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Short communication

Effect of dopaminergic medication on adenosine 2A receptor availability in patients with Parkinson's disease

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ABSTRACT

Objective: To assess the necessity of withdrawing dopaminergic medication in Parkinson's disease (PD) patients for accurate estimation of adenosine 2A receptor ($A_{2A}R$) availability using [^{11}C]TMSX PET imaging. This was accomplished by studying the short-term effect of the cessation of dopaminergic medication on $A_{2A}R$ availability in non-dyskinetic patients with PD treated with dopaminergic medication.

Methods: Eight PD patients (age 67.9 ± 5.6 years; 6 men, 2 women) without dyskinesia were enrolled in this study. $A_{2A}R$ availability was measured using PET imaging with a [7-methyl-^{11}C]-(E)-8-(3,4,5-trimethoxystyryl)-1,3,7-trimethylxanthine ([^{11}C]TMSX) radioligand after a short term cessation of dopaminergic medication (12hrs for levodopa, 24hrs for dopamine agonists and MAO-B inhibitors). Repeated PET imaging was performed while the patients were back 'on' their regular dopaminergic medication (median 13 days after first imaging). Conventional MRI was acquired for anatomical reference. Specific binding of [^{11}C]TMSX was quantified as distribution volume ratios (*DVR*) for caudate, pallidum and putamen using Logan graphical method with clustered gray matter reference region.

Results: No significant differences were observed for the *DVR*s in all three striatal regions between 'on' and 'off' medication states. Strong correlations were also observed between the two states. Statistical equivalence was found in pallidum (TOST equivalence test, $p = 0.045$) and putamen (TOST equivalence test, $p = 0.022$), but not in caudate *DVR* (TOST equivalence test, $p = 0.201$) between the two medication states.

Conclusions: Our results show that dopaminergic medication has no significant short-term effect on the availability of A_{2A} receptors in putamen and pallidum of patients with PD. However, relatively poor repeatability was demonstrated in the caudate.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized with the loss of nigrostriatal dopaminergic neurons. Dopamine from these neurons regulates striatal output through direct and indirect pathways to globus pallidus. Adenosine A_{2A} receptors ($A_{2A}R$) form an antagonistic heterodimer with dopamine D_2 receptors (D_2R) in the striatal structures. Within striatum, most of these heterodimers are expressed on the medium spiny neurons of the indirect pathway [1]. Their interaction with D_2R contributes significantly to the regulation of motor functions by maintaining a balanced inhibitory output from external globus pallidus to subthalamic nucleus and other downstream structures [2].

In PD, degeneration of dopaminergic neurons leads to altered $A_{2A}R$ expression and an imbalance of striatal output. Post-mortem and *in-vivo* PET studies have shown an increase in $A_{2A}R$ binding in putamen among dyskinetic PD patients [1,3]. Moreover, simultaneous $A_{2A}R$ antagonism with D_2R activation has been shown to reduce 'off time' without increasing dyskinesias [4]. This mechanism of action forms the basis for the use of FDA approved $A_{2A}R$ antagonist, istradefylline, as an add-on therapy for PD patients on levodopa/carbidopa [5]. Recent studies have also shown improvement in postural symptoms and gait deficits with istradefylline in mid to advanced stage PD patients [6,7]. However, the availability of $A_{2A}R$ in patients with more advanced PD has not been

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thoroughly studied. The main impediment in studying advanced PD patients is the exacerbation of motor symptoms while they are 'off' of their regular medication for 12–24hrs before the scan. Yet, this withdrawal is important to accurately distinguish between the acute effect of dopaminergic medication and the long-term effect of pathogenic mechanisms on $A_{2A}R$ functions. Thus, the aim of this study was to investigate the effect of short-term cessation of dopaminergic medication on $A_{2A}R$ availability in striatal regions in PD patients without dyskinesia. This would aid in determining whether reliable radioligand binding estimates can be obtained with [^{11}C]TMSX PET imaging without the cessation of anti-parkinsonian medication. This would, further, make it feasible to study $A_{2A}R$ binding in more advanced PD patients who would not tolerate even short-term cessation of their antiparkinsonian medication.

