PET Imaging of Mitochondrial Complex I with $^{18}$F-BCPP-EF in the Brains of MPTP-Treated Monkeys

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$^{18}$F-BCPP-EF was applied to assess mitochondrial complex I (MC-I) activity in the brains of Parkinson disease model monkeys prepared by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and also presynaptic dopamine parameters. **Methods:** $^{11}$C-$\beta$-CFT for the dopamine transporter; $^{11}$C-3,4-dihydroxy-phenyl-L-alanine (\(\beta\)-L-DOPA), L-6-$^{18}$F-fluorodopa ($^{18}$F-FDOPA), or 6-$^{11}$C-methyl-tyrosine ($^{11}$C-6MeTyr) for dopamine synthesis; or 2-tert-butyl-4-chloro-5-([2-($^{18}$F-fluoroethoxy)-ethoxy]-pyridin-3-ylmethoxy)-2H-pyridazin-3-one ($^{18}$F-BCPP-EF) for MC-I was intravenously injected into normal and MPTP monkeys in order to analyze their uptake in the striatum. **Results:** Significant reductions in presynaptic dopamine parameters and MC-I activity were detected in the striatum of MPTP monkeys. Correlations were observed between MC-I activity and dopamine transporter as well as between MC-I activity and dopamine synthesis in the striatum. The order of detectability of impaired MC-I activity was $^{11}$C-6MeTyr >>> $^{11}$C-L-DOPA $\geq$ $^{18}$F-FDOPA. **Conclusion:** $^{18}$F-BCPP-EF has potential as a PET probe for the quantitative imaging of MC-I damage in the living brains of Parkinson disease model monkeys using PET.

**Key Words:** PET-monkey; dopamine transporter; dopamine synthesis; mitochondria complex I

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Parkinson disease (PD) is characterized by the progressive degradation of nigrostriatal pathways with the selective loss of dopaminergic neurons in the substantia nigra pars compacta, resulting in movement disorder such as resting tremor, akinesia, bradykinesia, rigidity, and postural instability. These clinical symptoms of PD were previously reported to be induced after 40%–50% loss of the neurons in the substantia nigra pars compacta; there was also a reduction of dopamine to about 20% of normal levels in the striatum (1). Although the direct cause of the selective neurodegeneration in PD has not yet been identified, mitochondrial dysfunction has emerged as a common aspect of its pathogenesis (2,3). In mammalian cells, the electron transport chain in mitochondria consists of 5 complexes (from I to V) that produce adenosine triphosphate, with complex I (MC-I) being the first and rate-limiting step of overall respiratory activity and oxidative phosphorylation under physiologic conditions. Impaired electron transport and oxidative phosphorylation due to MC-I deficiency may account for the neuronal cell death in PD. The loss of MC-I catalytic activity has been detected in multiple tissues from sporadic PD patients with increased oxidative stress (3).

The exposure of humans to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces a syndrome that mimics the core neurologic symptoms of PD and selectively dopamine neurodegeneration in the substantia nigra pars compacta (4). MPTP is metabolized by monoamine oxidase B located in glia cells, to 1-methyl-4-phenylpyridinium ions, which are selectively taken up through the dopamine transporter (DAT) and stored in vesicles via uptake by vesicular monoamine transporter 2. These ions then induce their toxic effects in dopaminergic neurons by inhibiting adenosine triphosphate synthesis and generating free radicals.

PET has been applied to the quantitative and noninvasive imaging of biologic functions, and several abnormalities in the nigrostriatal dopamine pathway have been imaged in PD patients using PET (5). In addition to dopamine, we recently designed a PET probe, 2-tert-butyl-4-chloro-5-([2-($^{18}$F-fluoroethoxy)-ethoxy]-pyridin-3-ylmethoxy)-2H-pyridazin-3-one ($^{18}$F-BCPP-EF), for the quantitative imaging of MC-I in vivo and confirmed that it was suitable for imaging MC-I function in the living brains of rats (6) and monkeys (7,8).

In the present study, we prepared an MPTP-induced PD model of cynomolagus monkeys (Macaca fascicularis) (9,10), and $^{18}$F-BCPP-EF was evaluated based on its ability to detect changes in MC-I activity in the living brain after a treatment with MPTP. Presynaptic dopamine damage was monitored using $^{11}$C-$\beta$-CFT for DAT and $^{11}$C-3,4-dihydroxy-phenyl-L-alanine (\(\beta\)-L-DOPA), L-6-$^{18}$F-fluorodopa ($^{18}$F-FDOPA), and 6-$^{11}$C-methyl-tyrosine ($^{11}$C-6MeTyr) for dopamine synthesis, and the relationship between presynaptic dopaminergic damage and impaired MC-I activity was also assessed.

