Adenosine A2A Receptors Measured with [11C]TMSX PET in the Striata of Parkinson’s Disease Patients

Masahiro Mishina1,2,3,*, Kiichi Ishiwata1, Mika Naganawa1,4, Yuichi Kimura1,5, Shin Kitamura2,6, Masahiko Suzuki1,7, Masaya Hashimoto1,7, Kenji Ishibashi1,8, Keiichi Oda1, Muneyuki Sakata1, Makoto Hamamoto2,3, Shiro Kobayashi9, Yasuo Katayama9, Kenji Ishii1

1 Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo, Japan, 2 The Second Department of Internal Medicine, Nippon Medical School, Bunkyo-ku, Tokyo, Japan, 3 Department of Neurology, Nippon Medical School Chiba Hokusoh Hospital, Inzai-shi, Chiba, Japan, 4 Department of Diagnostic Radiology, PET Center, Yale University, New Haven, Connecticut, United States of America, 5 Biophysics Group, Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Japan, 6 Department of Internal Medicine, Nippon Medical School Musashi Kosugi Hospital, Kawasaki, Kanagawa, Japan, 7 Department of Neurology, The Jikei University School of Medicine, Minato-ku, Tokyo, Japan, 8 Department of Neurology and Neurosurgical Graduate School, Tokyo Medical and Dental University, Tokyo, Japan, 9 Department of Neurosurgery, Nippon Medical School Chiba Hokusoh Hospital, Inzai-shi, Chiba, Japan

Abstract

Adenosine A2A receptors (A2ARs) are thought to interact negatively with the dopamine D2 receptor (D2R), so selective A2AR antagonists have attracted attention as novel treatments for Parkinson’s disease (PD). However, no information about the receptor in living patients with PD is available. The purpose of this study was to investigate the relationship between A2ARs and the dopaminergic system in the striata of drug-naïve patients and PD patients with dyskinesia, and alteration of these receptors after antiparkinsonian therapy. We measured binding ability of striatal A2ARs using positron emission tomography (PET) with [7-methyl-11C]-8-(3,4,5-trimethoxyxystyril)-1,3,7-trimethylxanthine ([11C]TMSX) in nine drug-naive patients with PD, seven PD patients with mild dyskinesia and six elderly control subjects using PET. The patients and eight normal control subjects were also examined for binding ability of dopamine transporters and D2Rs. Seven of the drug-naive patients underwent a second series of PET scans following therapy. We found that the distribution volume ratio of A2ARs in the putamen were larger in the dyskinesic patients than in the control subjects (p < 0.05, Tukey-Kramer post hoc test). In the drug-naive patients, the binding ability of the A2ARs in the putamen, but not in the head of caudate nucleus, was significantly lower on the more affected side than on the less affected side (p < 0.05, paired t-test). In addition, the A2ARs were significantly increased after antiparkinsonian therapy in the bilateral putamen of the drug-naïve patients (p < 0.05, paired t-test) but not in the bilateral head of caudate nucleus. Our study demonstrated that the A2ARs in the putamen were increased in the PD patients with dyskinesia, and also suggest that the A2ARs in the putamen compensate for the asymmetrical decrease of dopamine in drug-naïve PD patients and that antiparkinsonian therapy increases the A2ARs in the putamen. The A2ARs may play an important role in regulation of parkinsonism in PD.

Introduction

Parkinson’s disease (PD) is a progressive degenerative neurological disorder characterized clinically by resting tremor, bradykinesia, cogwheel rigidity, and postural instability [1]. These symptoms result primarily from the loss of dopaminergic neurons in the substantia nigra and can be reduced by levodopa, which replenishes dopamine, and dopamine agonists.

Adenosine is an endogenous modulator of synaptic function in the central nervous system [2], and its effects are mediated by at least four receptor subtypes: A1, A2A, A2B, and A3 [3]. Adenosine A1 receptors (A1Rs) are widely distributed throughout the entire brain, while adenosine A2A receptors (A2ARs) are enriched in the basal ganglia [4,5]. The A2AR is known to stimulate adenylyl cyclase and interacts with the dopamine D2 receptor (D2R) negatively at the level of second messengers and beyond [5]. The A1R is known to inhibit adenylyl cyclase.

