Cis-4-[^{18}F]fluoro-D-proline detects neurodegeneration in patients with akinetic-rigid parkinsonism

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ABSTRACT

OBJECTIVE: To investigate whether the amino acid PET-tracer cis-4-[^{18}F]fluoro-D-proline (D-cis-[^{18}F]FPro) shows increased uptake in the basal ganglia of patients with neurodegenerative akinetic-rigid parkinsonism. D-cis-[^{18}F]FPro is a sensitive PET tracer for inflammation-associated neurodegeneration in animal models. We hypothesized that D-cis-[^{18}F]FPro might also be a sensitive marker of alterations of the basal ganglia in parkinsonian syndromes.

METHODS: Ten subjects with neurodegenerative akinetic-rigid parkinsonism (5 with idiopathic Parkinson's disease and 5 with atypical parkinsonian syndromes) were imaged with D-cis-[^{18}F]FPro and compared to 13 patients with brain tumors who had no basal ganglia involvement. PET images 20-50 min after injection were evaluated and tracer uptake in the basal ganglia was quantified using volume-of-interest (VOI) analysis with basal ganglia to background ratios. Disease severity was assessed with UPDRS III and correlated with D-cis-[^{18}F]FPro uptake.

RESULTS: In patients with parkinsonism, VOI analysis revealed mild but significantly increased D-cis-[^{18}F]FPro uptake in the basal ganglia, pronounced in the lenticular nucleus. Disease severity correlated with D-cis-[^{18}F]FPro uptake in the right pallidum ($r = -0.687$, $p = 0.041$).

CONCLUSIONS: Data suggest that D-cis-[^{18}F]FPro is a sensitive marker of inflammation-associated degenerative processes in parkinsonian syndromes.

Keywords: Parkinson's Disease; parkinsonism; neurodegeneration; PET; D-cis-[^{18}F]FPro

INTRODUCTION

Neurodegenerative parkinsonian syndromes, including Parkinson's disease (PD), multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD), are characterized by progressive dopaminergic depletion and degeneration of the basal ganglia. A number of recent studies suggests that microglia-mediated inflammation plays a key role in the neurodegenerative processes involved in these disorders [1]. Cis-4-[^{18}F]fluoro-D-proline (D-cis-[^{18}F]FPro) is a promising PET tracer for the evaluation of inflammation-mediated neurodegenerative processes. In animal models of cortical ischemia or cerebral gliomas, histology revealed that D-cis-[^{18}F]FPro can be used to image neurodegenerative processes, which were related to microglia activation, remote of the primary lesion [2, 3]. However, D-cis-[^{18}F]FPro reliably crosses the intact blood-brain barrier [4], therefore allowing examination of neurodegenerative processes in neurological diseases with intact blood-brain barrier.

Based upon the above, we performed a pilot study to test the hypothesis that D-cis-[^{18}F]FPro might constitute a marker of neurodegenerative processes in parkinsonian syndromes. Specifically, we hypothesized that D-cis-[^{18}F]FPro uptake is increased in the basal ganglia of patients with parkinsonisms (compared to tumor patients with intact basal ganglia who served as control subjects). We furthermore tested whether disease duration and severity correlate with uptake of D-cis-[^{18}F]FPro.

METHODS

Subjects

In a prospective study, we recruited ten subjects with degenerative parkinsonian syndromes (5 with PD, 4 with MSA, 1 CBD) from the Department of Neurology at the University Hospital Cologne; diagnoses were assessed using current clinical consensus criteria. Disease severity was assessed with the Unified PD rating scale (UPDRS) part III in the 'OFF' state after medication withdrawal. Parkinsonian subjects were compared to 13 subjects with intracranial neoplasms in whom tumor growth (or edema) neither affected the basal ganglia nor the reference region. All brain scans of control subjects were visually assessed for changes of D-cis-[^{18}F]FPro uptake in the basal ganglia or any reference regions. Additionally, brain tumors must not include the motor cortex. The latter subjects were part of another study [5]. Clinical and demographic characteristics of all subjects are summarized in Table 1. This study was approved by the ethics committee of the medical faculty of the Heinrich Heine University Düsseldorf (application number 2452) and federal authorities. All subjects gave informed consent before study participation.

