance with the behavioural observations, showing roughly only 30% use of the contralateral forepaw at this time point for A53T-α-syn (n=8, p=0.045), while the WT-α-syn rats showed no such difference (n=7, p=0.949). No significant correlations between PET data and behaviour were observed. All counts using stereological methods, and subsequent comparison, are still ongoing.

Fig 1: Positron emission tomography and behavioural studies. (A) Graphical schematic of WT-α-syn and A53T-α-syn viral vectors. (B) Cylinder tests at 10wpi detected motor deficits in A53T-α-syn overexpressing rats (n=8) but not WT-α-syn rats (n=7). PET scans were obtained from WT-α-syn and A53T-α-syn rats 12wpi, using a tracer substrate of aromatic L-amino acid decarboxylase (AADC; 18F-FMTyr) (C), or a dopamine transporter ligand (DAT, 18F-LBT999) (D). (C) Neither for WT-α-syn (n=3) nor for A53T-α-syn (n=2) a difference was observed in AADC metabolism (FMT). (D) In contrast the DAT tracer (LBT999) showed a significant difference in A53T-α-syn (n=4) but not WT-α-syn animals (n=3).

Discussion/Conclusion

We have created two AAV-overexpression rat models of PD; WT-α-syn and A53T-α-syn. Only the latter showed significant DA deficiency and neuronal loss detectible by LBT PET imaging, this is in concordance with behavioural tests. However, in our experiment this difference might also arise from the lower number of subjects in the WT-α-syn group. Additionally, the inter-animal variability seems to be more prominent in the WT-α-syn as compared to A53T-α-syn.

Research by Lazaro et al. [6] has shown that A53T mutated α-synuclein has increased presence of oligomers in the nucleus compared to the wildtype protein in HEK cells, and additionally A53T α-synuclein cells might possibly secrete oligomers [6]. Taken together, this might explain the more deleterious effect of A53T α-synuclein we found.

Our parametric data suggest that the DAT tracer, LBT999, is more sensitive to detect a mild PD phenotype as compared to the AADC tracer, FMT. This phenomenon has previously been described, and is possibly due to a combination of reduced nerve terminal DAT binding sites and downregulation of DAT in surviving neurons, in an attempt to increase DA availability [7]. More FMT scans will have to be done to increase numbers and compensate for ineffective benserazide blocking. Further analysis of PET data will allow correlation of PET data to behavioural and histological measurements.

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Mots-Clés: PET, Parkinson, Rodent, DAT, AADC, Dopamine