Recently, A2AR antagonists have attracted attention as potential non-dopaminergic therapies for PD. For example, caffeine is a nonselective adenosine receptor antagonist and is known to reduce the risk of developing PD [6,7]. In addition, theophylline, which is also a nonselective adenosine receptor antagonist, was expected to be a promising agent for the treatment of PD [8]. However, findings from clinical trials of both caffeine and theophylline have been unimpressive [9,10]. The selective A2AR antagonists was developed as novel nondopaminergic agents for PD [11] and provides antiparkinsonian benefit without causing or worsening dyskinesia, which is one of the most inconvenient side effects of dopaminergic therapy [12]. A postmortem study reported that the density of A2AR binding...
sites in PD was comparable to that found in the normal subjects, while the density in the basal ganglia was lower in patients with Huntington's chorea than in normal subjects [13]. However, another study using in situ hybridization and autoradiography suggested that A2ARs were involved in the development of dyskinesia following long-term levodopa therapy in PD [14]. Therefore, A2ARs may be involved in the appearance of the side effects of antiparkinsonian agents. Although the A2AR has attracted much attention in PD therapies, clinical evidence describing A2ARs in living PD patients, such as drug-naive patients, is lacking.

We developed PET ligands for mapping adenosine receptors, and we successfully visualized A1Rs with [1-methyl-11C]β-dicyclopropylmethyl-1-methyl-3-propylxanthine ([11C]MPDX) [15,16] and A2ARs with [7-methyl-11C]-E-9-(3,4,5-trimethoxy styryl)-1,3,7-trimethylxanthine ([11C]TMSX, Fig. 1) [17,18,19,20]. [11C]TMSX has a xanthine structure and is an analog of istradefylline. In addition, the specific binding of [11C]TMSX to A2ARs was confirmed with a theophylline challenge [21]. We performed test-retest studies and optimized the kinetics for [11C]TMSX in normal subjects, thus confirming good reproducibility of [11C]TMSX PET in the putamen. In this study, we investigated A2ARs in the striata of drug-naive PD patients and PD patients with dyskinesia, and alteration of A2ARs after antiparkinsonian therapy using [11C]TMSX PET.

Results

Figure 2 shows representative PET images for normal subjects, a drug-naive PD patient before and after anti-parkinsonian therapy, and a PD patient with dyskinesia.

Group comparison

There was no significant difference with age among the three groups, i.e. the drug naive PD patients (five men and four women, mean age 64.6±5.7 years, Table 1), the PD patients with dyskinesia (two men and five women, mean age 65.0±7.3 years, Table 2) and the normal subjects (three men and three women, mean age 60.7±8.5 years). Rate of female was slightly larger in the drug-naive patients (five men and four women, mean age 64.6±5.7 years), the drug-naive patients (five men and four women, mean age 65.0±7.3 years), and the normal subjects (five men and five women, mean age 60.7±8.5 years). In the PD, duration of disease was longer in the patients with dyskinesia (11.1±7.2 years) than the drug naive patients (20±1.2 years, p<0.001, unpaired t-test). There was no significant difference with the Unified Parkinson’s Disease Rating Scale part III (UPDRS-III) between the drug naive patients (17±13.2) and the patients with dyskinesia (12.6±11.2).

The distribution volume ratio (DVR) for [11C]TMSX in the bilateral putamen of patients with dyskinesia was larger than that of normal controls (p<0.05), although that of drug-naive patients was comparable with that of normal controls (Table 1). In the head of the caudate nucleus, the DVR of patients with dyskinesia was slightly larger than that of other two groups, although not significant.

In both the bilateral putamen and the head of the caudate nucleus, the uptake ratio index (URI) of [11C]raclopride ([11C]RAC), a marker for postsynaptic D2R, in the bilateral striata was lower in the patients with dyskinesia than in the normal controls (Table 3). The URI of [11C]raclopride ([11C]RAC), a marker for postsynaptic D2R, in the bilateral striata was lower in the patients with dyskinesia than in the normal controls (Table 3).

Asymmetry in drug-naive state

When we examined the asymmetrical symptoms in the early phase of PD as they relate to the PET images, we found that the DVR for [11C]TMSX in the putamen was significantly lower on the more affected side than on the less affected side in the PD patients (Table 4). In contrast, the head of the caudate nucleus showed no significant differences between the more affected side and the less affected side according to DVRs for [11C]TMSX.

The URI for [11C]CFT in the putamen was significantly lower on the more affected side than on the less affected side in the drug-naive patients, and the URI for [11C]RAC was significantly higher on the more affected side than on the less affected side (Table 4). In the head of the caudate nucleus, there was no significant difference between the more affected side and the less affected side in the URI for either [11C]CFT or [11C]RAC.

Figure 3 demonstrates the lack of significant correlation among the DVR for [11C]TMSX, the URI for [11C]CFT and the URI for [11C]RAC in the bilateral striata of drug-naive patients. Specifically, there was no significant correlation among the following variables; the DVR for [11C]TMSX versus the URI for [11C]CFT (Figure 3A and 3D), the DVR for [11C]TMSX versus the URI for [11C]RAC (Figure 3B and 3E), and the URI for [11C]CFT versus the URI for [11C]RAC (Figure 3C and 3F).

