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Evaluation of nigrostriatal damage and its change over weeks in a rat model of Parkinson's disease: small animal positron emission tomography studies with [^{11}C]β-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, [^{11}C]-2β-carbomethoxy-3β-(4-fluorophenyl)-tropane ([^{11}C]β-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 [^{11}C]β-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 [^{11}C]β-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.

Keywords: Parkinson's disease; Rat model; Medial forebrain bundle; Dopamine transporter; [^{11}C]β-CFT

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

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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].

[^{18}F]-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 [^{11}C]-2β-carbomethoxy-3β-(4-fluorophenyl)-tropane ([^{11}C]β-CFT), which has a high affinity and selectivity for DAT [27–29]. The accumulation of [^{11}C]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 [^{11}C]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). [^{11}C]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 counterclockwise turns minus clockwise turns.

2.4. PET Scans

Imaging studies with [^{11}C]β-CFT were conducted 2 days after the behavioral test in each rat. [^{11}C]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 [^{11}C]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 and 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