2. Methods

2.1. Subjects

Eight patients with PD (two females/six males, mean age 67.9 ± 5.6 years, mean disease duration 5.0 ± 3.2 years) were enrolled for the study from the neurology outpatient clinics of Turku University Hospital. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland. Written informed consent was obtained from all study participants as per the principles of the Declaration of Helsinki. All patients fulfilled the clinical diagnostic criteria of idiopathic PD without dyskinesia with a mean (±SD) Unified Parkinson's Disease Rating Scale (UPDRS) III score of 28.8 (±6.4). All study subjects had normal cognitive status (Mini Mental State Examination [MMSE] 28.8 [±0.7]). The mean levodopa equivalent dose (LED) was 595.6 (±383.3) mg. Seven patients were on a combination of MAO-B inhibitors and dopamine agonists while one was on dopamine agonist alone. Six of the eight patients were also on levodopa (Supplementary Table 1). Exclusion criteria included significant comorbidities or any active neurologic condition apart from PD.

2.2. Clinical assessment

UPDRS, LED, MMSE and disease duration were recorded as part of the clinical evaluation. Clinical examinations were performed before and after the temporary withdrawal of dopaminergic medication prior to each PET scan.

2.3. [^{11}C]TMSX PET and MRI imaging

The production of [7-methyl-^{11}C]-(E)-8-(3,4,5-trimethoxystyryl)-1,3,7-trimethylxanthine ([^{11}C]TMSX) radioligand was performed using methods detailed in our earlier study [8]. PET scanning was performed using ECAT high resolution research tomograph PET scanner (HRRT, CTI PET Systems, Knoxville, TN, USA) with a spatial resolution of 2.5 mm in radial and axial directions. Patients were instructed to avoid caffeinated beverages 24 h prior to the scan due to the antagonistic properties of caffeine on adenosine receptors. A 6-min transmission scan using ^{137}Cs point source was performed for attenuation correction. Thereafter, a 60-min dynamic emission scan with 27 timed frames (6 × 10, 1 × 30, 5 × 60, 5 × 150, and 8 × 300 s; total 3600 s) was acquired. Brain MRI was performed using Philips Gyroscan Intera 1.5 T Nova Dual scanner (Philips, Netherlands) acquiring 3D axial T1 weighted sequence for anatomical reference. The detailed PET and MRI acquisition protocol has been described earlier [9]. The patients halted their PD medication 12 h (standard levodopa) or 24 h (prolonged release dopamine agonists and/or MAO-B inhibitors) prior to the first PET scan and returned to their normal dosing regimen after the scan. A repeated PET scan with identical protocol but without the cessation of dopaminergic medication was acquired within a median of 13 days (Interquartile range [IQR] = 7.8–14.0) from the first scan. MR images were processed and parcellated with Freesurfer software to generate caudate, putamen and pallidum regions of interests (ROI) [10]. MRIs were then co-registered with PET images using statistical parametric mapping software (SPM8, Wellcome Centre for Human Neuroimaging, UK) running on Matlab 2017a (The Mathworks, Inc. USA). To further assess the differences in radioligand binding within caudate, ROIs on caudate subdivisions were drawn manually on MR images with superimposed PET scans using an in-house developed software (Carimas, v2.10). Anterior commissure was utilized as the anatomical landmark for caudate demarcation into anterior and posterior regions. Region specific binding of [^{11}C]TMSX was quantified as distribution volume ratios (*DVR*) using Logan reference tissue-input method within 20–60 min time interval. This was applied to ROI specific time activity curves (TAC) using the clustered gray matter reference region derived from supervised clustering algorithm (SVCA) using modified Super-PK software, as validated previously [9]. Areas under the curve (AUC) of the reference region TAC were calculated to evaluate the robustness of the reference region. Global mean *DVR* was calculated using volume weighted mean of the whole brain mask. Bilateral mean *DVR*s were calculated for caudate, putamen, pallidum and for the whole striatum. For the anterior and posterior subdivisions of caudate, bilateral mean, and contralateral and ipsilateral (with respect to the clinically more affected side) *DVR*s were calculated.

2.4. Statistical analysis

Statistical analyses were performed using R software (v4.0.3). Normality distribution of the data was evaluated with Shapiro-Wilk test. Paired *t*-test was performed to check for differences in the aforementioned *DVR* and AUC values between the 'on' and 'off' medication states. Pearson's test for correlation was used to test the effectiveness of pairing, and two one sided test (TOST) method for paired data was used for the evaluation of equivalence of *DVR*s and AUCs between the two different medication states. Threshold for negligible difference (epsilon) was set to 0.04 for TOST equivalence test. This difference was obtained from an earlier study evaluating the effect of dopaminergic therapy on $A_{2A}R$ availability in drug naïve patients [3]. P-values <0.05 from two-tailed tests were regarded statistically significant for all analyses.

