Motor behavior correlates with striatal $^{[18F]}$-DOPA uptake in MPTP-lesioned primates


Submitted
Abstract

The MPTP-lesioned monkey is a well-known, animal model for Parkinson’s disease (PD). MPTP causes damage to dopaminergic cell groups resulting in motor dysfunction similar to PD. PET scans using [18F]-DOPA are applied to determine presynaptic striatal dopaminergic activity. In a patient with PD, striatal uptake of [18F]-DOPA is decreased and striatal uptake values correlate inversely with motor scores. We have correlated uptake values of [18F]-DOPA to motor impairment in MPTP-lesioned monkeys in various stages of neuronal degeneration.

Eight rhesus monkeys received MPTP infusions. Motor signs were rated regularly and correlated to striatal uptake of [18F]-DOPA as measured with PET. MPTP caused the expected parkinsonian motor signs which were accompanied by reduced striatal uptake of [18F]-DOPA. There were significant correlations between the two endpoints. In conclusion, striatal [18F]-DOPA uptake correlates inversely with the severity of motor impairment in MPTP-lesioned non-human primates.

Keywords: MPTP, Parkinson’s disease, primates, [18F]-DOPA, PET, movement disorders
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Introduction

Parkinson’s disease (PD) is a movement disorder, associated with degeneration of dopaminergic neurons from the substantia nigra pars compacta. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) destroys monoaminergic neurons. As dopaminergic neurons are especially sensitive to this neurotoxin, MPTP induces depletion of dopamine in the striatum. Brain damage induced by MPTP in monkeys induces many of the behavioural and biochemical features of PD\textsuperscript{3, 7, 8, 28, 29}.Behavioural deficits include akinesia, rigidity and flexed posture and sometimes resting tremor. MPTP-lesioned non-human primates are a widely used animal model for PD\textsuperscript{3, 5, 10, 24}.

\textit{In vivo} measurements with positron emission tomography (PET) or single-photon emission computed tomography (SPECT) in this primate model provide an opportunity to study the pathophysiology and biochemistry of the dopaminergic system in time, in humans as well as in animal models\textsuperscript{5, 10, 14, 25, 26, 33}. [18F]-DOPA (6-[18F]-fluoro-L-3,4-dihydroxyphenylalanine) is a fluoro analogue of the naturally occurring amino acid derivative and dopamine precursor dihydroxyphenylalanine (DOPA). It is metabolised into [18F]-fluoro-dopamine by the enzyme Amino Acid Decarboxylase (AADC). Several human studies have shown that dopaminergic function, measured by [18F]-DOPA PET scans is negatively associated with clinical severity in patients with PD\textsuperscript{4, 21, 25, 27}. Also, the degree of clinical asymmetry is correlated with asymmetry in [18F]-DOPA uptake in the putamen of patients with PD\textsuperscript{4}. [18F]-DOPA uptake is correlated with post mortem determined dopamine cell counts and dopamine levels in humans\textsuperscript{32}. Both SPECT and PET scanning with respectively [123I]-\(\beta\)-CIT ([123I]-2-\(\beta\)-carbomethoxy-3-\(\beta\)-(4-iodophenyl)-tropane, imaging the dopamine transporter) and with [18F]-DOPA or [18F]-FMT (6-[18F]-fluoro-L-m-tyrosine, analogue of L-dopa) have been applied to investigate the dopaminergic system \textit{in vivo} in MPTP-lesioned monkeys\textsuperscript{15-18, 28}.

MPTP-treated monkeys display motor deficits in combination with reduced uptake of the mentioned radiolabeled dopaminergic markers. Some studies have analysed the correlation between neuroimaging measures and behavioural measures in these monkeys. Uptake of [123I]-\(\beta\)-CIT and [18F]-FMT both correlated well with motor symptoms in MPTP-lesioned monkeys\textsuperscript{16, 17, 19, 28}. [18F]-DOPA uptake in the striatum of MPTP-lesioned parkinsonian monkeys is also altered\textsuperscript{12-14}. So far, a systematic analysis of correlation between behaviour and presynaptic dopaminergic imaging has not yet been reported despite the extensive use of [18F]-DOPA in the study of PD.