PET Imaging with D-cis-[^{18}F]FPro and data reconstruction

D-cis-[^{18}F]FPro was prepared with a radiochemical yield of 36 ± 7%, a radiochemical purity > 98%, and a specific radioactivity of > 18.5 TBq/mmol, as previously described [2]. All subjects fasted for at least 12 h prior to the PET studies. Transmission scans with ^{68}Ge/^{68}Ga rotating line sources were performed before tracer injection and used for attenuation correction. After intravenous injection of 244 ± 48 MBq D-cis-[^{18}F]FPro, cerebral PET scans were acquired in list mode on a Siemens ECAT Exact HR+ scanner (32 rings; axial field of view, 15.5 cm). After correction for random and scattered coincidences, dead-time, and decay, data were iteratively reconstructed (63 image planes, ordered-subsets expectation maximization, 6 iterations, 16

subsets) using the ECAT 7.2 software. The reconstructed image voxel size was 2 x 2 x 2.4 mm and a static image obtained 20 - 50 minutes after tracer injection was used for further analysis.

MR imaging

Magnetic resonance images of the brain (including T1-weighted images before and after application of a contrast agent and T2-weighted images) were acquired for anatomical comparison.

PET image analysis

We analyzed the PET images with the relevant toolboxes of PMOD 3.9. All PET images were normalized to the Montreal Neurological Institute (MNI) space including rigid matching of each subject's PET to the anatomical MRI and MRI segmentation. Left and right basal ganglia volumes-of-interest (VOIs) and their corresponding substructures (caudate, pallidum, putamen) were defined using the built-in Hammers N30R83 atlas. In accordance with previous studies on brain tumors with D-cis-[^{18}F]FPro using lesion-to-brain ratios (LBR) [2, 3, 5], we defined a reference region for calculation of target-to-reference ratios: a VOI centered upon the brainstem was used as reference region; as additional reference regions, we analyzed basal ganglia uptake in relation to uptake in the cerebellum and occipital cortex as defined from the built-in atlas. As all reference regions are potentially affected by pathology of parkinsonism, we assured that standard uptake values (SUV) did not differ between patients with parkinsonism and control subjects ($p > 0.05$ for all regions); additionally SUV of the considered reference regions did not differ between groups of parkinsonism (PD versus MSA; $p > 0.05$ for all regions). The ratios of activity in the target tissue divided by the activity in the reference region were used for statistic comparison.

Statistics

We analyzed the data with the Statistical Package for the Social Sciences (SPSS) version 24. Group data are presented as mean ± standard deviation. Group comparisons were performed with Student's t-tests, and chi-square tests as appropriate; a normal distribution of the data was assessed with Shapiro-Wilk tests, Q-Q plots, and box plots. For VOI-based PET analysis, a repeated measures ANOVA with 'brain region' as repeated measure, and the between-subjects factor 'group' was calculated. Significance was accepted at $p < 0.05$.

RESULTS

The ANOVA revealed significantly increased D-cis-[^{18}F]FPro uptake in the basal ganglia of parkinsonian subjects compared to control subjects ($p = 0.049$), with regional group differences in the left pallidum (1.446 ± 0.253 vs. 1.188 ± 0.210; $p = 0.019$), right pallidum (1.379 ± 0.224 vs. 1.123 ± 0.123; $p = 0.003$), and right putamen (1.579 ± 0.251 vs. 1.369 ± 0.164; $p = 0.032$) (Table 2). Exemplary images are shown in Figure 1 and supplementary Figure 1. Despite a significant age difference between the two groups, we did not find a correlation of age with regional D-cis-[^{18}F]FPro uptake in the control sample (all p-values > 0.05), and age was not a significant co-variate when included in the ANOVA ($p = 0.765$). Increased uptake in the right and left pallidum of parkinsonian subjects was also observed when using occipital (left: 0.900 ± 0.119 vs. 0.782 ± 0.131; $p = 0.045$, right: 0.862 ± 0.119 vs. 0.746 ± 0.122; $p = 0.041$) and cerebellum (left: 1.054 ± 0.167 vs. 0.884 ± 0.119; $p = 0.013$, right: 1.009 ± 0.163 vs. 0.841 ± 0.093; $p = 0.007$) as reference region.