Post-therapeutic state

In the seven PD patients with follow-up studies, the DVR for [11C]TMSX in the bilateral putamen was significantly increased in the post-therapeutic state relative to the drug-naive state (Table 5). The URIs for both [11C]CFT and [11C]RAC in the bilateral

---

Figure 1. Structure of [11C]TMSX.

doi:10.1371/journal.pone.0017338.g001
putamen was significantly decreased in the post-therapeutic state than the drug-naive state.

In the head of the caudate nucleus bilaterally, the URIs for $[^{11}\text{C}]$CFT and $[^{11}\text{C}]$RAC were significantly decreased in the post-therapeutic state than the drug-naive state, but there was no significant difference in the DVR for $[^{11}\text{C}]$TMSX.

**Discussion**

As previous studies reported [13,14,23], our PET study demonstrates that the putaminal binding ability of A2ARs was increased in the PD patients with dyskinesia, and that there was no significant difference in the striatal binding ability of A2ARs between drug-naive PD patients and normal controls. However, in drawing attention to the asymmetrical symptoms in drug-naive PD, our study suggests that A2ARs were asymmetrically down-regulated in the putamen but not in the head of the caudate nucleus. The function of A2ARs is thought to be opposite to that of D2Rs [5]. PET studies using $[^{11}\text{C}]$RAC have shown elevated binding of D2Rs in the putamen in early PD [24,25,26], due to decreased endogenous dopamine and weak D2R affinity for $[^{11}\text{C}]$RAC [27]. However, a postmortem study reported that the density of the D2R binding sites was higher in PD patients than normal controls [28]. Therefore, D2Rs are thought to be up-regulated in the putamen of the patients with advanced PD. Past studies indicated that 80% loss of dopaminergic neurons in the substantia nigra was needed to be developed to symptoms of PD [29]. We reported on the asymmetrical compensation of the sigma1 receptor for the reduction of dopamine in the putamen in early PD [30]. Our study in the drug-naive state suggested that A2ARs might play an important role in inhibition of asymmetrical parkinsonism in PD along with D2Rs and sigma1 receptors.

In the patients with PD, DATs in the putamen were reduced in the diskinesic patients relative to the drug-naive patients, and reduced in the post-therapeutic state relative to the drug-naive state. These findings were considered to reflect the progression of PD. The uptake of $[^{11}\text{C}]$RAC in the putamen was reduced in the diskinesic patients relative to the drug-naive patients, and reduced

---

**Figure 2. PET images for normal subjects, a drug-naive patient with Parkinson's disease (PD), and a PD patient with dyskinesia.** The normal subjects are a 63-year-old female for $[^{11}\text{C}]$TMSX, and a 63-year-old male for $[^{11}\text{C}]$CFT and $[^{11}\text{C}]$RAC (A). The drug-naive patient is a 60-year-old male with right-dominant parkinsonism, and underwent two series of PET scans before (B) and after anti-parkinsonian therapy (C). The patients did not develop dyskinesia at a second series of PET scan in the post-therapeutic state. The DVR of $[^{11}\text{C}]$TMSX was smaller in the left putamen than in the right and was increased after treatment with anti-parkinsonian therapy for 14 months. The uptake of $[^{11}\text{C}]$CFT was reduced in the putamen, especially on the left. The uptake of $[^{11}\text{C}]$RAC was preserved in the striatum and was larger in the left putamen than in the right. The relative uptake of $[^{11}\text{C}]$CFT and $[^{11}\text{C}]$RAC in the putamen was lower in the post-therapeutic state than in the drug-naive state. The PD patient with dyskinesia is a 65-year-old male with right-dominant parkinsonism (D). His disease duration was 19 years. The DVR of $[^{11}\text{C}]$TMSX was increased in the striata. The uptake of $[^{11}\text{C}]$CFT was significantly decreased in the putamen.

doi:10.1371/journal.pone.0017338.g002
in the post-therapeutic state relative to the drug-naive state. These findings are consistent with the compensatory change of D2Rs. We think that there are two possible reasons. First, [11C]RAC may have competed with the D2R agonists and increased endogenous dopamine due to levodopa, thus decreasing the binding of [11C]RAC to D2Rs. Second, the antiparkinsonian therapy may have abrogated the compensation of D2R due to the decrease in the dopamine. These factors can affect the binding of [11C]RAC in the striata, and it is difficult to detect the up-regulation of D2R using [11C]RAC PET in the patients with PD.

