Adenosine 2A receptor availability in dyskinetic and nondyskinetic patients with Parkinson disease

A.F. Ramlackhansingh, MRCP
S.K. Bose, PhD
I. Ahmed, MRCP
F.E. Turkheimer, PhD
N. Pavese, MD
D.J. Brooks, MD, DSc

ABSTRACT

Objective: To investigate striatal adenosine A2A receptor availability in patients with Parkinson disease (PD) with and without levodopa-induced dyskinesias (LIDs). While providing effective relief from the motor symptoms of PD, chronic levodopa use is associated with development of LIDs. A2A receptors are expressed on the bodies of indirect pathway medium spiny striatal neurons and on dopamine terminals and play a role in modulating dopamine transmission. A2A antagonists have antiparkinsonian activity by boosting levodopa efficacy. We aimed to study A2A receptor availability in patients with PD with and without LIDs using PET and \[^{11}C\]SCH442416, an A2A antagonist.

Methods: Six patients with PD with and 6 without LIDs were studied withdrawn 12 hours from medication. Their PET findings were compared with 6 age-matched healthy controls. Using spectral analysis, \[^{11}C\]SCH442416 regional volumes of distribution (\(V_T\)) were computed for the caudate, putamen, and thalamus and binding potentials (BPND) reflecting the ratio of specific: nonspecific uptake were compared between groups.

Results: A2A binding in the caudate and putamen of subjects with PD with LIDs was far higher (\(p = 0.026\) and \(p = 0.036\), respectively) than that of subjects with PD without LIDs, which lay within the control range. Thalamic A2A availability was similar for all 3 groups.

Conclusion: Patients with PD with LIDs show increased A2A receptor availability in the striatum. This finding is compatible with altered adenosine transmission playing a role in LIDs and provides a rationale for a trial of A2A receptor agents in the treatment of these motor complications.

Neurology® 2011;76:1811–1816

GLOSSARY

ANCOVA = analysis of covariance; GPe = external pallidum; GPI = globus pallidus interna; H&Y = Hoehn & Yahr; LED = levodopa effective dose; LEU = levodopa equivalent unit; LID = levodopa-induced dyskinesia; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD = Parkinson disease; PPE = preproenkephalin; ROI = region of interest; SNpr = substantia nigra pars reticulata; UPDRS = Unified Parkinson’s Disease Rating Scale.

Parkinson disease (PD) is the second most prevalent neurodegenerative disorder worldwide. The prodrug levodopa remains the most effective oral therapy and is eventually offered to most patients with PD. Oral levodopa, however, is not without complications. Chronic exposure in PD is associated with the development of involuntary movements known as levodopa-induced dyskinesias (LIDs) in around 50% of patients after 5 years and 90% after 10 years. LIDs complicate management and have been linked to increased mortality and weight loss.

The pathogenesis of LIDs is thought to be linked to changes in the output of the striatum to the internal pallidum via direct and indirect pathways. Indirect pathway neurons express adenosine A2A receptors. Cynomolgus monkeys, lesioned with the nigral toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), that subsequently develop dyskinesia after exposure to levodopa, showed significantly higher striatal A2A mRNA levels when compared to nondyskinetic monkeys and a normal control group. Human postmortem studies also demonstrate increased striatal A2A mRNA expression in patients with PD with dyskinesias.
We hypothesize that dyskinetic patients with PD would have higher striatal A2A receptor availability compared to nondyskinetic subjects. We used \( ^{11} \text{C}\)SCH442416 PET as an in vivo marker of A2A availability. This nonxanthine radioligand binds selectively and reversibly to striatal A2A receptors with nanomolar affinity. Preclinical studies suggest that it is suitable for the in vivo imaging of adenosine A2A receptors with PET because of its high affinity and selectivity, good signal-to-noise ratio, and low levels of radioactive metabolites in the brains of rats and nonhuman primates. \(^9^{,}^{10}\)

**METHODS Subjects.** The size of the PD cohorts and control group was determined by power calculations based on the previously established low variance of the striatal binding potential for \( ^{11} \text{C}\)SCH442416 (SD of \( B_{PD} \) = 0.2 in controls). Six subjects with PD and 6 without dyskinesias were calculated to provide 80% power at a 5% error rate to detect an effect size of \( \Delta B_{PD} \) = 0.4.