In the group of parkinsonian subjects, disease severity (as measured with UPDRS III) correlated with D-cis-[^{18}F]FPro uptake in the right pallidum ($r = -0.687$, $p = 0.041$), but not with uptake in other basal ganglia subregions. Disease duration did not correlate with D-cis-[^{18}F]FPro uptake.

PD patients often present with asymmetric clinical symptoms. Upon visual assessment, four of five subjects from the subgroup of PD patients exhibited increased D-cis-[^{18}F]FPro uptake concordant with clinical symptom manifestation, i.e. patients with right dominant parkinsonian symptoms had higher D-cis-[^{18}F]FPro accumulation in the left basal ganglia and patients with more symmetric symptom expression also showed a symmetric uptake of the tracer (Figure 2).

DISCUSSION

We here present pilot data on increased D-cis-[^{18}F]FPro uptake in the basal ganglia of parkinsonian patients. Particularly, the pallidum and putamen showed a significant increased uptake of D-cis-[^{18}F]FPro, whereas tracer accumulation in the caudate did not differ significantly from controls. In the subgroup of PD patients, D-cis-[^{18}F]FPro uptake was concordant with clinical symptom expression in four out of five patients.

To date, accumulation of D-cis-[^{18}F]FPro has been studied and compared to histopathology in animal models of cortical infarction and cerebral glioma [2, 3]. Interestingly, increased D-cis-[^{18}F]FPro uptake was not only restricted to the ischemic area itself, but was also detected in remote and structurally connected thalamic nuclei. Histopathological evaluation revealed an activation of astrocytes and microglia in these thalamic areas with focally increased D-cis-[^{18}F]FPro uptake, most likely reflecting secondary neurodegenerative processes [2, 3]. In parkinsonism and in particular in PD, neurodegeneration affects primarily brain stem nuclei - i.e. dopaminergic neurons of the substantia nigra - which causes dopaminergic depletion of the striatum. Neuronal death of substantia neurons is accompanied by axonal degeneration which might even proceeds degeneration of the cell soma (so called ‘dying back’ theory) [6]. Additionally, microglia activation is seen in the striatum of patients with neurodegenerative parkinsonism in human PET studies [7, 8] as well as in histopathological studies of humans and animal models [9].

Thus, we suggest that increased D-cis-[^{18}F]FPro uptake in the basal ganglia of patients with neurodegenerative parkinsonism reflects activated astrocytes and inflammation-associated processes [10, 11] due to remote neuronal death of substantia neurons. The normal

accumulation of D-cis-[^{18}F]FPro in the caudate is in line with the described rostro-caudal gradient of dopaminergic deficiency in neurodegenerative parkinsonism with the caudate being typically less affected [12, 13].

The immanent role of astrocytes and microglia in neurodegenerative parkinsonism for disease maintenance and propagation is increasingly recognized [14]. Thus far, PET imaging of activated microglia in neurodegenerative parkinsonism was mostly performed with [^{11}C]PK11195, a ligand of the translocator protein 18kDa (TSPO) [7, 8]. In these studies, increased [^{11}C]PK11195 in the striato-nigral pathway was observed, which was stable over the time of two years in PD patients [7]. In a direct comparison of [^{11}C]PK11195 and D-cis-[^{18}F]FPro, the latter was more sensitive in detecting regions with activated microglia and astrocytes than [^{11}C]PK11195 [2]. PET imaging with D-cis-[^{18}F]FPro might therefore be more suitable for assessing inflammation-associated processes in degenerative parkinsonism.