Striatal GABAergic medium spiny neurons (MSNs) represent more than 90% of striatal neurons, and A2ARs are highly

### Table 1. Demographic and clinical data of drug-naive patients with Parkinson’s disease.

| No | Age (years) | Gender | Duration from onset (years) | Symptom | Hoehn & Yahr | UPDRS-III Pre-therapy | Duration of therapy (month) | Antiparkinsonian agents at post-therapy PET | LED (mg) | UPDRS-III Post-therapy |
|----|-------------|--------|-----------------------------|---------|--------------|----------------------|--------------------------|-----------------------------------------------|----------|-----------------------|
| 1  | 72          | F      | 2.0                         | R>L     | 1            | 5                    | 7.0                      | Levodopa and carbidopa            | 100      | 19                    |
| 2  | 60          | M      | 1.3                         | R>L     | 2            | 8                    | 14.2                     | Pramipexole and amantadine        | 75       | 6                     |
| 3  | 58          | F      | 3.0                         | R>L     | 4            | 41                   | 15.9                     | Pramipexole, levodopa and carbidopa | 450      | 25                    |
| 4  | 68          | F      | 0.5                         | R>L     | 2            | 23                   | 15.9                     | Pramipexole, amantadine, levodopa and carbidopa | 137.5 | 4                     |
| 5  | 64          | M      | 1.2                         | L>R     | 1            | 6                    | 12.9                     | Pramipexole                      | 150      | 7                     |
| 6  | 56          | M      | 0.7                         | L>R     | 2.5          | 10                   | 20.8                     | Pramipexole and amantadine        | 200      | 21                    |
| 7  | 68          | M      | 3.0                         | L>R     | 3            | 18                   | 14.7                     | Ropinirole, selegiline, droxidopa, levodopa and benserazide | 825.5 | 21                    |
| 8  | 71          | M      | 4.0                         | R>L     | 1.5          | 12                   | 14.5±13.2                | 276.9±272.2                     | 14.7±8.7 |
| 9  | 64          | F      | 2.0                         | L>R     | 2.5          | 36                   | 12.6±11.2                | 5.6±2.0                          | 548.9±267.7 |

UPDRS-III; Unified Parkinson’s Disease Rating Scale part III. LED; levodopa-equivalent dose.

### Table 2. Demographic and clinical data of dyskinesic patients with Parkinson’s disease.

| No | Age (years) | Gender | Duration from onset (years) | Symptom | Hoehn & Yahr | UPDRS-III | UPDRS-IV | Antiparkinsonian agents | LED (mg) |
|----|-------------|--------|-----------------------------|---------|--------------|-----------|----------|------------------------|----------|
| 1  | 73          | F      | 5                            | R>L     | 2            | 8         | 5        | Pramipexole, Levodopa, carbidopa and entacapone | 416.7 |
| 2  | 65          | M      | 19                           | R>L     | 3            | 9         | 6        | Cabergoline, trihexyphenidyl hydrochloride, droxidopa levodopa and carbidopa | 667.0 |
| 3  | 66          | F      | 10                           | R>L     | 3            | 7         | 5        | Trihexyphenidyl hydrochloride, zonisamide, levodopa and benserazide hydrochloride | 300.0 |
| 4  | 50          | F      | 2                            | R>L     | 2            | 11        | 3        | Pramipexole, levodopa and carbidopa | 750.0 |
| 5  | 66          | F      | 9                            | L>R     | 3            | 13        | 4        | Pramipexole, levodopa and carbidopa | 225.0 |
| 6  | 65          | M      | 11                           | L>R     | 2            | 3         | 7        | Pramipexole, cabergoline, amantadine, levodopa and carbidopa | 983.5 |
| 7  | 70          | F      | 22                           | L>R     | 4            | 37        | 9        | Pramipexole, trihexyphenidyl hydrochloride, amantadine, levodopa and carbidopa | 500.0 |

UPDRS-III; Unified Parkinson’s Disease Rating Scale part III. LED; levodopa-equivalent dose.
ribonucleic acid (mRNA) of A2ARs and \([3H]SCH-58261\)-specific binding to A2ARs were increased in the putamen in PD patients with mild dyskinesia. Another study showed that mRNA levels for the A2ARs were elevated in the putamen of levodopa-treated MPTP monkeys [33,34]. Therefore, an alteration in A2AR expression may be involved in the dendritic atrophy in the MSNs.

