Imaging of Dopamine Transporter in Rodents Using PET and SPECT

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1 INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder, which includes, among other pathologies, death of dopaminergic cells in the substantia nigra. In this study, dopamine transporter (DAT) imaging of different rodent PD models was performed with clinically used ^{123}I-β-CIT and SPECT or ^{18}F-FE-PE2I and PET. With small animal imaging scanners, the striatum can be visualized and ligand binding quantified using both methodologies; however, PET has superior sensitivity in comparison to SPECT. Moreover, PET is more suitable to dynamic imaging.

DAT imaging was preformed on three different rodent models of PD. Unilateral AAV-α-synuclein intracranial infusion of human gene to substantia nigra causes aggregates in brain similar to PD. Local infusion of 6-OHDA causes dopaminergic and noradrenergic neuronal loss in the brain. MPTP is a neurotoxin which causes permanent PD like symptoms by destroying dopaminergic neurons in substantia nigra. Characteristic depletion of dopamine and it's metabolites in striatum was observed in the studied models.

Modern imaging modalities offer a translational method to study functional changes of PD in rodents. Quantitative results can be obtained in vivo quickly and cost-efficiently over several time points, enabling longitudinal follow-up of disease progression within individual animals, and offering a valuable tool for preclinical research and efficacy studies.

2 METHODS

Animals: All animal experiments were carried out according to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals, and approved by the National Animal Experiment Board, in AAALAC accredited laboratory.

MPTP PD model: MPTP (Toronto Research Chemicals) was given twice a day at the dose of 15 mg/kg in saline i.p. at 3 h intervals on two consecutive days (Days 0 and 1), resulting to total amount of 60 mg/kg.

6-OHDA PD model: Male Wistar rats were anesthetized with isoflurane and placed in a stereotactic frame. Infusion of 6-OHDA (4 µg/µl) was done into the right striatum. A total of 5 µl of 6-OHDA, equal to 20 µg, was infused at a speed of 0.5 µl/min and was equally distributed in 4 sites at the following coordinates: AP +1.0, ML +2.8, DV -6.0, -5.5, -5.0 and -4.4 mm. After the surgical procedure, the skin was closed and rats were allowed to recover from anesthesia.

AAV PD model: Male Wistar rats were anesthetized with isoflurane and placed in a stereotactic frame. Viral vector infusion was done using AAV1/2 expressing human A53T α-synuclein (total of 2 µl, 0.2 µl/min) at the following coordinates: AP -5.2, ML 2.1, DV -7.5 mm from bregma. After the procedure skin was closed and disinfected.

SPECT imaging: The animals were anesthetized with isoflurane and injected i.v. with ca. 20 MBq (mice) or 30 MBq (rats) of ^{123}I-β-CIT (Map Medical). SPECT imaging was started 60 min post injection with NanoSPECT/CT Plus (Mediso). Imaging protocol consisted of planar tomography image which was used as a reference to choose brain imaging area. After choosing the imaging area, helical SPECT scan was performed from the same coordinates using 140 s/time frame. High resolution multipinhole apertures were used. After SPECT imaging helical CT was performed. Reconstruction of SPECT images was done using HiSPECT. Image processing and analysis was performed using InVivoScope software.

PET imaging: The animals were anesthetized using isoflurane (4 – 5% induction, 1.5 – 2% maintenance), cannulated in lateral tail vein and positioned in the scanner (BioPET/CT, Sedecal). A scout scan was performed to determine the scanning area. PET scan was started and ^{18}F-FE-PE-2I was administrated as a fast bolus. List mode PET scan for 60 min was acquired, after which a CT scan was performed. Image reconstruction was done using three dimensional ordered subset expectation maximization (3D OSEM) with 1 iteration, 25 subsets including attenuation correction. Imaging data was fitted to simple reference tissue model using PMOD software (v3.7).

HPLC analysis: dopamine (DA), 3,4-dohydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA) and serotonin (5-HT) concentrations from striatum were determined using HPLC with electrochemical detection. Tissue samples were homogenized in 0.1 M perchloric acid with MSE Soniprep 150 ultrasonic disintegrator. Tissue homogenates were centrifuged and supernatants were filtered through polypropylene membrane and diluted with 0.1 M perchloric acid. The analytes were separated on a Zorbax SB-Aq reversed-phase column with a Zorbax SB-Aq pre-column in an isocratic run. The neurotransmitter levels were normalized as pg/g wet tissue.

3 RESULTS

As a summary, SPECT imaging and HPLC analysis of dopaminergic system in striatum was evaluated in three separate rodent models of PD. Decrease in DAT ligand binding suggesting dopaminergic neuronal death was seen in all studied models. Further, reduction in neurotransmitter concentration (DA, DOPAC and HVA) in striatum was analyzed using HPLC (Figure 1).

PET imaging was performed in the same PD models as studied with SPECT. When MPTP exposed mice were imaged with DAT tracer, ^{18}F-FE-PE2I (Figure 2), 70% ($p<0.0001$) reduction in the striatal BP_{ND} was observed suggesting dopaminergic neuronal death characteristic to PD.

In 6-OHDA model PET BP_{ND} of healthy striatum was 1.14 in comparison to lesioned side which was calculated to be 0 (Figure 3). Similar time activity curves were seen in lesioned striatum and cerebellum, which has minimal expression of DAT.

PET imaging was also used to follow longitudinally the disease progression after unilateral AAV-α-synuclein infusion. The rats were imaged on weeks 4, 8 and 16 after infusion. No disease progression was observed (Figure 4). To compare PET and SPECT results, one rat (AAV-α-synuclein, at 4 weeks) was imaged using both techniques (Figure 5). Higher sensitivity and specificity were obtained using PET, which could be seen as better contrast between striatum and extra striatal signal. In addition to DAT, ^{123}I-β-CIT binds also to serotonin transporter (SERT), which was seen as midbrain signal.

