Evaluation of nigrostriatal damage and its change over weeks in a rat model of Parkinson’s disease: small animal positron emission tomography studies with $^{[11C]}\beta$-CFT

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Abstract

Introduction: The cardinal pathological feature of Parkinson’s disease (PD) is progressive loss of dopaminergic neurons. Since dopamine transporter (DAT) is a protein located presynaptically on dopaminergic nerve terminals, radioligands that bind to these sites are promising radiopharmaceuticals for evaluation of the integrity of the dopamine system. This study using positron emission tomography (PET) tracers, $^{[11C]}\beta$-carbomethoxy-3$\beta$-(4-fluorophenyl)-tropane ($^{[11C]}\beta$-CFT, radioligand for DAT), was aimed at evaluating the degree of nigrostriatal damage and its change over weeks in a rat model of PD.

Methods: The brains of these rats were unilaterally lesioned by mechanical transection of the nigrostriatal dopamine pathway at the medial forebrain bundle (MFB). Behavioral studies were carried out by apomorphine (APO) challenge prior to and 1, 2 and 4 weeks after MFB axotomy. Small animal PET scans were performed 2 days after the behavioral test. Immunohistochemistry was conducted 4 days after the last PET scan.

Results: Compared with the contralateral intact side, a progressively decreased $^{[11C]}\beta$-CFT binding was observed on the lesioned side which correlated inversely with the APO-induced rotations. Postmortem immunohistochemical studies confirmed the loss of both striatal dopamine fibers and nigral neurons on the lesioned side.

Conclusion: These findings not only demonstrate that the neuronal degeneration in this model is relatively slow, but also suggest $^{[11C]}\beta$-CFT is a sensitive marker to monitor the degree of nigrostriatal damage and its change over weeks. This marker can be used prospectively to study the progression of the disease, thereby making detection of early phases of PD possible.

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1. Introduction

Parkinson’s disease (PD) is an age-related neurodegenerative disorder characterized by clinical symptoms of resting tremor, rigidity, bradykinesia and postural instability. The cardinal pathological feature is the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), leading to dopamine deficiency in the striatum [1,2]. It is estimated that 50% of the nigral neurons or 80% of the striatal dopamine needs to be lost before PD symptoms appear [3–5]. Early diagnosis of PD therefore is important for selecting the effective methods for slowing the degeneration of the nigrostriatal dopaminergic system and for reducing the functional decline of patients.

Functional imaging of dopamine neuron systems with positron emission tomography (PET) allows in vivo assessment of the degree of nigral neuronal loss and its change over time. It can be used prospectively to study the progression of the disease, thereby making detection of early
phases of PD possible [6–10]. Currently, radioligands available for PET to monitor progression in PD include excellent tracers both for the presynaptic system [11–14] and for the postsynaptic structures [15–17].

[18F]-6-Fluoro DOPA has been regarded as the “gold standard” for noninvasive assessment of presynaptic dopaminergic integrity in vivo [18,19] as its uptake correlates with total number of dopaminergic neurons in both humans [20] and nonhuman primates [21]. However, due to the compensatory up-regulation of aromatic l-amino acid decarboxylase in the early phase of PD, it is far from an ideal ligand for evaluation of disease progression [22]. Recently, chemicals targeted to the dopamine transporter (DAT) are increasingly considered to be a more accurate marker [11,13,23,24] than other modes. DAT is a protein located presynaptically on dopaminergic nerve terminals and it regulates the dopamine concentration in the synaptic cleft through reuptake of dopamine into presynaptic neurons [25,26]. Among the most selective ligands for DAT are cocaine analogs, such as [11C]-2β-carbomethoxy-3β-(4-fluorophenyl)-tropane ([11C]-β-CFT), which has a high affinity and selectivity for DAT [27–29]. The accumulation of [11C]-β-CFT correlates with dopamine neuron loss in the SN. Furthermore, it has been proven to be a highly sensitive marker (for monitoring disease progression and detecting early phases of PD [30,31].

The purpose of this study was to use [11C]-β-CFT to evaluate the severity of the nigrostriatal lesion and its change over weeks in a rat model of PD induced by mechanical transection of the nigrostriatal dopamine pathway at the medial forebrain bundle (MFB). [11C]-β-CFT mall animal PET images were acquired at different stages before and after lesions in the same rats, while the extent of the lesion in individual rats was quantified by endpoint immunohistochemical studies.