Our study showed that the binding of \([11C]TMSX\) to A2ARs was increased in the putamen in the PD patients with mild dyskinesia. A postmortem study suggested that the messenger ribonucleic acid (mRNA) of A2ARs and \([3H]SCH-58261\)-specific binding to A2ARs were increased in the putamen of PD patients with dyskinesia following long-term levodopa therapy [14]. Another study showed that mRNA levels for the A2ARs were elevated in the putamen of levodopa-treated MPTP monkeys compared with controls, although autoradiography with \([3H]SCH-58261\) could not show significant difference [23].

### Table 3. The DVRs for \([11C]TMSX\) and URIs for \([11C]CFT\) and \([11C]RAC\) in the striata of normal controls, drug-naive patients with Parkinson’s disease (PD) and PD patients with dyskinesia.

| Radiopharmaceutical | Normal Control | Drug-naive PD | PD with dyskinesia |
|----------------------|----------------|---------------|-------------------|
| Putamen              |                |               |                   |
| \([11C]TMSX\)        | 1.47±0.11      | 1.48±0.10     | 1.58±0.15         |
| \([11C]CFT\)         | 2.67±0.49      | 1.14±0.33\(^1\) | 0.65±0.24\(^2,3\) |
| \([11C]RAC\)         | 3.10±0.42      | 3.43±0.55     | 2.86±0.70         |
| Head of the caudate nucleus |        |               |                   |
| \([11C]TMSX\)        | 1.38±0.08      | 1.17±0.09     | 1.44±0.15         |
| \([11C]CFT\)         | 2.52±0.54      | 1.83±0.35\(^4\) | 1.36±0.39\(^5,6\) |
| \([11C]RAC\)         | 2.70±0.48      | 2.50±0.37     | 1.84±0.49\(^6,11\) |

Values are mean ± SD (Drug-naive PD: \(n=9\); PD with dyskinesia: \(n=7\); normal: \(n=6\) for \([11C]TMSX\) and \(n=8\) for \([11C]CFT\) and \([11C]RAC\)). Binding of \([11C]TMSX\) was evaluated as the distribution volume ratio (DVR) and binding of \([11C]CFT\) or \([11C]RAC\) was expressed as the uptake ratio index (URI).

Our study also showed that the binding of \([11C]TMSX\) was increased in the putamen after the antiparkinsonian therapy. The finding may reflect alteration in compensation for the decrease of dopamine in the patients with PD. The drug-naive patients in this study did not develop dyskinesia by the time of second series of PET scans. Therefore, our study suggests that the increase in putaminal A2ARs after antiparkinsonian therapy preceded the development of dyskinesia in patients with PD. Dyskinesia may be involved in the additional gain of the A2ARs. We may predict the onset of dyskinesia using sequential \([11C]TMSX\) PET examination. Moreover, a recent study suggested that dyskinesia may involve not only A2ARs but also A1Rs [35]. Further PET studies with \([11C]MPDX\) and \([11C]TMSX\) will be needed to clarify this issue.

In conclusion, \([11C]TMSX\) PET demonstrated that the distribution of A2ARs was increased in the putamen in PD patients with dyskinesia. A2ARs was asymmetrically down-regulated in the putamen in drug-naive patients with PD, and the asymmetrical regulation of A2ARs seems to be involved in compensation for the decrease in dopamine. Our study also showed that the A2ARs were increased in the putamen after antiparkinsonian therapy.

### Materials and Methods

#### Subjects

We studied nine drug-naive patients with PD. Table 1 summarizes the clinical profiles of the patients. All patients were right-handed. Their PD diagnoses were based on their medical histories, physical and neurological examinations, laboratory tests, and magnetic resonance imaging (MRI) studies, to rule out other diseases [1,36,37]. They had no medical histories of bronchial asthma and did not take theophylline regularly. To confirm early diagnosis of PD, each patient was also examined for DATs and D2Rs by PET using \([11C]CFT\) and \([11C]RAC\), respectively. Specifically, we confirmed low binding of \([11C]CFT\) and normal or high uptake of \([11C]RAC\) in the putamen of all patients [38,39,40]. After PET examinations for drug naïve state, we also...
confirmed that the patients with PD were markedly ameliorated the parkinsonian symptoms by antiparkinsonian therapy, and that their diagnoses of PD remained unchanged in more than two years of observation. The patients did not experience hallucinations or dementia [41]. UPDRS-III was used to evaluate motor disability. Seven of the nine patients were re-examined with [11C]TMSX, [11C]CFT and [11C]RAC PET 14.5 ± 4.1 months after starting antiparkinsonian therapy (Patients 1–7 in Table 1). The daily levodopa equivalent dose (LED) was calculated as follow: levodopa (mg) + levodopa (mg) ×1/3 (if on entacapone) + levodopa (mg) ×0.02 × selegiline (mg) + pramipexole (mg) ×100 + ropinirole (mg) ×16.7 + cabergoline (mg) ×67 + pergolide (mg) ×100 + bromocriptine (mg) ×10. The LED for patients that underwent follow-up studies ranged from 75.0 to 825.5 mg (276.9 ± 272.2 mg) at the time of post-therapeutic PET scanning. No patients developed dyskinesia during the period of this study. The administration of antiparkinsonian agents was not stopped before obtaining the PET scans at the therapeutic state.

