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Evaluation of dopamine transporters and D2 receptors in hemiparkinsonian rat brains in vivo using consecutive PET scans of [^{18}F]FPCIT and [^{18}F]fallypride

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HIGHLIGHTS

- The dopaminergic integrity in unilateral 6-OHDA was evaluated by dual PET tracers.
- The brain uptake and BP_{ND} of [^{18}F]FPCIT was greatly decreased.
- The brain uptake and BP_{ND} [^{18}F]fallypride was slightly increased.
- DAT are down-regulated and D2R are up-regulated.

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ABSTRACT

The aim of this study was to investigate dopaminergic function in unilaterally lesioned 6-OHDA rats by dual PET radioligands: [^{18}F]FPCIT (a dopamine transporter imaging radioligand) and [^{18}F]fallypride (a dopamine D2 receptors imaging radioligand). As a result, the brain uptake of [^{18}F]FPCIT was significantly reduced and that of [^{18}F]fallypride was increased in the ipsilateral striatum (lesion side) of the 6-OHDA rats. These findings implicated that dopamine transporter is down-regulated and dopamine D2 receptor is up-regulated in this hemiparkinsonian rat model.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease that is neuropathologically characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta followed by projecting neurons into striatum. This pathway give rise to an abnormality in the striatal dopaminergic systems, for example a deficiency of dopamine transporters (DAT) or dopamine receptors (DR) has been observed (Innis et al., 1993; U.K. Rinne et al., 1990). Quantification of DAT density using molecular imaging is a very sensitive method to detect any loss of striatal DAT in early stage of PD. However, it is often hard to differentiate PD from other Parkinson's syndrome such as multi-system atrophy and vascular Parkinson syndrome because DAT density is typically lower in both PD and in Parkinson's syndrome (Booij et al., 1999; Marshall and Grosset, 2003). Therefore, assessing the alteration of DAT and D2R is necessary in order to exact diagnose these patients.

A number of radioligands for positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been developed to assess the integrity of the dopaminergic

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system. Several radioligands, such as [^{123}I]iodobenzamide ([^{123}I]IBZM), [2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo [3,2,1]oct-2-yl]methyl](2-mercaptoethyl)amino]ethyl]amino] ethanethiolato(3-)-*N*2,*N*2′,*S*2,*S*2′]oxo-[1*R*-(*exo*–*exo*)-[^{99m}Tc]technetium ([^{99m}Tc]TRODAT-1), 3,5-dichloro-*N*-{[(2*S*)-1-ethylpyrrolidin-2-yl]methyl}-2-hydroxy-6-methoxybenzamide ([^{11}C]raclopride) and *N*-(3-iodoprop-2*E*-enyl)-2β-carbomethoxy-3β-(4-methylphenyl)nortropane ([^{11}C]PE2I) have been used in studies with human subjects. However, these tracers have some limitations which include a short half-life ([^{11}C]raclopride, [^{11}C]PE2I), and a relatively low spatial resolution (i.e., [^{123}I]IBZM, and [^{99m}Tc]TRODAT-1). Therefore, radioligands based on F-18 are needed for improved evaluation of the integrity of the dopaminergic system because these radiocompounds have a longer half-life, high solution, high sensitivity and high affinity to the target (Okarvi, 2001). However, there is no report that investigated the relationship between DAT and D2R in vivo using [^{18}F]PET radioligands in the same subject. Among the [^{18}F]based radioligands, [^{18}F]FPCIT and [^{18}F]fallypride were reported to be the most promising PET radioligands for assessing the pre- and post-synaptic dopamine functions due to their high sensitivity and selectivity of DAT and D2R.

The 6-hydroxydopamine (6-OHDA) unilaterally lesioned rats are a very useful a hemiparkinsonian model for studying dopaminergic functions (Ungerstedt, 1971). The main advantage of this model is that it is easy to evaluate the severity of the disease using a simple behavioral test in a particular circling behavior after administering of dopaminergic drugs.

The aim of the present research is to evaluate the relationship between DAT and D2R in 6-OHDA unilateral lesioned rats using consecutive [^{18}F]FPCIT and [^{18}F]fallypride PET.

2. Material and methods

2.1. Animals

All animal studies were approved by the committee for the care and use of laboratory animals at Yonsei University College of Medicine. Five 6-OHDA unilaterally lesioned male Sprague-Dawley rats (434.32 ± 12.30 g, mean ± SD) and five control male Sprague-Dawley rats (402.15 ± 11.53 g, mean ± SD) were used for in vivo imaging of microPET. The rats were housed in a temperature and humidity controlled room with a 12 h light/dark cycle, with free access to food and water.