Twelve patients with PD were recruited from local Movement Disorders clinics. All patients fulfilled UK Parkinson’s Disease Society Brain Bank clinical diagnostic criteria for idiopathic PD. Patients with significant comorbidities, including a previous history of any other neurologic condition, dementia, or depression, were excluded. The patient group consisted of 2 separate cohorts: 6 subjects had peak dose levodopa-induced choreic dyskinesias and a further 6 subjects were dyskinesia-free on this medication. The levodopa effective dose (LED) for each subject with PD was calculated as follows: 100 mg levodopa = 2 mg cabergoline = 1 mg pramipexole or 1 mg pergolide = 5 mg ropinirole = 4 mg rotigotine patch = 0.5 mg rasagiline. The conversion factors below were used for controlled release levodopa and levodopa/carbidopa/entacapone combination: modified release cocarldopa 25/100 (carbidopa 25 mg, levodopa 100 mg) = 65 mg levodopa; 50 mg levodopa/carbidop/entacapone combination = 65 mg levodopa. Six age-matched healthy controls were also recruited. Table 1 summarizes the clinical features of all patients with PD and healthy controls.

**Standard protocol approvals, registrations, and patient consents.** Approval for this study was obtained from the Research and Ethics Committee of the Hammersmith, Queen Charlotte, and Chelsea and Acton Hospitals Trust, London, UK, reference number 05/Q0406/69. Permission to administer \( ^{11} \text{C}\)SCH442416 was obtained from the Administration of Radioactive Substances Advisory Committee (ARSAC), UK, reference number 262-336-22789. Signed informed consent was obtained from all participants, according to the Declaration of Helsinki. Funding was provided from the Parkinson’s Disease Society.

**Clinical assessment.** Disease duration, Hoehn and Yahr (H&Y) staging, LED, and the Unified Parkinson’s Disease Rating Scale (UPDRS) were recorded as part of the clinical assessment of all subjects with PD. Examinations were performed when the patients were in an “off” condition after temporary withdrawal of their medication.

**PET procedure.** PET scanning was performed at the Cyclotron Building, Hammersmith Hospital, London, UK, using a Siemens ECAT EXACT HR+ scanner. This scanner has an axial view of 15.5 cm. Sixty-three transaxial image planes were acquired at 2.46-mm slices with a reconstruction axial resolution of 5.4 mm and a transaxial resolution of 5.6 mm. \(^9\) A 10-minute transmission scan was acquired before the emission study using a single rotating photon point source of \(^{15}\)Cs for subsequent attenuation and scatter correction. This was followed by the injection of an average of 612 MBq of \( ^{11} \text{C}\)SCH442416 over 10 seconds, 30 seconds after the start of a 90-minute dynamic emission scan (34 frames). All PET data were acquired in 3-dimensional mode, corrected for attenuation, detector efficiency, random events, and scatter, and reconstructed into tomographic images using filtered back-projection.

Prior to scanning, all subjects had radial arterial cannulation under local anesthesia. Continuous online sampling was performed for the first 15 minutes, with discrete blood samples taken at baseline, 5, 10, 15, 20, 30, 40, 50, 60, 75, and 90 minutes. An aliquot of each discrete sample was rapidly centrifuged to obtain corresponding plasma and radioactivity concentrations that were measured in an NaI(TI) well counter for blood and plasma separately. The continuous blood counts were corrected using the plasma/blood ratio to derive a plasma input function. The plasma was further analyzed for radiolabeled metabolites using high-performance liquid chromatography and the plasma input function was corrected accordingly to produce a final parent radioligand input function to be used for quantification. A post hoc frame-by-frame realignment procedure was applied to compensate for head movement in the scanner. To reduce the influence of redistribution of radiotracer producing erroneous realignments, \(^{12}\) nonattenuation-corrected images were used. Nonattenuation-corrected images are considered to be more useful for the realignment algorithm because these images include a significant scalp and skull bone marrow signal compared with attenuation-corrected images. Frames were realigned to a single, reference frame acquired 40 minutes postinjection, which had a high signal-to-noise ratio, using a mutual informa-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Study population</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F Age, y Disease duration, y H&amp;Y stage LEU, mg/d UPDRS</td>
<td></td>
</tr>
<tr>
<td>Controls (n = 6), mean ± SD</td>
<td>3/3 65 ± 6.2 NA NA NA</td>
</tr>
<tr>
<td>PD without LID (n = 6), mean ± SD</td>
<td>4/2 67.7 ± 4.4 6.2 ± 3.4 1.9 ± 0.4 299 ± 112.2 54.7 ± 19.7</td>
</tr>
<tr>
<td>PD with LID (n = 6), mean ± SD</td>
<td>2/4 65.3 ± 11.4 13.2 ± 5.6* 2.8 ± 0.3* 1,029 ± 477.9* 64.7 ± 9.0</td>
</tr>
</tbody>
</table>

Abbreviations: H&Y – Hoehn & Yahr; LEU – levodopa equivalent unit; LID – levodopa-induced dyskinesia; NA – not applicable; PD – Parkinson disease; UPDRS – Unified Parkinson’s Disease Rating Scale.