We also studied advanced PD patients with mild dyskinesia. Table 2 summarizes the clinical profiles of the patients. All patients were right-handed. The LED for patients ranged from 225.0 to 983.5 mg (548.9 ± 267.7 mg) at the time of PET scanning. The Unified Parkinson’s Disease Rating Scale part IV (UPDRS-IV) was used to evaluate drug side effects such as dyskinesia. The dyskinesia of the patients was mild, and they could acquire PET examinations without any trouble caused by undesirable movements during the PET scanning.

For [11C]TMSX PET scanning, the control group consisted of six volunteers. For [11C]CFT and [11C]RAC PET scanning, another control group was used, which consisted of eight volunteers (five men and three women, mean age ± SD, 62.3 ± 6.9 years). The subjects in these control groups were all

Figure 3. Scattergrams for binding parameters of [11C]TMSX, [11C]CFT and [11C]RAC in the striata of drug-naïve patients. The binding parameter for [11C]TMSX was expressed as DVR, and parameters for [11C]CFT and [11C]RAC were expressed as URI. There was no significant correlation among these variables.

doi:10.1371/journal.pone.0017338.g003
right-handed. They did not have a history of neurological disease or any abnormalities on physical or neurological examinations. In addition, they did not take medications known to affect brain function and did not have a history of alcoholism. The normal subjects for [11C]TMSX PET had no medical histories of bronchial asthma.

The study protocols were approved by the Ethics Committee of the Tokyo Metropolitan Institute of Gerontology. Written informed consent was obtained from all subjects who participated in this study.

### [11C]TMSX PET

PET was performed in the Tokyo Metropolitan Institute of Gerontology Positron Medical Center with an SET-2400W PET scanner (Shimadzu Co., Kyoto, Japan) [46]. Image manipulations were carried out on a MacBookPro computer with medical image processing software Dr. View/Linux R2.5 (AJS Inc., Tokyo, Japan) implemented in CentOS 5.4 (The CentOS Project, http://www.centos.org/) and Parallels Desktop 5 (Parallels Holdings, Ltd., Renton, WA, US).

All subjects avoided caffeine consumption over 12 hours before obtaining the [11C]TMSX PET scans. The [11C]TMSX was prepared as previously described [42]. Specific activity at the time of injection ranged from 40.6 to 119.5 GBq/μmol (69.6±20.8 GBq/μmol). After a transmission scan with a rotating 68Ga/68Ge rod source for attenuation correction, each subject received an intravenous injection of 300 MBq of [11C]TMSX. Beginning 75 minutes after the injection, an emission scan was performed using the 3D mode, which lasted 15 minutes.

The [11C]TMSX was prepared as previously described [42]. Specific activity at the time of injection ranged from 5.7 to 280.0 GBq/μmol (63.1±57.4 GBq/μmol). Transmission data were acquired with a rotating 68Ga/68Ge rod source for attenuation correction. Each subject received an intravenous injection of 300 MBq of [11C]TMSX. Beginning 75 minutes after the injection, an emission scan was performed using the 3D mode, which lasted 15 minutes.

Circular regions of interest (ROIs) 10 mm in diameter were positioned on the PET images over the bilateral putamen, the head of the caudate nucleus and the cerebellar hemisphere. For a semi-quantitative analysis of the [11C]TMSX and [11C]RAC, we calculated URI in the putamen and the head of the caudate nucleus [49] as follows:

\[
URI = \frac{AS - AC}{AC}
\]

where \(AS\) is the radioactivity in the ipsilateral putamen or the head of the caudate nucleus, and \(AC\) is the radioactivity of the bilateral cerebellar hemispheres.

### Statistics

Among the drug naïve PD patients, the PD patients with dyskinesia and the normal subjects, One-way analysis of variance with Tukey-Kramer post hoc test were used to compare DVRs for [11C]TMSX PET, URIs for [11C]CFT and [11C]RAC PET, and age. Where only two groups were compared, unpaired \(t\)-test was used. Group difference in gender was calculated with the use of the chi-square test of proportions. In the drug naïve patients, striata from the more affected and the less affected side were compared, and the effect of therapeutic treatment on the striata was evaluated by the paired \(t\)-test. Regression analyses were used for comparison among binding parameters of [11C]TMSX, [11C]CFT and [11C]RAC. The level of significance was set to \(p<0.05\). Statistical values were computed using the software package JMP version 9.0.0 (SAS Institute Inc., Cary, NC, USA) on a Macintosh computer.