**MATERIALS AND METHODS**

**Animals**

Animals were maintained and handled in accordance with the recommendations of the U.S. National Institutes of Health and the guidelines of the Central Research Laboratory, Hamamatsu Photonics. The following experiments were approved by the Ethical Committee of the Central Research Laboratory, Hamamatsu Photonics.

Five male normal and 5 MPTP-treated cynomolagus monkeys (Macaca fascicularis; age range, 2.8–3.3 y; weight range, 2.0–3.5 kg) were subjected to PET scans (9,10). MPTP at doses ranging from 0.2 to 0.4 mg/kg of free base in phosphate-buffered saline was injected intravenously once per week over a 4-mo period until a stable parkinsonian syndrome was observed. The total doses of MPTP administered ranged from 1.5 to 3.0 mg/kg. To avoid the possibilities of spontaneous
recovery and direct inhibition of \(^{18}\)F-BCPP-EF binding to MC-I by MPTP. MPTP administration was halted for 2 mo, from the last MPTP treatment to the first PET measurements.

MR images of the monkeys were obtained with a 3.0-T MR system (Signa Excite HDxt 3.0T; GE Healthcare) under pentobarbital anesthesia.

**PET Ligand Syntheses**

\(^{11}\)C-\(\beta\)-CFT was labeled by the \(^{11}\)C-N-methylation of its nor-compound with \(^{11}\)C-methyl iodide \((11)\). Radioactive purity was greater than 98%, and specific radioactivity was 42.0 ± 7.9 GBq/\(\mu\)mol. \(\beta\)-\(^{11}\)C-L-DOPA was synthesized using a combination of organic synthesis and multienzymatic procedures \((12)\). Radiochemical purity was better than 98%, and specific radioactivity was 25.4 ± 8.5 GBq/\(\mu\)mol. \(^{18}\)F-FDOPA was labeled using chiral phase-transfer alkylation \((13)\). Radiochemical and enantiomeric purities were greater than 99% and 92%, respectively. Specific radioactivity was 50.5 ± 6.6 GBq/\(\mu\)mol. \(^{11}\)C-6Me\(\text{Tyr}\) was labeled using the rapid Pd(0)-mediated cross-coupling reaction of \(^{11}\)C-methyl iodide and corresponding alkenyl boronate precursor \((10,14)\). Radiochemical purity was greater than 99%, and specific radioactivity was 40.0 ± 10.1 GBq/\(\mu\)mol. \(^{18}\)F-BCPP-EF was radiolabeled by the nucleophilic \(^{18}\)F-fluorination of the corresponding precursor \((6-8)\). Radiochemical purity was greater than 98%, and specific radioactivity was 139.6 ± 37.0 GBq/\(\mu\)mol.

**PET Measurements**

After overnight fasting, monkeys were tracheostomized, immobilized with pancuronium bromide (0.05 mg/kg intramuscularly), and artificially ventilated (Cato; Drager). Anesthesia was continued with 0.4–0.8 vol% isoflurane in a \(\text{N}_2\text{O}/\text{O}_2/\text{N}_2\) (1:1:1) gas mixture. A venous cannula was added to some plasma samples (sample/methanol = 1/1), which were centrifuged, and the supernatants obtained were developed using thin-layer chromatography plates (AL SIL G/UV; Whatman) with a mobile phase of ethyl acetate. The ratio of unmetabolized fraction \((R = 0.66)\) was determined using a phosphoimaging plate (FLA-7000; Fuji Film). The input function of unmetabolized \(^{18}\)F-BCPP-EF was calculated using the data obtained by correction of the ratio of the unmetabolized fraction to total radioactivity, which was used as the arterial input function. A kinetic analysis of \(^{18}\)F-BCPP-EF was performed using the Logan graphical analysis \((7,8)\).

The quantitative analysis of \(^{11}\)C-\(\beta\)-CFT was performed with a simplified reference tissue model to calculate the nondisplaceable binding potential \((B_{\text{ND}})\) \((17)\) using the time–activity curve in the cerebellum as an input function. Quantitative analyses of \(\beta\)-\(^{11}\)C-L-DOPA, \(^{18}\)F-FDOPA, and \(^{11}\)C-6Men\(\text{Tyr}\) were conducted by a multitime graphical analysis \((MTGA)\) \((10,18,19)\) applying the time–activity curve in the cerebellum as an input function.