This pilot study exhibits several restrictions: the number of subjects was limited, a mixed population of parkinsonian subjects was analyzed, and the control group had brain neoplasms. Nevertheless, despite these factors that were likely to limit the statistical power of this study, we observed a significant increase of D-cis-[^{18}F]FPro uptake in the basal ganglia in the group of subjects with neurodegenerative parkinsonism. We aimed at a proof-of-principle, and in spite of statistical limitations, the positive outcome clearly suggests that further studies on uptake of D-cis-[^{18}F]FPro in neurodegenerative diseases are warranted. Furthermore, the two study groups were not matched for age. However, age was neither significant in a correlation analysis nor when included as a co-variate in the ANOVA. We did not include perfusion studies as potential confounder for differences in D-cis-[^{18}F]FPro uptake. Future studies should aim for

larger and more homogenous study samples, allowing for expanded statistical analyses, for example performing a correlation analysis of D-cis-[^{18}F]FPro binding with laterality of akinetic-rigid symptoms.

In conclusion, we observed significantly increased accumulation of D-cis-[^{18}F]FPro in the basal ganglia of subjects with neurodegenerative akinetic-rigid parkinsonism. Data suggest that D-cis-[^{18}F]FPro constitutes a marker for inflammation-associated neurodegeneration.

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TABLES AND FIGURES

Table 1: Demographic and clinical characteristics of subjects

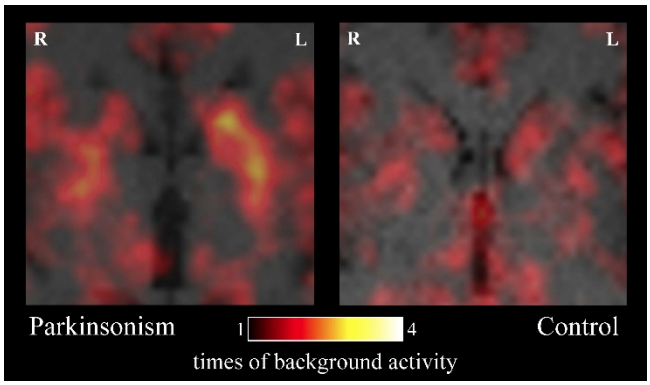
	Parkinsonian patients n = 10	Control patients n = 13	P-value
Age [y]	66.2 ± 5.9	55.5 ± 12.3	**0.024**
Sex (male / female)	6 / 4	10 / 3	0.650
Diagnosis	5x PD 4x MSA 1x CDB	5x glioblastoma 2x astrocytoma WHO grade III 1x oligodendroglioma WHO grade III 1x glioma, unspecified 1x astrogliosis 3x metastasis	-
Injected dose [MBq]	260.9 ± 37.4	231.2 ± 51.5	0.156
Disease duration [y]	4.5 ± 3.5	-	-
UPDRS III	44.7 ± 18.8	-	-

Abbreviations: UPDRS = unified Parkinson's disease rating scale

Table 2: Uptake of D-cis-[^{18}F]FPro in the basal ganglia, expressed as ratio to the brainstem

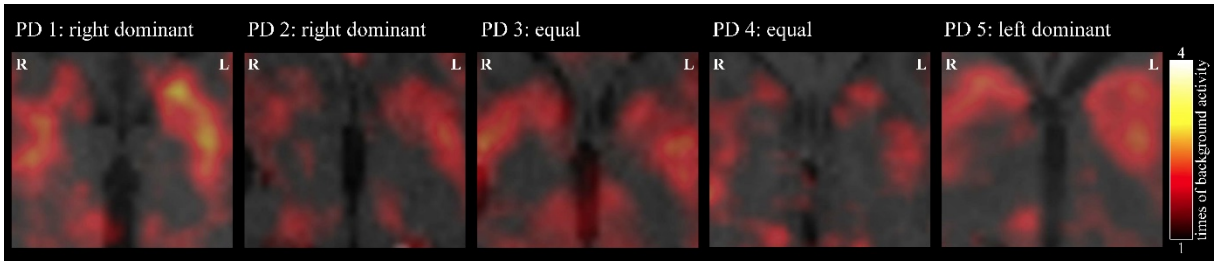
	Parkinsonian patients n = 10	Control patients n = 13	P-value
Right hemisphere			
Caudate	1.150 ± 0.312	1.068 ± 0.280	0.531
Putamen	1.579 ± 0.252	1.369 ± 0.164	**0.032**
Pallidum	1.379 ± 0.224	1.123 ± 0.123	**0.003**
Left hemisphere			
Caudate	1.194 ± 0.300	1.118 ± 0.236	0.521
Putamen	1.566 ± 0.279	1.431 ± 0.173	0.181
Pallidum	1.446 ± 0.253	1.188 ± 0.210	**0.019**