* \( p < 0.05 \) 2-tailed Mann-Whitney U test.
tion algorithm,13 and the transformation parameters were then applied to the corresponding attenuation-corrected dynamic images. The nonattenuation-corrected images were denoised using a level 2, order 64 Battle Lemarie wavelet filter.14

[11C]SCH442416 was manufactured and supplied by Hammersmith Immanet, GE Healthcare.

MRI procedure. Each subject had a volumetric T1-weighted MRI performed with a Philips 1.5 T Eclipse system for the purpose of defining the cerebellum, caudate, putamen, and thalamus for sampling. The individual MRIs were coregistered to the PET-summed images using an automated multiresolution optimization procedure.13 The anatomic segmentations obtained were used to define the above regions of interest (ROIs). ROIs were then applied onto the dynamic PET image to obtain the average time-activity curves of radioactivity concentrations. These were then used for quantification.

PET quantification. Spectral analysis together with the metabolite-corrected plasma input function was used to analyze the kinetic components of the regional time-activity curves.16,17 In brief, spectral analysis decomposes the regional time-activity course of the radioactivity concentration into separate kinetic components that are functions of the input function and of the tissue compartments with different residence times. It makes no a priori assumptions about the number and nature of kinetic compartments present. The regional total volume of distribution ($V_T$) can be obtained by adding the spectral analysis components detected into a suitable kinetic range that encompasses the free tracer in tissue plus its binding, both nonspecific or specific to the A2A receptors.14,16 Finally, the measure of interest, which is the binding potential of the specifically bound radioligand relative to the nondisplaceable radioligand in tissue ($BP_{ND}$),14 is calculated from the volumes of distribution as follows:

$$BP_{ND} = \frac{V_T}{V_T \text{ target region}} \left(1 - \frac{1}{V_T \text{ cerebellum}}\right)$$

The cerebellum (which is devoid of A2A receptors in humans) was used as a reference region and therefore $V_T \text{ cerebellum}$ was used as an estimate of the free and nonspecific binding of [11C]SCH442416 throughout the brain. All preprocessing and quantification steps were performed using in-house routines written in Matlab 6.5 (The MathWorks Inc., Natick, MA).

Statistical analysis. Clinical features of the PD groups (age, disease duration, LED, H&Y staging, and UPDRS score) were interrogated using nonparametric Mann-Whitney U testing. Regional group differences in $BP_{ND}$ were also tested with the Mann-Whitney U statistic. The threshold for statistical significance was set at $p = 0.05$.

In order to investigate the possible confounding effects of between-group differences in disease severity, disease duration, and dose of dopaminergic treatment on regional $BP_{ND}$ we performed repeated-measures analysis of covariance (ANCOVA) using age at scanning, disease duration, levodopa equivalent unit (LEU), UPDRS scores, and H&Y staging as covariates. Additionally, we checked the relationship between each of these parameters and [11C]SCH442416 $BP_{ND}$ values by Spearman rho correlation studies. Spearman rho correlations were also performed to determine the relationship between dyskinesia severity, rated with the UPDRS, and regional [11C]SCH442416 $BP_{ND}$.

All statistical tests were performed using SPSS (v 16, SPSS Inc., IBM, Chicago, IL).

RESULTS Subjects with LIDs had longer disease duration ($p = 0.036$), higher LED ($p = 0.006$), and higher H&Y staging ($p = 0.005$) than non-LID subjects. Mean UPDRS scores, however, did not differ between PD groups ($p = 0.172$) (table 1).

The regional cerebral [11C]SCH442416 $BP_{ND}$ of all groups are detailed in table 2. The data revealed large increases in caudate and putamen mean $BP_{ND}$ of the dyskinetic patients with PD compared with the nondyskinetic patients with PD and the control group ($p < 0.05$). Thalamic A2A binding was equivalent across all 3 groups. The figure shows [11C]SCH442416 summed images from a healthy subject, a patient with PD without dyskinesias, and a patient with PD with dyskinesias.