### Acknowledgments

The authors thank Dr. T. Oda for the production of [11C]TMSX and Ms. H. Tsukinari for caring for the subjects undergoing PET scanning.
Author Contributions

Conceived and designed the experiments: MM K. Ishiwata K. Ishii. Performed the experiments: MM K. Ishiwata M. Hashimoto K. Ishibashi KO K. Ishii. Analyzed the data: MM MN Y. Kimura M. Sakata. Contributed reagents/materials/analysis tools: MM K. Ishiwata MN S.

References

1. Lees AJ, Hardy J, Revus T (2009) Parkinson’s disease. Lancet 373: 2055–2066.
2. Dunwiddie TV, Masino SA (2001) The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 24: 31–55.
3. Fredholm BB, AP II, Jacobson KA, Klott KN, Linden J (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol Rev 53: 527–532.
4. Svenningsson P, Hall H, Soball G, Fredholm BB (1997) Distribution of adenosine receptors in the postmortem human brain: an extended autoradiographic study. Synapse 32: 327–335.
5. Fredholm BB, Svenningsson P (2003) Adenosine–adenosine receptors: development of a concept and some comments on therapeutic possibilities. Neurology 61: 85–90.
6. Ross GW, Abbott RD, Petrovitch H, Morens DM, Grandinetti A, et al. (2000) Association of coffee and caffeine intake with the risk of Parkinson disease. JAMA 283(26): 2674–2679.
7. Ascherio A, Zhang SM, Hernan MA, Kawachi I, Colditz GA, et al. (2001) Prospective study of caffeine consumption and risk of Parkinson’s disease in men and women. Ann Neurol 50: 56–63.
8. Kostic VS, Svetel M, Sternic N, Dragasevic N, Przedborski S (1999) Increased density of dopamine D2 receptors in the putamen, but not in the caudate nucleus in early Parkinson’s disease: a PET study with [11C]raclopride. J Neurosci 19: 156–161.
9. Antonini A, Leenders KL, Voutoulopoulou P, Maguire R, Missonnier J, et al. (1997) Complementary PET studies of striatal neuronal function in the differential diagnosis between multiple system atrophy and Parkinson’s disease. Brain 120( Pt 12): 2187–2195.
10. Ishihashi K, Ishii K, Oda K, Minuzawa H, Ishiwata K (2010) Competition between [11C]raclopride and endogenous dopamine in Parkinson’s disease. Nucl Med Commun 31: 159–166.
11. Piggott MA, Marshall EF, Thomas N, Lloyd S, Court JA, et al. (1999) Striatal dopaminergic markers in dementia with Lewy bodies, Alzheimer’s and Parkinson’s diseases: rostrocaudal distribution. Brain 122( Pt 8): 1449–1468.
12. Jankovic J (2005) Progression of Parkinson’s disease: are we making progress in charting the course? Arch Neurol 62: 351–352.
13. Miyashita M, Ishiwata K, Ishii K, Kitamura S, Kimura Y, et al. (2005) Function of sigma receptor in Parkinson’s disease. Acta Neurol Scand 112: 103–107.
14. Mori A, Shindou T (2003) Modulation of GABAergic transmission in the striatopallidal system by adenosine A2A receptors: a potential mechanism for the antiparkinsonian effects of adenosine A2A antagonist. Neurology 61: 844–848.
15. Schiffman SN, Jacobs O, Vanderhaeghen JJ (1991) Striatal restricted adenosine A2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: in situ hybridization histochemistry study. J Neurochem 57: 1062–1067.
16. McNell TH, Brown SA, Rafal JF, Shouloud I (1988) Atrophy of medium spiny 1 striatal dendrites in advanced Parkinson’s disease. Brain Res 455: 148–152.
17. Zaja-Milatovic S, Milatovic D, Schantz AM, Zhang J, Montine KS, et al. (2005) Dendritic degeneration in neostriatal medium spiny neurons in Parkinson’s disease. Neuroscience 64: 543–557.
18. Xiao D, Cassin JJ, Healy B, Burdett TC, Chen J-F, et al. (2010) Deletion of adenosine A1 or A2A receptors reduces L-3,4-dihydroxyphenylalanine-induced dyskinesia in a model of Parkinson’s disease. Brain Res, in press.
19. Koller WC, Montgomery EB (1997) Issues in the early diagnosis of Parkinson’s disease. Neurology 49: S10–25.