Our aim in this study was to study the correlation between motor symptoms and striatal uptake of [18F]-DOPA PET in MPTP-lesioned monkeys in a quantitative manner.
Materials and methods

Subjects

A total of eight male, right-handed rhesus monkeys (*Macaca mulatta*) were studied under an approved protocol that met all institutional guidelines and requirements stated in the ‘Principles of Laboratory Care’ (NIH publication No. 85-23, revised 1985). The monkeys were over the age of 7 years, weighing between 6 and 12 kg and were individually housed in cages under standard conditions. Diet consisted of lab chow supplemented with fruit while water was *ad libitum* available. The monkeys participated in a drug trial investigating a potentially neuroprotective substance, namely CGP 3466B. See for detailed results. This study was approved by the local ethics committee for animals of the University Medical Center Groningen, Groningen, The Netherlands.

MPTP treatment

A two-phase bilateral MPTP lesion approach was used to generate bilateral parkinsonism. This treatment induces moderate to severe parkinsonian symptoms with a significant reduction in limb movements and decreased striatal [18F]-DOPA uptake. Under total anaesthesia a first dose of 2.5 mg MPTP was administered into the carotid artery contralateral to the dominant limb (unilateral stage) and, eight weeks later, a second dose of 1.25 mg MPTP was infused into the other carotid artery (bilateral stage). The second dose was lower to limit incapacitation of the animals. This procedure induces a stable PD model with little recovery.

Four out of the eight monkeys received a potentially neuroprotective agent, the anti-apoptotic compound CGP 3466B (also known as TCH346), at several dose levels for the next fourteen days after the second injection. CGP 3466B blocks glyceraldehyde 3-phosphate dehydrogenase (GAPDH), an enzyme involved in the apoptotic pathway. The other four monkeys received saline. It was assumed that while this compound may retard neurodegeneration in this model, it does not alter the relationship between dopaminergic function and behaviour.

PET procedures

All monkeys were scanned in the healthy stage (1 week prior to the first MPTP lesion), between the first and the second lesion (7 weeks after the first lesion) and ca 5 weeks after the second lesion.

Animals were fasted overnight prior to the PET scan. During handling and transportation they were sedated with 3.1 mg/kg (±)-ketamine (Nimatek, Eurovet, Bladel, the Netherlands) intramuscular (IM), which also functioned as anaesthetic induction (4.7 mg/kg). An intravenous (IV) cannula was positioned into the vena saphena magna for
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administration of drugs and radiotracer. Atropine (0.06–0.09 mg/kg IM; Centrafarm, Etten-Leur, The Netherlands) and sodium pentobarbital (8–11 mg/kg IV; Sanofi, Maassluis, The Netherlands) were administered, after which the trachea was intubated, and the monkey was ventilated with 1–2% (v/v) vaporised isoflurane (Abbott, Kent, UK) in a carrier gas of $O_2:N_2O$ (50:50). The peripheral decarboxylase inhibitor carbidopa was administered orally (3 mg/kg) 30 minutes before $[^{18}\text{F}\text{-}]$DOPA administration. The monkeys’ heads were fixated in a stereotactic frame and positioned centrally in the field of view of a Siemens ECAT Exact HR+ scanner (Siemens, Munich/Erlangen, Germany). This tomograph has a resolution of 5 mm full width at half maximum in the centre of the field of view and an axial field of view of 15.5 cm. A transmission scan with $^{68}\text{Ge}$ was performed for attenuation correction. An average of 98.2 MBq (range: 22 to 167) of $[^{18}\text{F}\text{-}]$DOPA in six ml of physiological saline was injected IV over one minute. Scanning was initiated immediately and 21 frames were acquired (10 x 30, 3 x 300, 4 x 600, 4 x 900 sec, totalling 120 minutes). One monkey’s PET scan failed in the unilateral phase due to a technical problem.