Repeated-measures ANCOVA using age at scanning, disease duration, LEU, UPDRS scores, and H&Y staging as covariates showed no contributions of those factors to the between-group differences in mean striatal $BP_{ND}$ ($p > 0.05$).

Spearman rho nonparametric statistic found no correlation between striatal [11C]SCH442416 uptake and age ($r = 0.081$, $p = 0.802$), disease duration ($r = 0.176$, $p = 0.584$), LED ($r = 0.350$, $p = 0.265$), and UPDRS score ($r = 0.158$, $p = 0.624$). Additionally, we found no correlation between striatal [11C]SCH442416 uptake and dyskinesia severity as measured by UPDRS subscale IV ($r = 0.081$, $p = 1$).

DISCUSSION In this study we demonstrated higher uptake of [11C]SCH442416 in the caudate and putamen of patients with more advanced PD with LIDs compared to earlier cases without LIDs and to healthy controls. No difference in striatal A2A binding was found between patients with PD without LIDs and healthy controls. In the human brain, the striatum contains one of the highest concentrations

<table>
<thead>
<tr>
<th>Region</th>
<th>Controls</th>
<th>PD without LID</th>
<th>PD with LID</th>
<th>Control vs PD with LID</th>
<th>PD without LID vs PD with LID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate, mean ± SD</td>
<td>0.53 ± 0.24</td>
<td>0.4 ± 0.24</td>
<td>0.96 ± 0.46</td>
<td>$p = 0.041$</td>
<td>$p = 0.026$</td>
</tr>
<tr>
<td>Putamen, mean ± SD</td>
<td>0.97 ± 0.21</td>
<td>0.97 ± 0.33</td>
<td>1.67 ± 0.62</td>
<td>$p = 0.0087$</td>
<td>$p = 0.036$</td>
</tr>
<tr>
<td>Thalamus, mean ± SD</td>
<td>0.12 ± 0.18</td>
<td>0.13 ± 0.14</td>
<td>0.14 ± 0.18</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: LID = levodopa-induced dyskinesia; NS = not significant; PD = Parkinson disease.
of A2A receptors. The increase in A2A receptor availability that we have detected in vivo mirrors the results of histopathologic studies where A2A receptor mRNA expression was shown to be elevated in brains of dyskinetic patients with PD and in dyskinetic animal lesion models of PD. The increased striatal \( ^{11}\text{C}\text{SCH442416} \) binding observed in our dyskinetic patients is most likely to reflect adaptive changes to long-term exposure to levodopa, as observed in animal models of PD; however, we cannot exclude other possible explanations including low striatal adenosine levels resulting in increased A2A availability or a compensatory receptor upregulation.

Increased striatal A2A expression could result in the production of dyskinesias by inhibiting traffic through the indirect pathway to the external pallidum (GPe). The indirect pathway links the striatum with the globus pallidus interna (GPi)/substantia nigra pars reticulata (SNpr) complex via the external pallidum and subthalamic nucleus. The GPi then projects directly to the ventral thalamus with GABAergic output and has a final inhibitory effect on motor activity. Indirect striatal neurons projecting to GPe are GABAergic, as are the neurons projecting from the GPe to the subthalamic nucleus, while the STN to GPi projections are glutamatergic. The net effect of the indirect striatal output is, therefore, facilitatory on GPi and inhibitory on thalamocortical projections. Dopamine (D2) receptors are expressed by neurons in the indirect pathway, their stimulation being necessary for the activity of this system. In contrast, the direct pathway GABAergic connecting striatum to GPi inhibits this nucleus and so facilitates thalamocortical activity.

A2A receptors are found on the cell bodies of striatal indirect pathway neurons projecting to the GPe. They are found in conjunction with D2 receptors forming heteromers. Adenosine binding to heteromerized A2A receptors results in adenylyl-cyclase activation and an intramembrane receptor interaction with D2 receptors, antagonizing their activity. The overall effect of increased A2A agonism is thus thought to be an increase in striatal-GPe pathway activity and its inhibitory effect on thalamocortical pathways. This would be reversed by administration of A2A blockers. If our PET finding of increased striatal A2A availability in dyskinetic PD represents a reduction in endogenous adenosine levels occupying these sites then underactivity of the indirect pathway would follow, leading in turn to increased and potentially disorganized thalamocortical traffic.

A2A activation is also thought to be a requirement for dyskinesia priming mechanisms. In MPTP primate models of PD, animals that did not develop dyskinesias had lower levels of A2A receptor binding and gene expression compared to those animals that developed dyskinesias.