20. Gelb DJ, Oliver E, Gilman S (1999) Diagnostic criteria for Parkinson disease. Arch Neurol 56: 33–39.
21. Kasavina Y, Ruottinen HM, Nagen K, Lekhoinen P, Oikonen V, et al. (2000) Upregulation of putaminal dopamine D2 receptors in early Parkinson’s disease: a comparative PET study with [11C]raclopride and [11C]N-methylspiperone. J Nucl Med 41: 653–662.
22. Ribeiro MJ, Vidalhiet M, Loch G, Dupel G, Nguyen JP, et al. (2002) Dopaminergic function and dopamine transporter binding assessed with positron emission tomography in Parkinson disease. Arch Neurol 59: 580–586.
23. Ishihashi K, Saino Y, Muramatsu S, Kanemaru K, Oda K, et al. (2010) VALiDRG of cardiac 123I-SHRG scintigraphy in patients with Parkinson’s disease who were diagnosed with dopamine PET. Eur J Nucl Mol Imaging 37: 3–11.
24. McKeith IG, Dickson DW, Lowe J, Emre M, O’Brien JT, et al. (2005) Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 63: 1863–1872.
25. Ishiwata K, Wang WF, Kimura Y, Kawamura K, Ishii K (2003) Preclinical studies on [14C]TMSX for mapping adenosine A2A receptors by positron emission tomography. Ann Nucl Med 17: 205–211.
26. Ishiwata K, Naguchi J, Wakabayashi S, Shindou J, Ogai N, et al. (2000) [14C]-labeled KF18446: a potential central nervous system adenosine A2A receptor ligand. J Nucl Med 41: 345–354.
27. Ishii K, Kimura Y, Vries EFd, Elinga PH (2007) TRACER tracers for mapping adenosine A2A receptors as probes for diagnosis of CNS disorders. CNS Agents Med Chem 7: 57–77.
28. Spaeth A, Ishiwata K (2009) Adenosine receptor ligands and PET imaging of the CNS. Handbook Exp Pharmacol, pp 617–642.
29. Ishiwata K, Kimura Y, Oda K, Ishii K, Sakata M, et al. (2010) Development of PET radiopharmaceuticals and their clinical applications at the Positron Medical Center. Geriatr Gerontol Int 10(Suppl 1): S100–196.
30. Misumi M, Ishiwata K, Kimura Y, Nagaiwa M, Oda K, et al. (2007) Evaluation of distribution of adenosine A2A receptors in normal human brain measured with [18F]14C]methyl-9-phenyl-2-methoxyxystatine. J Nucl Med 46: 32–37.
31. Ishiwata K, Kimura Y, Vries EFd, Elinga PH (2007) STRACER tracers for mapping adenosine A2A receptors as probes for diagnosis of CNS disorders. CNS Agents Med Chem 7: 57–77.
32. Ishiwata K, Wang WF, Kimura Y, Kawamura K, Ishii K (2003) Preclinical studies on [14C]TMSX for mapping adenosine A2A receptors by positron emission tomography. Ann Nucl Med 17: 205–211.
33. Ishiwata K, Naguchi J, Wakabayashi S, Shindou J, Ogai N, et al. (2000) [14C]-labeled KF18446: a potential central nervous system adenosine A2A receptor ligand. J Nucl Med 41: 345–354.
34. Misumi M, Senda M, Kimura Y, Toyama H, Ishiwata K, et al. (2000) Intrahemispheric correlation between static scan and distribution volume images for [11C]humanPET. ANN NUCLE MED 41: 193–190.
35. Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, et al. (1996) Distribution volume ratios without blood sampling from graphical analysis of PET data. J Cereb Blood Flow Metab 16: 834–840.
36. Fujitaka T, Watanuki S, Yamamoto M, Miyake M, Seo S, et al. (1997) Performance evaluation of a large axial field-of-view PET scanner: SET-2400W. Ann Nucl Med 11: 307–313.
37. Kamawaka K, Oda K, Ishiwata K (2003) Age-related changes of the [11C]CFT binding to the striatal dopamine transporters in the Fischer 344 rats: a PET study. Ann Nucl Med 17: 249–253.
38. Langer O, Doll F, Landkuv C, Sandell J, Swahn C, et al. (1999) Precursor synthesis and radiolabelling of the dopamine D2 receptor ligand [11C]raclopride from [11C]methoxytrifluoroacetic acid. J Labelled Comp Radiopharm 42: 1183–1193.
39. Ishikawa K, Ishii K, Oda K, Minuzawa K, Ishiwata K (2006) Regional analysis of age-related decline in dopamine transporters and dopamine D2-like receptors in human striatum. Synapse 63: 282–290.