Heart rate, electrocardiogram, respiration rate and $O_2$ saturation were monitored throughout the experiment.

**PET data analysis**

PET data were reconstructed to a 128x128x63 matrix with a plane separation of 0.2425 cm and a bin size of 0.2250 cm.

Using the Clinical Applications Programming Package (Siemens, Munich/Erlangen, Germany), regions of interest (ROIs) were placed by hand on PET data collected from 60 – 120 minutes. Elliptical ROIs were placed over the left and right striatum, and as a reference, on the posterior half of the brain. The reference region included both cortical and white matter and was of a deliberately large size to minimise intersubject variability in placement.

The rate of specific uptake into the striatum ($K_{mv}$ values) relating tracer uptake to the reference brain region, was calculated. In our case, instead of the occipital lobe, the back of brain is used as a reference region. Mean striatal uptake was calculated as the average of left striatal uptake and right striatal uptake.

**Assessment of motor symptoms**

Monkeys were observed and videotaped for 1 hour each test day: at 10 and 3 days prior to the first MPTP treatment (naive stage), 6 and 7 weeks after the first MPTP treatment (unilateral stage) and 3, 7, 14, 21, 28 and 35 days after the second MPTP treatment (bilateral stage). Given the lack of significant differences, the several time points in each stage were averaged per stage.
A single, trained, ‘blind’ investigator (GA) evaluated motor symptoms using two different rating scales. The first scale was a qualitative assessment of parkinsonian symptoms using a rating scale for non-human primates\textsuperscript{23}, with minor modifications for bilaterally treated MPTP monkeys\textsuperscript{31, 34}. This clinically oriented rating scale included the items: tremor, gait, akinesia/bradykinesia, balance, rigidity/posture and food intake. The total parkinsonian symptoms score was obtained as the sum of all items.

The second rating scale was a quantitative assessment of goal-directed limb movements\textsuperscript{3, 34}. Decreased values indicate increased parkinsonian signs. Lateralised limb movements consisted of the collection of all limb movements on either of both body halves.

**Statistics**

For the statistical analysis, the three measurements in the eight subjects were treated as independent because there had always been a new lesion between any two measurements, thereby violating the assumption of dependence within subjects. Because one PET measurement was missing, this resulted in a total of 23 observations. Because data were not normally distributed according to the Shapiro Wilk test, we calculated Spearman’s rho, two-tailed. A p value < 0.05 was considered significant.

**Results**

\textbf{[\textsuperscript{18}F]-DOPA uptake}

Before injection of MPTP, mean [\textsuperscript{18}F]-DOPA uptake for left and right striatum was 0.00208 ±0.00025 (K\textsubscript{rel} ± st.dev., see table 1). An example of a transaxial slice through the striatum is shown in figure 1.

Unilateral administration of MPTP resulted in a significant decrease of left [\textsuperscript{18}F]-DOPA striatal uptake, see table 1 (p = 0.01; paired t-test, two-tailed). In contrast, [\textsuperscript{18}F]-DOPA uptake in the right striatum was increased, but not significant (p = 0.16; paired t-test, two-tailed). After administration of MPTP into the right carotid artery, [\textsuperscript{18}F]-DOPA uptake in the right striatum was decreased in the four monkeys receiving saline, although not as much as after the first application of MPTP. [\textsuperscript{18}F]-DOPA uptake in the right striatum stayed within the normal range in the CGP 3466B treated group. No effect of the second application of MPTP was noticed on [\textsuperscript{18}F]-DOPA uptake in the left striatum.
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Figure 1: Transaxial slice through the striatum in the non-lesioned state.