3. Results

The mean administered dose of [^{11}C]TMSX was 473.6 MBq (±51.9) for the first, and 500.4 MBq (±20.7) for the second scan, with no significant differences between them ($p = 0.19$). None of the patients experienced severe off-symptoms and remained fully ambulatory during the temporary withdrawal of their PD medication.

No difference was found in the global mean *DVR*s between the two imaging time points (median [IQR] % difference, 0.12 [-0.93- 0.28], $p = 0.76$). Significantly effective pairing was found in the global mean *DVR*s between the scans ($r = 0.96$, $p < 0.001$), and the *DVR* values between the two medication states were statistically equal ($p < 0.001$).

No differences were found in the SVCA gray matter reference region TAC AUCs between the two imaging time points (median [IQR] % difference, −1.31 [-4.27- 7.95], $p > 0.99$) (Fig. 1a). Significantly effective pairing was found in the reference region TAC AUCs between the two scans ($r = 0.86$, $p = 0.006$) representing strong association (Fig. 1b). The reference region AUCs of the TACs for both time points were also found to be statistically equal ($p < 0.001$).

No differences were found in the mean striatum *DVR* values between 'on' and 'off' medication states ($p = 0.43$) (Fig. 1c). Strong correlation ($r = 0.71$, $p = 0.048$) (Fig. 1d) and statistical equivalence ($p = 0.047$) were found in the mean striatal *DVR* values between 'on' and 'off' medication states. In the ROI specific analyses no significant differences in [^{11}C]TMSX binding were observed when the study subjects were 'on' and 'off' dopaminergic medication in caudate (median [IQR] % difference, 1.40 [-1.88-6.21], $p = 0.25$), pallidum (median [IQR] % difference, 0.00 [-1.37-2.95], $p = 0.59$) and putamen (median [IQR] % difference, 0.00