2. Materials and methods

2.1. Animals

Experiments were performed on eight adult male Wistar rats (Laboratory Animal Services Center, Capital Medical University, Beijing, China) weighing 240–260 g. Animals were housed in groups of three to four in a cage containing sawdust bedding, with free access to rat chow and water in a laboratory equipped with a 12:12-h (08:00–20:00) light/dark cycle. Room temperature and humidity were maintained at 23±0.5°C and 60%, respectively. They were allowed to acclimate to their environment for 3 days before the experiments. All procedures were reviewed and approved by the University Animal Care and Use Committee and were consistent with the NIH Guidelines for the Care and Use of Laboratory Animals.

2.2. Unilateral MFB axotomy

For unilateral MFB axotomy, rats were anesthetized with 350 mg/kg chloral hydrate intraperitoneally and positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) with the tooth bar set at −3.3 mm. Lesions were performed using a retractable Scouten wire knife (David Kopf Instruments) as described previously [32,33]. Briefly, the knife was lowered through a drill hole 3.8 mm posterior to and 2.4 mm right of bregma to a ventral position of 8.5 mm below the dura, and the blade was extended by 2.0 mm toward the midline. The knife was slowly raised 3.0 mm and subsequently lowered back to its original position, the blade was then retracted and the knife was withdrawn.

2.3. Behavioral tests

The rotational behavior was measured prior to and 1, 2 and 4 weeks postlesion in each animal. Rats were first placed into bowls of 30 cm in diameter attached to a rotometer (Animal Rotation Meter, Columbus Instruments, USA) and were allowed to rest for 5 min to adapt to the testing environment. Then they were injected intraperitoneally with 0.5 mg/kg apomorphine (APO, Sigma, USA). Measurement of rotational activity began 5 min after injection. The animals were tested for 30 min in a quiet and dark environment. The rotometer recorded the number of clockwise turns (ipsilateral to the lesion) and counterclockwise turns (contralateral to the lesion). The net number of turns was that of counter-clockwise turns minus clockwise turns.

2.4. PET Scans

Imaging studies with [11C]-β-CFT were conducted 2 days after the behavioral test in each rat. [11C]-β-CFT was synthesized from its corresponding precursors as described previously [34], with a radiochemical purity of more than 95%. Each rat was injected with 3–4 mCi [11C]-β-CFT through the tail vein. After an uptake period of 30 min, the rats were anesthetized with 6% chloral hydrate intraperitoneally and scanned in a high-resolution eXplore Vista PET/CT scanner (GE Healthcare, USA). Each rat was scanned for 20 min in a prone position with its brain centered in the axial transaxial fields of view. PET images were reconstructed with a 3D ordered subsets expectation maximization algorithm with corrections for decay, detector deadtime, scatter and random coincidences. The final image resolution in the central FOV was less than 1 mm at full width half maximum. For semiquantitative evaluation, regions of interest (left and right striatum) were drawn onto coronal slices according to the standard rat brain atlas by Paxinos and Watson [35].

2.5. Immunohistochemistry

Four days after the last PET scan, the rats were deeply anesthetized with 350 mg/kg chloral hydrate and then transcardially perfused with 100 ml 0.9% saline followed by 200 ml 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4). The brains were removed, postfixed overnight and cryoprotected in 30% sucrose. Tissues were sliced into 30-μm-thick coronal sections using a freezing
microtome (CM 1850; Leica). The sections through the SN (4.80–6.30 mm posterior to bregma) and the striatum (0.3–1.3 mm posterior to bregma) were obtained according to the rat atlas of Paxinos and Watson [35]. They were stored in PBS solution for immunocytochemical processing. Every sixth section through the SN or the striatum was selected for immunohistochemical detection of the dopaminergic neuronal marker tyrosine hydroxylase (TH), and the rest of the sections were used in other experiments not reported here. Briefly, following quenching of endogenous peroxidase activity (using a solution of 3% hydrogen peroxide in PBS) and blocking of nonspecific secondary antibody binding (using 3% normal horse serum/0.1% Triton X-100 in PBS), sections were incubated overnight in the primary antibody (mouse anti-TH, 1:10,000 dilution, Sigma) at 4°C; after thorough washing, the sections were incubated in the biotinylated horse antimouse secondary antibody (1:200 dilution, Vector) for 2 h at room temperature and then incubated with avidin–biotin peroxidase complex for 30 min at 37°C. Immunoreactions were visualized with a standard diaminobenzidine-hydrogen peroxide chromogen reaction. The sections were mounted on gelatin-coated slides and coverslipped with mounting medium. Negative control study was performed by omission of the primary antibody.