Table 1: Striatal uptake of $^{[18F]}$-DOPA (mean of all monkeys ± standard deviation) for the three experimental stages.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>left</th>
<th>right</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>$0.00211 \pm 0.00039$</td>
<td>$0.00204 \pm 0.00025$</td>
<td>$0.00208 \pm 0.00025$</td>
</tr>
<tr>
<td>Left</td>
<td>$0.00055 \pm 0.00097$</td>
<td>$0.00271 \pm 0.00098$</td>
<td>$0.00163 \pm 0.00059$</td>
</tr>
<tr>
<td>Bilateral</td>
<td>$0.00035 \pm 0.00041$</td>
<td>$0.00175 \pm 0.00130$</td>
<td>$0.00118 \pm 0.00066$</td>
</tr>
</tbody>
</table>

Mean uptake is the average of left and right uptake.

Motor scores

Before injection of MPTP, all monkeys displayed normal motor behaviour. No signs of parkinsonism were detected and likewise mean parkinsonian symptoms scores were 0. Monkeys spent more time on right than on left limb movements (see table 2).

Administration of MPTP into the left carotid artery induced right-sided parkinsonism in all monkeys as described previously, resulting in an increase of mean parkinsonian symptoms scores. The amount of limb movements on the right body half decreased, while the amount of left sided limb movements remained in the same range as in the non-lesioned stage.

In the saline treated group the second infusion of MPTP into the right carotid artery induced parkinsonism on the left side of the body, thus increasing the mean parkinsonian symptoms scores. In the four monkeys receiving CGP 3466B, no parkinsonism developed on the left side of the body, and mean parkinsonian symptoms scores remained unchanged. These effects have been described in detail elsewhere. The severity of parkinsonian signs on the right body side was unaffected by the second MPTP treatment. Time spent on left limb movements was reduced in the saline group, but not in the CGP 3466B treated group. Also, no effect was noticed of the second infusion of MPTP on right limb movements.
Table 2: Overview of behavioural data (mean ± standard deviation).

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Limb movements</th>
<th>Total parkinsonian symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>left</td>
<td>right</td>
</tr>
<tr>
<td>None</td>
<td>10.28 ±5.7</td>
<td>18.18 ±9.7</td>
</tr>
<tr>
<td>Left</td>
<td>10.11 ±4.7</td>
<td>3.94 ±3.4</td>
</tr>
<tr>
<td>Bilateral</td>
<td>6.26 ±6.0</td>
<td>3.76 ±2.5</td>
</tr>
</tbody>
</table>

The higher the value of parkinsonian symptoms, the worse the condition. Concerning the limb movements: the higher the value, the better the condition.

Sd = standard deviation;

Correlation between motor symptoms and \([18F]\)-DOPA uptake

Mean parkinsonian symptoms scores were significantly correlated with mean striatal uptake of \([18F]\)-DOPA, according to Spearman’s rho (see figure 1). A significant relationship was also found between left striatal \([18F]\)-DOPA uptake and right limb movements (see figure 2) and between right striatal \([18F]\)-DOPA uptake and left limb movements (see figure 3 and table 3).

Figure 2: Correlation between mean striatal \([18F]\)-DOPA uptake (\(K_{\text{m}}\)) and total parkinsonian symptoms scores.
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Figure 3: Correlation between left striatal $[^{18}\text{F}]$-DOPA uptake ($K_{e1}$) and right limb movements.

Table 3: Correlation between striatal $[^{18}\text{F}]$-DOPA uptake ($K_{e1}$) and behavioural data. Observations were pooled over all experimental stages.

<table>
<thead>
<tr>
<th></th>
<th>$\rho$ (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean striatal $[^{18}\text{F}]$-DOPA uptake vs. total parkinsonian symptoms scores</td>
<td>0.521 (0.01)</td>
</tr>
<tr>
<td>Left striatal $[^{18}\text{F}]$-DOPA uptake vs. right limb movements</td>
<td>0.499 (0.05)</td>
</tr>
<tr>
<td>Right striatal $[^{18}\text{F}]$-DOPA uptake vs. left limb movements</td>
<td>0.418 (0.05)</td>
</tr>
</tbody>
</table>

$\rho = $ Spearman’s Rho
Discussion

This study attempted to correlate motor symptoms with striatal \[^{18}F\]-DOPA uptake in the MPTP lesioned monkey in a systematic and quantitative fashion.