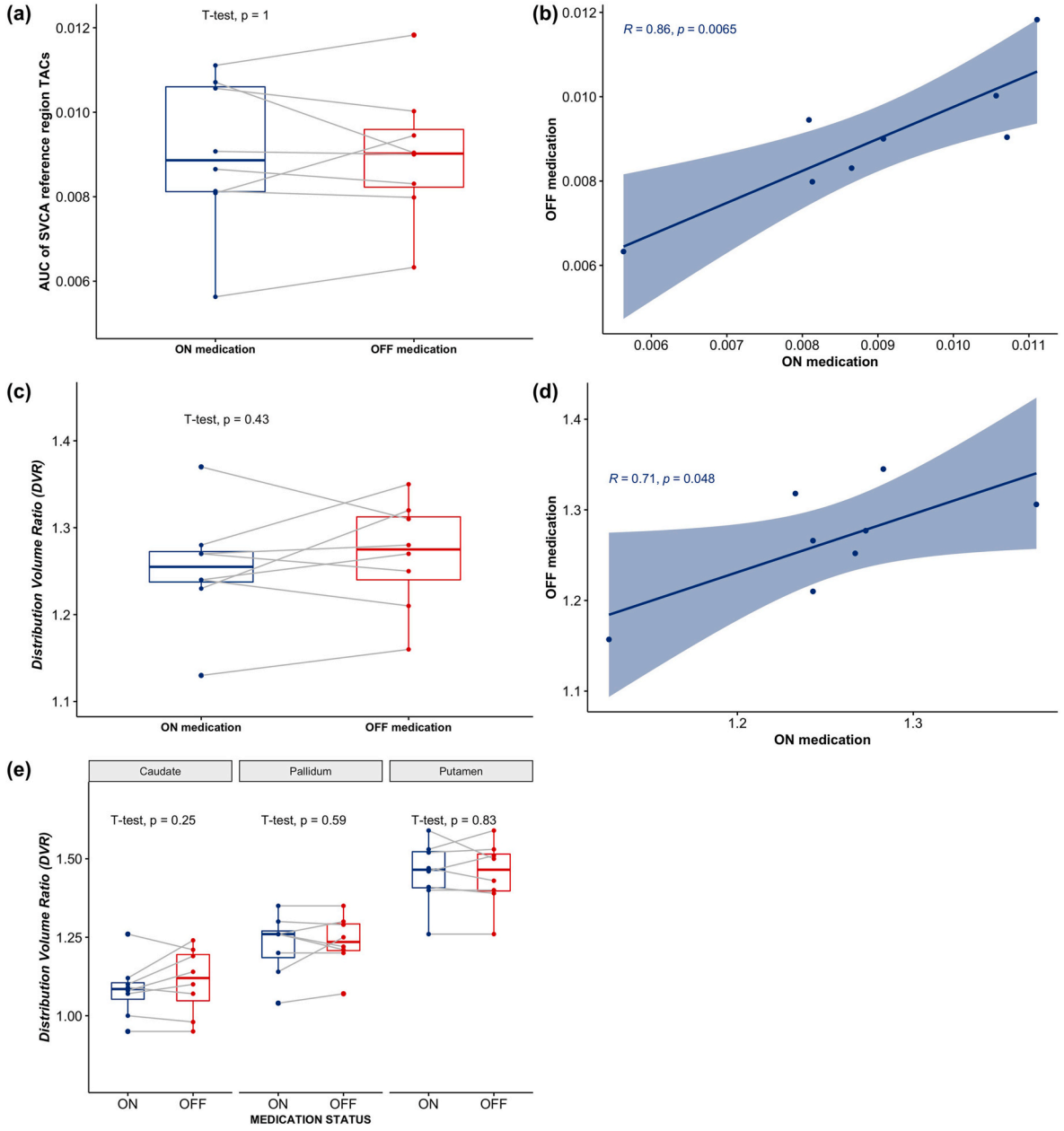

Fig. 1. Box and scatter plots comparing [^{11}C]TMSX PET parameters while PD patients were 'on' dopaminergic medication (ON medication) and 'off' it (OFF medication) (a) Box plots representing areas under the curve (AUC) of time activity curves (TAC) while the subjects were ON medication and OFF medication (b) Scatter plot representing correlation (Pearson's) between the subjects' reference region TAC AUC values while ON and OFF medication. (c) Box plot representing mean striatal *DVR* values from subjects while ON and OFF medication states. (d) Scatter plot representing correlation (Pearson's) for mean striatal *DVR* values from subjects while ON and OFF medication states. (e) Box plot showing *DVR* values for caudate, pallidum and putamen from subjects while ON and OFF dopaminergic medication.

[-1.74-1.34], p = 0.83) (Fig. 1e). Significantly effective pairing was found between 'on' and 'off' medication states in caudate (r = 0.83, p = 0.011), pallidum (r = 0.85, p = 0.007) and putamen (r = 0.89, p = 0.003). Statistical equivalence was found in pallidum (p = 0.045) and putamen (p = 0.022) between the two medication states. However, no statistical equivalence was found in caudate *DVR* values between the time points (p = 0.201).

Finally, considering that caudate *DVR* values were not equivalent between the two medication states, and that the clinical presentation in earlier stages of the disease is usually asymmetric, caudate subdivisions with contralateral and ipsilateral sides were evaluated for possible differences between scans. Neither significant difference nor equivalence was found in $A_{2A}R$ availability in any of the caudate subdivisions between the medicated and non-medicated state. Effective pairing was found only for the ipsilateral posterior caudate (Table 1).

4. Discussion

Overall, we observed no significant difference between 'on' and 'off' medication states in the availability of $A_{2A}R$ in any of the striatal structures. The correlations for $A_{2A}R$ binding in striatal regions between the two scans while 'on' and 'off' medication were generally high. Pallidum and putamen were found to be statistically equivalent between the two medication states. The binding of $A_{2A}R$ in the entire caudate was not statistically equivalent between the two time points. We thence evaluated subdivisions of the caudate for exploratory purposes and

Table 1
Correlations, differences and equivalences in [^{11}C]TMSX binding in caudate subdivisions while on dopaminergic medication and off dopaminergic medication, presented with Pearson correlation, paired *t*-test, median (interquartile range) percentual differences and Paired two one sided *t*-test (TOST) for equivalence of distribution volume ratios (*DVR*). Caudate subdivisions of ipsilateral and contralateral sides in the brain are related to the primarily more affected side of the motor symptoms. P-values <0.05 are regarded as statistically significant.