The number of dopaminergic neurons was determined by counting the average of TH- immunoreactive (TH-ir) neurons in the six to eight SNpc sections at 20× magnification (Olympus V ANOX-T, Japan). The optical density (OD) of TH-ir terminals in the striatum was measured using the Leica QWin image analysis system version 2.8 (Leica Imaging Systems, Cambridge, UK).

2.6. Statistical analysis

Changes in $[^{11}C]\beta$-CFT binding on the lesioned side were determined as a percentage of those on the contralateral control side. Correlation between two parameters was evaluated by the coefficient of determination ($R^2$). One-way analysis of variance with least-significant differences test for multiple comparisons was used where appropriate. All results were presented as means±S.E.M., and $P<.05$ was accepted as statistically significant.

3. Results

Four out of eight rats were discarded because the rotational behavior and $[^{11}C]\beta$-CFT binding were almost unchanged at 4 weeks after MFB axotomy.

3.1. MicroPET imaging

Fig. 1A shows representative coronal $[^{11}C]\beta$-CFT images of the same rat brain prior to and 1, 2 and 4 weeks after MFB axotomy. Prior to lesion, marked
accumulation of $[^{11}\text{C}]\beta$-CFT was observed in the striatal regions with no significant difference between the left and right striata. After the lesion, $[^{11}\text{C}]\beta$-CFT binding showed a significant decrease in the right striatum. The uptake ratio decreased to 53.45±11.44% ($P<0.01$), 13.01±8.38% ($P<0.01$) and 5.63±3.35% ($P<0.01$) at 1, 2 and 4 weeks, respectively, compared with the intact left side (Fig. 1B).

### 3.2. Correlation of $[^{11}\text{C}]\beta$-CFT uptake ratio with rotational behavior

Fig. 2 shows the net number of rotations induced by APO challenge in rats prior to and 1, 2 and 4 weeks after MFB axotomy. Before MFB axotomy, slight contralateral and ipsilateral rotations could be seen in the rats and the net number of rotations was 1±6.31 turns per 30 min. After MFB axotomy, APO induced remarkable contralateral rotations, while the ipsilateral rotations almost decreased to zero. The net number of rotations for the rats was 78.75±13.71, 154.75±22.99 and 174.25±26.31 turns per 30 min, respectively, at 1, 2 and 4 weeks postlesion. Statistical analysis of the data from the microPET and behavioral studies revealed a significant inverse correlation between the decrease in $[^{11}\text{C}]\beta$-CFT uptake ratio (right/left) and the net number of APO-induced rotations ($R^2=0.85$, $P<0.01$; Fig. 3) after the lesion.

### 3.3. Striatal and nigral TH immunoreactivity

To further evaluate the integrity of the dopamine system after MFB axotomy, all four lesioned rats underwent postmortem immunohistochemical analysis. Compared with the intact left side, both striatal TH-ir fibers and nigral TH-ir neurons decreased dramatically on the lesioned side ($P<0.01$, Fig. 4), which suggested that the MFB was transected successfully.

### 4. Discussion

This is the first study to use longitudinal $[^{11}\text{C}]\beta$-CFT PET imaging to assess the degree of nigrostriatal damage and its change over weeks in a partially lesioned rat model of PD induced by unilateral mechanical transection of MFB. Recently, this model has been used as a useful hemi-
Parkinson model for investigation of the disease mechanisms and for assessment of the effect of therapies [33,36,37]. Many studies have shown that such a lesion paradigm produces a precisely defined lesion of dopaminergic nigrostriatal tract that results in the retrograde degeneration of SN dopaminergic neurons. Compared with the 6-hydroxydopamine (6-OHDA) lesioned rat model, the neuronal degeneration caused by unilateral MFB axotomy is relatively slow and has been suggested to mimic the progressive neuronal loss in PD patients [32,38].