We found significant correlations between: (i) total parkinsonian symptoms scores and mean striatal \[^{18}F\]-DOPA uptake; (ii) lateralised limb movements and contralateral \[^{18}F\]-DOPA uptake in MPTP lesioned monkeys. This finding of significant correlations between motor scores and dopamine function in MPTP-lesioned monkeys is in agreement with previous studies using \[^{123}I\]-\(\beta\)-CIT SPECT \(^{16}\) and \[^{18}F\]-FMT PET \(^{17},^{18}\).

In the non-lesioned phase we found a mean \(K_{\text{ref}}\) of 0.002. This is in the same range as the results of others \(^{11},^{22}\). Differences of absolute or relative striatal tracer uptake values between our data and those of others may be explained by different doses of premedication given, or different time points of data collection and ROI size \(^{20}\). Some of our \(K_{\text{ref}}\) values were negative. As \(K_{\text{ref}}\) is a random variable, the ‘spot estimate’ can be negative if the true \(K_{\text{ref}}\) is 0 or very low.

Because all monkeys were right-handed, right limb movements were higher than left limb movements in the naïve phase. In the bilateral phase \[^{18}F\]-DOPA uptake in the right striatum was decreased in the four monkeys receiving saline, although not as much as after the first application of MPTP into the left carotid artery. This can be explained by the lower dose given the second time.

Figure 4: Correlation between right striatal \[^{18}F\]-DOPA uptake (\(K_{\text{ref}}\)) and left limb movements.
Motor behavior correlates with striatal $^{18}$F-DOPA uptake in MPTP-lesioned primates

A wide range in motor dysfunction and $^{18}$F-DOPA uptake is advantageous for a regression analysis between these parameters. The two different treatment regimes of the monkeys resulted in a different degree of the MPTP lesions and therefore, different decreases in striatal $^{18}$F-DOPA uptake and different degrees of parkinsonian motor signs. Besides, severity of parkinsonism after the first lesion was variable between monkeys, suggesting large variation in individual susceptibility to the neurotoxic effects of MPTP. As time progressed, more variability occurred in motor symptoms scores and $^{18}$F-DOPA uptake, partly being induced by the administration of CGP 3466B to a subset of monkeys. As we have shown previously, administration of CGP 3466B may influence the degree of nigral damage after injection of MPTP. Thus the CGP 3466B treatments increase the range of PET and behavioural parameters. By those treatment regimes, we were able to reach a wide range in motor dysfunction and $^{18}$F-DOPA uptake.

We assumed that CGP 3466B does not alter the relationship between striatal dopaminergic uptake and behaviour, as both are altered in a consistent manner. This is supported by the finding that the correlations between motor scores and striatal uptake were similar in all treatment groups. However, this assumption has not been tested by experimental data and a possible effect of CGP 3466B on the mutual relation between the two parameters cannot completely be ruled out.

In our statistical analyses, we considered all data points as independent observations, although these are not completely independent from each other. However, the internal relationship disappears after administration of MPTP. The inter-animal and inter-treatment variances have been treated as random error.

In this study we found a significant correlation between total parkinsonian symptoms scores and mean striatal $^{18}$F-DOPA uptake and between lateralised limb movements and contralateral $^{18}$F-DOPA uptake in a valid animal model for PD: MPTP lesioned monkeys. This correlation between striatal $^{18}$F-DOPA uptake and behavioural measures was consistent in different degrees of severity of MPTP-induced parkinsonism.

Therefore, the present data show that striatal $^{18}$F-DOPA uptake correlates with behavioural measures in MPTP-treated monkeys.

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