Caudate and its subdivisions	Pearson r (p)	Paired t-test p	median (IQR) % difference	Paired TOST p
Caudate	**0.827 (0.011)**	0.252	1.40 (−1.88-6.21)	0.201
Anterior Caudate	0.538 (0.169)	0.677	1.51 (−3.41-4.16)	0.295
Posterior Caudate	0.650 (0.080)	0.529	0.51 (−6.58-3.91)	0.154
Caudate (contralateral)	0.377 (0.356)	0.494	−1.05 (−4.14-2.11)	0.353
Anterior Caudate (contralateral)	0.445 (0.269)	0.471	−1.62 (−4.61-1.81)	0.404
Posterior Caudate (contralateral)	0.459 (0.252)	0.592	−0.14 (−6.49-5.87)	0.195
Caudate (ipsilateral)	0.648 (0.081)	0.857	−1.02 (−3.11-5.44)	0.200
Anterior Caudate (ipsilateral)	0.621 (0.100)	0.955	−0.09 (−2.53-6.13)	0.202
Posterior Caudate (ipsilateral)	**0.810 (0.015)**	0.495	−2.20 (−6.18-2.49)	0.127

found no significant difference nor equivalence in any of the caudate subdivisions. Similarly, correlations were also weaker in the caudatal subdivisions. This may be attributed to higher variation due to small ROI size. In order to draw robust conclusions about the effect of short-term cessation of dopaminergic medication on the caudate, studies with larger cohorts are needed.

Increase in [^{11}C]TMSX binding in the putamen of previously drug naïve PD patients about one year after the initiation of antiparkinsonian therapy has been shown in an earlier PET study [3]. Our patients, however, were already on stable medication and had a higher overall disease duration. Dopamine agonists, especially apomorphine but also the non-ergot agonists rotigotine and pramipexole, have been shown to act as negative allosteric modulators of $A_{2A}R$ agonists [11]. Majority of our study subjects were on a combination of levodopa/carbidopa, pramipexole and MAO-inhibitors. Thus, the negative allosteric effects of pramipexole on $A_{2A}R$ availability may have been masked by other dopaminergic medications.

In this study we sought to investigate the acute effect of dopaminergic medication on $A_{2A}R$ binding. Since our cohort comprised of both early and moderate stage PD patients, we cannot rule out the possibility of a more chronic effect of dopaminergic medication on the availability of $A_{2A}R$, as our patients had been medicated with antiparkinsonian drugs for average 4.93 years. It is also pertinent to mention that in common practice, PD patients are often on multiple dopaminergic medications simultaneously. We cannot eliminate the likelihood of each of these medications having a differential effect on $A_{2A}R$ availability. However, our aim was to evaluate the effect of dopaminergic medication in a typical clinical setting, primarily, to assess the feasibility of not taking advanced PD patients 'off' their medication to obtain reliable $A_{2A}R$ binding estimates. Finally, Logan graphical method with cerebral cortex as reference region has shown an acceptable test-retest variability of 5% in putamen for [^{11}C]TMSX [12]. Moreover, we have previously shown that our methodology with the SVCA gray matter reference region has good agreement with *DVR* estimates acquired with arterial input function [9].

5. Conclusion

In summary, our results demonstrate that cessation of dopaminergic medication in PD patients does not change $A_{2A}R$ availability in putamen and pallidum. However, results for caudate and its sub-divisions are inconclusive. Future studies with larger sample size are therefore recommended to elucidate the effect of dopaminergic medication on caudate. Taken together, our findings suggest that PD patients may not need to be taken off their dopaminergic medication for reliable estimates of $A_{2A}R$ availability specifically in putamen and pallidum.

Ethics approval

The study protocol was accepted by the Ethics Committee of the Hospital District of Southwest Finland and abided to the principles of the Declaration of Helsinki.

Data statement

The raw data used in the preparation of this article can be shared in anonymized format by request of a qualified investigator.

Authors' contributions

Conceptualization: [Eero Rissanen], [Laura Airas], [Juha Rinne]; Methodology: [Jouni Tuisku], [Riitta Parkkola], [Semi Helin]; Formal analysis and investigation: [Imran Waggan], [Markus Matilainen]; Writing - original draft preparation: [Imran Waggan]; Writing - review and editing: [Imran Waggan], [Eero Rissanen], [Laura Airas], [Juha Rinne], [Jouni Tuisku]; Funding acquisition: [Laura Airas]; Resources: [Laura Airas]; Supervision: [Eero Rissanen], [Juha Rinne], [Laura Airas]

All authors have approved this version of the manuscript.

Declaration of competing interest

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2021.03.030.

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