In the current experiments, a progressively decreased $[^{11}\text{C}]\beta$-CFT binding was noted in the right striatum, compared with the intact left counterpart. This phenomenon possibly represented a combination of progressively reduced DAT binding sites with loss of dopamine nerve terminals as well as progressive down-regulation of DAT in surviving terminals. Down-regulation of the DAT in presynaptic surviving terminals was considered to be an adaptive change to preserve the synaptic dopaminergic concentration in early disease, but this may ultimately result in increased dopamine turnover and higher oscillations in synaptic dopamine concentration, thereby possibly predisposing toward the occurrence of motor complications as disease progresses [22,39]. The decreased $[^{11}\text{C}]\beta$-CFT binding was confirmed by endpoint immunohistochemical studies which showed that more than 80% nigral TH-ir neurons and striatal TH-ir fibers were lost at 4 weeks postlesion, whereas a slower degeneration has been reported by Brecknell et al. [32]. Brecknell et al. [32] used similar animal models to study the time course of cell death by immunohistochemical method for 16 weeks. They found that approximately 28% of cells died within the first week followed by a progressive cell loss which reached an end point at about 10 weeks, at which time 6–12% of the neurons remained. The discrepancy could be attributable to the small sample size and large variation between the animals in our experiment. Furthermore, the exact location of our axotomy is much closer to the cell bodies than that of Brecknell et al. [32].

The decreased $[^{11}\text{C}]\beta$-CFT binding was significantly correlated with the rotational behavior induced by APO injection. APO is a dopamine receptor agonist capable of stimulating both $D_1$ and $D_2$ receptors [40]. The marked dopamine depletions in parkinsonian rats causes functional supersensitivity of dopamine receptors (especially $D_2$ receptors) at the postsynaptic terminals in the denervated side of the striatum. The administration of APO induces contralateral rotation. Our results indicated that a more than 90% decrease in presynaptic dopaminergic biomarkers seemed to be necessary for the appearance of significant contralateral rotational behavior (more than five turns per minute). This result was in agreement with previous studies which revealed that major and almost complete dopaminergic degeneration was necessary to increase striatal dopamine $D_2$ receptor expression in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkeys and unilaterally 6-OHDA lesioned rats [41,42]. This view was also supported by several human postmortem studies which showed that 50–60% of the nigral neurons or 70–80% of the striatal dopamine must be lost before PD symptoms became obvious [3–5]. The lack of up-regulation of $D_2$ receptors at lower levels of cell degeneration may be partly due to compensatory increases in dopamine synthesis and release by spared cells.

In our present experiment, the $[^{11}\text{C}]\beta$-CFT binding in the denervated side of the striatum decreased to 53.45±11.44% of the intact left side by 1 week postlesion, at which time the number of APO-induced rotations was under three turns per minute which was considered to be a nonsignificant change by most studies. It suggested that mild loss of presynaptic dopaminergic components, which did not cause pronounced behavioral changes, was detectable by functional imaging techniques. Thus we could predict the degree of nigrostriatal damage and its change over weeks to some degree by measuring $[^{11}\text{C}]\beta$-CFT binding before any PD signs appear. However, the function of $[^{11}\text{C}]\beta$-CFT imaging in the assessment of preclinical symptom and in monitoring disease progression must be considered carefully because $[^{11}\text{C}]\beta$-CFT imaging may slightly overestimate the true loss of dopaminergic terminals in PD due to a compensatory down-regulation of the DAT [22]. Additionally, DAT could also be affected by age and other factors. A decline in DAT binding with increasing age has been observed in many neuroimaging studies [43–46].

In summary, unilateral MFB axotomy caused a progressively decreased $[^{11}\text{C}]\beta$-CFT binding in the denervated side of the striatum which correlated well with the APO-induced rotations. These findings demonstrate that $[^{11}\text{C}]\beta$-CFT is a sensitive marker to monitor the degree of nigrostriatal damage and its change over weeks, it can be used prospectively to study the progression of the disease, thereby making detection of early phases of PD possible. The results also suggest the neuronal degeneration in this model is a relatively slow process, which mimics the slow reduction of the number of dopaminergic neurons in the SNpc in PD patients. Therefore, this model serves as a powerful tool to help us to understand the pathogenic mechanisms of PD and to evaluate the potential treatment options for this disease, as well as the regenerative growth capacity of injured SN dopaminergic neurons.

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