In our small cohort of dyskinetic patients, we did not find a correlation between striatal \( ^{11}\text{C}\text{SCH442416} \) uptake and dyskinesia severity. However, we had limited power to detect such a correlation and confounds such as varying individual levodopa dosages and presynaptic dopamine storage capacities could have obscured such a relationship. Other neurotransmitter systems could also influence onset of dyskinesias. Preproenkephalin (PPE) is one such neuromodulator and increased PPE expression has been associated with the development of dyskinesia in MPTP monkeys. Interestingly, PPE mRNA expression in rat striatum is facilitated by A2A activation.

Our dyskinetic subjects had a longer duration of PD, had more severe H&Y stage, and were taking higher doses of levodopa than the nondyskinetic cases. It could be argued that the clinical differences observed between PD groups rather than the presence of dyskinesias were responsible for the higher
and $^{[11}C]SCH442416$ uptake in the LIDs group. Analysis was thus done in an attempt to control for these confounding variables. Nonparametric statistics were used for univariate testing because of the small sample size, not for non-normality, as the sample size did not allow meaningful normality testing. To test for the effect of confounding, we sought the most powerful approach, the repeated-measures ANCOVA, where regions of interest are the repeated factor. The repeated-measures ANCOVA, similarly to the analysis of variance, is generally robust to non-normality and was preferred to the nonparametric alternative (Friedman 2-way analysis of variance) as the latter cannot handle covariates.

Repeated-measures ANCOVA using age at scanning, disease duration, LEU, and H&Y staging as covariates showed no effect of any of these factors on regional group differences in BPND. Additionally, no correlations between any of these clinical features and $^{[11}C]SCH442416 - BDND$ were found. Finally, thalamic $^{[11}C]SCH442416$ BDND was similar between all groups despite the differences in levodopa exposure. Taken together, these findings support, but do not prove, that the increase in striatal $^{[11}C]SCH442416$ BDND observed in our dyskinetic patients is related to the presence of involuntary movements.

The study indicates that adenosine A2A sites are a potential pharmacologic target for the management of LIDs. Istradefylline and preladenant, 2 selective adenosine A2A antagonists, are currently being tested as potential symptomatic agents in PD. The results of the first clinical trials with istradefylline have recently become available.24-28 While the drug has tested as potential symptomatic agents in PD. The adenosine A2A antagonists, are currently being studied for the management of LIDs. Istradefylline and preladenant, 2 selective adenosine A2A antagonists, are currently being tested as potential symptomatic agents in PD. The results of the first clinical trials with istradefylline have recently become available.

Preladenant at the dose of 1 mg/kg inhibited levodopa-induced behavioral sensitization after repeated daily administration in rodent models of PD, suggesting a reduced risk of the development of dyskinesias.29,30 An increase in dyskinesias following adjunct istradefylline usage would fit with our hypothesis that raised striatal A2A binding reflects reduced adenosine agonism in our dyskinetic PD cases. If we are correct, adenosine agonists rather than antagonists would exhibit antidyskinetic activity. Preladenant at the dose of 1 mg/kg inhibited levodopa-induced behavioral sensitization after repeated daily administration in rodent models of PD, suggesting a reduced risk of the development of dyskinesias.29,30 An increase in dyskinesias following adjunct istradefylline usage would fit with our hypothesis that raised striatal A2A binding reflects reduced adenosine agonism in our dyskinetic PD cases. If we are correct, adenosine agonists rather than antagonists would exhibit antidyskinetic activity. Preladenant at the dose of 1 mg/kg inhibited levodopa-induced behavioral sensitization after repeated daily administration in rodent models of PD, suggesting a reduced risk of the development of dyskinesias.29,30 An increase in dyskinesias following adjunct istradefylline usage would fit with our hypothesis that raised striatal A2A binding reflects reduced adenosine agonism in our dyskinetic PD cases. If we are correct, adenosine agonists rather than antagonists would exhibit antidyskinetic activity.

**Neurology® Launches Subspecialty Alerts by E-mail!**

Customize your online journal experience by signing up for e-mail alerts related to your subspecialty or area of interest. Access this free service by visiting http://www.neurology.org/cgi/alerts/etoc.xhtml or click on the “E-mail Alerts” link on the home page. An extensive list of subspecialties, methods, and study design choices will be available for you to choose from—allowing you priority alerts to cutting-edge research in your field!