Figure 1. A) The striatal neurotransmitter levels were analyzed in different PD models using HPLC with electrochemical detection. Characteristic depletion in dopamine levels and metabolites was observed. Dopamine (DA), 3,4-dohydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA) and serotonin (5-HT). AAV-a-synuclein, n=15, 6-OHDA n=5 contralateral; n=17 ipsilateral, MPTP, n=5. Multiple t-test **** = $p < 0.001$. B) Representative images of ^{123}I-β-CIT SPECT DAT imaging. Depending on the used PD model unilateral or bilateral differences in ligand binding to striatum were seen. AAV-α-synuclein (B), 6-OHDA (C) and MPTP (D).

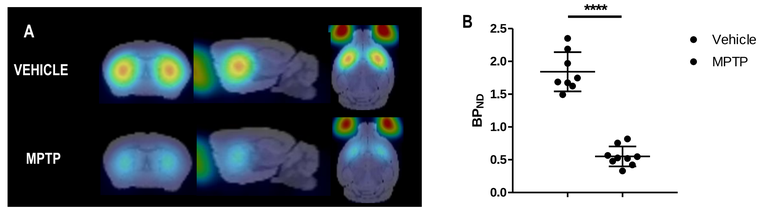

Figure 2. A) Mean PET images 30 – 60 min post injection shows lower ^{18}F-FE-PE2I uptake in the striatum of MPTP treated mice (n=9) compared to vehicle treated group (n=8). The PET images were co-registered with mouse MRI template. Sections shown in coronal, sagittal and horizontal view. B) Individual BP_{ND} results were calculated using SRTM. Values are shown as mean ±SD. MPTP treated mice showed 70% decrease (unpaired two-tailed T-test, **** $p < 0.0001$) in BP_{ND} when compared to vehicle treated group.

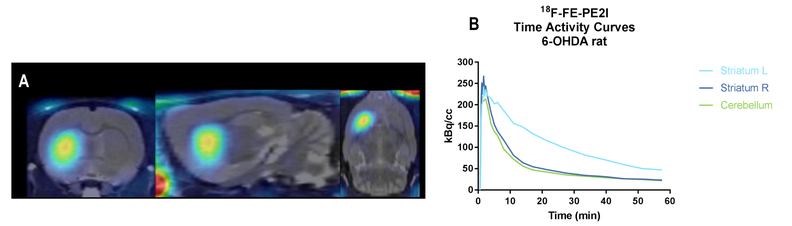

Figure 3. A) Mean PET images 30 – 60 min post injection shows lower ^{18}F-FE-PE2I uptake in the 6-OHDA lesioned (right) striatum compared to contralateral (left) side. The PET images were co-registered with rat MRI template. Sections are shown in coronal, sagittal and horizontal view. B) Time activity curves in striata and cerebellum. BP_{ND} were 1.14 and 0.00 in right and left striatum, respectively.

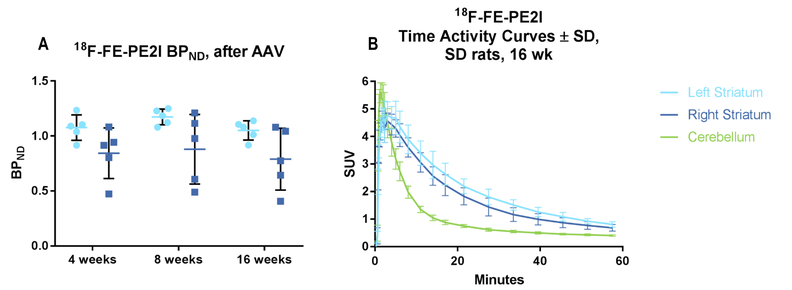

Figure 4. A) BP_{ND} in ipsilateral (right) and contralateral striatum (left) 4 – 16 weeks after intrastriatal AAV- α-synuclein infusion. Similar results were obtained over time. B) Individual BP_{ND} including mean values and SD were calculated using SRTM..

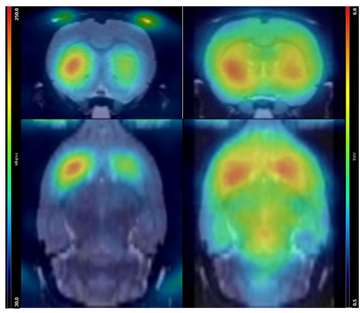

Figure 5. Example PET (left) and SPECT (right) images from the same rat. Scans were performed 4 weeks post unilateral AAV-α-synuclein infusion to right striatum. PET image was summarized from time frames 20 – 35 min post ^{18}F-FE-PE2I injection and SPECT image was taken as a static scan 60 – 85 minutes post ^{123}I-β-CIT injection. ^{18}F-FE-PE2I showed higher specificity and sensitivity to scan DAT in rodents compared to ^{123}I-β-CIT. This was seen as higher extrastriatal signal in the SPECT image as ^{123}I-β-CIT binds also to SERT which was seen as signal from midbrain.

4 CONCLUSION

Dopamine transporter imaging has been conducted in several PD models using both SPECT and PET techniques. Imaging of DAT was more sensitive with PET compared to SPECT. However, SPECT allows faster throughput due to longer usable time of radioligand and it can be also applied to image SERT. Both of these methodologies are also used in clinical diagnosis of PD patients.

In summary, fully translational imaging offers quantitative results quickly and cost-efficiently over several time points, enabling longitudinal follow-up of disease progression within individual making it valuable tool for preclinical research and efficacy studies.