Figure 1: Exemplary images of basal ganglia uptake of D-cis-[^{18}F]FPro



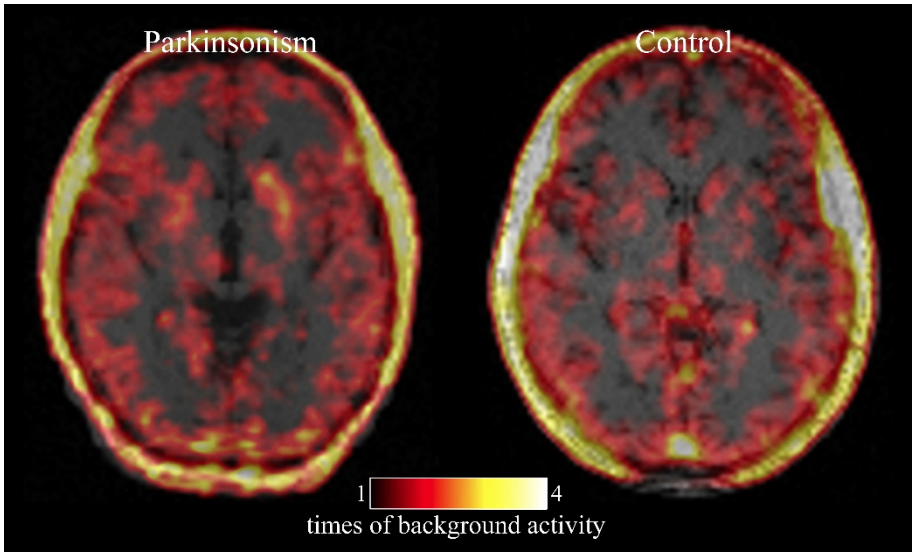
Axial slices of D-cis-[^{18}F]FPro PET (20 - 50 min after tracer injection), co-registered to the anatomical MRI at the level of the basal ganglia; right and left sides as indicated. Left panel shows a patient with Parkinson's disease (same patient as PD 1 in Figure 2), right panel shows a patient with astrogliosis in the right parahippocampal area (not shown). Images were normalized to background uptake (i.e, uptake in the brainstem). Increased D-cis-[^{18}F]FPro uptake is seen in the basal ganglia of the patient with Parkinson's disease, pronounced on the left side. Entire cerebral cross-sections are given as supplementary figure.

Figure 2: D-cis-[^{18}F]FPro uptake in the basal ganglia of PD patients



Axial slices of D-cis-[^{18}F]FPro PET (20 - 50 min after tracer injection), co-registered to anatomical MRI at the level of the basal ganglia; right and left side as indicated. Increased D-cis-[^{18}F]FPro uptake in the basal ganglia contralateral to clinically dominant side is seen in PD 1 and PD 2; PD 3 and PD 4 with equal symptom distribution show symmetrical D-cis-[^{18}F]FPro uptake. PD 5 with left dominant symptoms also shows symmetrical D-cis-[^{18}F]FPro uptake.

Supplementary Figure 1: Exemplary cerebral cross-sections of D-cis-[^{18}F]FPro uptake



Axial slices of D-cis-[^{18}F]FPro PET (20 - 50 min after tracer injection) at the level of the basal ganglia, co-registered to the anatomical MRI (same patients as presented in figure 1).