2.2. 6-OHDA lesion model

Before anesthesia, animals were pretreated with desipramine hydrochloride (DMI; 12.5 mg/kg i.p.; 12.5 mg/ml; 0.1 ml/100 g). Surgery was performed under deep i.p ketamin/xylazine anesthesia (at dose of 40 mg/kg and 5 mg/kg, respectively). Each animal was positioned in a stereotaxic apparatus (Stoelting Co., Illinois, USA). The 20 μg/4 μl/site of 6-hydroxy dopamine (6-OHDA; Sigma-Aldrich, St. Louis, MO) was injected into 2 sites (total of 40 μg 6-OHDA) in the right striatum according to the rat's brain atlas (Paxinos and Watson, 2007). 6-OHDA was injected into the following coordinates (relative to the bregma and dura): anterior–posterior (AP) +0.5 mm, medial–lateral (ML) 2.5 mm, dorsal–ventral (DV) −5.0 mm and AP −0.5 mm, ML 4.2 mm, DV −5.0 mm at a rate of 1 μl/min using a 26 G Hamilton syringe. The inserted needle was withdrawn from each location after 5 min, and skin was sutured.

2.3. Behavioral test

Rotational behavior was evaluated using a multichannel rotometer system (ROTORAT, MED Associates, Inc). Contralateral or ipsilateral rotational behaviors were induced by intraperitoneal injection of *d*-amphetamine (5 mg/kg) on day 14 after the 6-OHDA injection. Each animal was placed in a cylindrical test chamber for 60 min. Counter-clockwise rotations were used for analysis. Animals showing more than 100 rotations per minute were considered successful and used for further experiments (Schwarting and Huston, 1996).

2.4. Autoradiography study of [^{125}I] FPCIT

Autoradiography of [^{125}I] FPCIT was used to confirm the 6-OHDA model for this study. [^{125}I] FPCIT was prepared using the iodination destannylation method. Na[^{125}I] solution was supplied from PerkinElmer (Waltham, MA). FPCIT precursor was purchased from Future Chem (Seoul, Korea). Briefly, tributylstannyl precursor was dissolved in MeOH and then Na[^{125}I] was added. The reaction mixture was acidified to a pH of 4–5 with 1.0 M aqueous HCl, and then 30% H_2O_2 was added. After incubation for 30 min at ambient temperature, the reactant was purified by high performance liquid chromatography (HPLC) using the following condition; The reactant was loaded on a C18 column (Waters micro-Bondapack, 5 micro meter, 3.9 × 300 mm) and eluted using isocratic solvent system (30:70:0.1%—acetonitrile/water/TFA) with a flow rate of 1 ml/min. We obtained the product at 17.1 min and reconstituted it in 1 ml of PBS. It was then filtered through a 0.22 micro meter syringe filter.

Each animal received a single intravenous dose of approximately 2 MBq/kg body weight via a tail vein. After injections, each rat was returned to their cages. At 2 h post-injection, the rats were suffocated to death and the carcasses were frozen in the deep freezer. The frozen rat heads were separated from body and embedded in 3.5% carboxy methyl cellulose (CMC) and each block was frozen in a bath of acetone cooled with solid carbon dioxide. And set in an autocryotome (Nakagawa, Japan). Then, frozen rat heads were sliced into 40 micrometer-thick coronal sections. Sections were taken from various levels of each head using adhesive tape. The freeze-dried sections were covered with a protective membrane and were placed in contact with the imaging plate (BAS-SR2025, Fuji Photo Film). The plates were exposed in lead-sealed boxes at room temperature for 24 h. After exposure, the radioactivity recorded from the imaging plate was scanned using a bio-imaging analyzer system (BAS5000, Fuji Photo Film) and the images were sampled using multi-gauge image analysis software.

2.5. Preparation of radioligands

[^{18}F]FPCIT and [^{18}F]fallypride were synthesized according to the procedure previously described (Lee et al., 2007; Moon et al., 2010).

2.6. PET studies

PET scanning was performed with the Siemens Inveon small animal PET scanner (Siemens Medical Solutions). The scanner has a peak absolute system sensitivity of < 10% for an energy window of 250–750 eV, an axial field of view of 12 cm, and a transaxial field of view of 10 cm (Constantinescu and Mukherjee, 2009). Anesthesia was administered with 2.5% isoflurane and was maintained for 150 min of the PET experiment with 1.5% isoflurane. After cannulation in a tail vein, rats were positioned in the center of gantry. Tracer accumulation in the brain was