The correlation analyses against MC-I activity were conducted using right and left striatal regions separately from 1 monkey. Because some PET measurements and quantitative analyses failed, the final number of monkeys used in the analyses was 4 for control and 4 for MPTP-treated.

**Statistical Analysis**

Results are expressed as mean ± SD. Comparisons between conditions were performed using the paired, 2-tailed Student t test, and correlations were tested by a single regression analysis using the Kaleidagraph program (Synergy Software). A probability level of less than 5% \((P < 0.05)\) was considered to indicate statistical significance.

**RESULTS**

Figure 1 represents the typical PET images of a normal monkey and an MPTP-treated monkey. As shown in the top panels, PET measurements of DAT availability \((\text{\(^{11}\)C-\(\beta\)-CFT [Fig. 1A]) and dopamine synthesis (\(\beta\)-\(^{11}\)C-L-DOPA [Fig. 1B], \(^{18}\)F-FDOPA [Fig. 1C], and \(^{11}\)C-6Men\(\text{Tyr}\) [Fig. 1D]) clearly imaged the striatum of the normal monkey brain. The cerebral uptake of \(^{18}\)F-BCPP-EF in normal monkeys showed homogeneous and symmetric patterns in the 2 hemispheres and enabled discrimination of the cortical and basal ganglion regions (Fig. 1E).

Presynaptic DAT availability in the striatum, which was assessed using \(^{11}\)C-\(\beta\)-CFT, was markedly lower in MPTP monkeys than in normal animals (bottom panels of Fig. 1A). In addition, dopamine synthesis in the striatum, as evaluated using \(\beta\)-\(^{11}\)C-L-DOPA (Fig. 1B), \(^{18}\)F-FDOPA (Fig. 1C), and \(^{11}\)C-6Men\(\text{Tyr}\) (Fig. 1D), was also significantly lower in MPTP monkeys than in normal animals (66.7%, 83.1%, and 46.6%, respectively, of the corresponding normal).

The metabolic rates of \(^{18}\)F-BCPP-EF in plasma were rapid; only 52%, 19%, and 10% of nonmetabolites were detected 10, 30, and 60 min, respectively, after the injection without formation of any lipophilic...
metabolites. The striatal total distribution volume (Vt) of 18F-BCPP-EF was significantly lower in MPTP monkeys than in normal animals (71.7% that of the corresponding normal) (Fig. 1E). The plot of the Vt of 18F-BCPP-EF against the BPND of 11C-6MeTyr revealed that dopamine synthesis rates assessed using 11C-6MeTyr provided greater correlation coefficients than those assessed using 18F-FDOPA, 11C-L-DOPA and 18F-FDOPA (Fig. 2B). As shown in Figure 3, the correlation analyses between MPTP-induced percentage reductions of dopamine parameters and percentage reduction of 18F-BCPP-EF in the striatum of the living monkey brain revealed that dopamine synthesis rates assessed using 11C-6MeTyr were 0.974, P < 0.001; 11C-L-DOPA (R² = 0.634, P < 0.01), and 18F-FDOPA (R² = 0.844, P < 0.001) were a much better correlation that that of DAT measured using 11C-6MeTyr (R² = 0.451, P < 0.05).

DISCUSSION

The present study demonstrated the ability of 18F-BCPP-EF as a PET probe to detect MPTP-induced changes in MC-I activity in parallel with assessments of presynaptic dopamine parameters. The most remarkable results of the present study was that 18F-BCPP-EF detected MPTP-induced impairments in MC-I activity in the living brain of PD model monkeys using PET. MC-I is the first and rate-limiting step of overall respiratory activity and oxidative phosphorylation from MC-I to MC-V, and impaired electron transport and oxidative phosphorylation due to MC-I deficiency may account for the neuronal cell death in PD. A small reduction in MC-I activity of approximately 25% is sufficient to decrease adenosine triphosphate synthesis and mitochondrial respiration in brain mitochondria. MC-I dysfunction in PD patients has been identified in mitochondria obtained from postmortem brains and biopsy tissue samples (2,3); however, MC-I dysfunction in the living brain had not been demonstrated noninvasively before the present study.

As we have reported previously, 18F-BMS-747158-02 developed for myocardial imaging (20) is not suitable for MC-I imaging in the living brain because of its high affinity and high lipophilicity (6). 18F-fluorobenzyl triphenylphosphonium cation was reported to bind to mitochondria, reflecting their membrane potential with voltage-dependency (21); however, this PET probe has not been applied for imaging of mitochondrial function in the living brain. In contrast, our previous reports demonstrated the first PET probe as 18F-BCPP-EF for MC-I imaging in the living brain (6–8).

Furthermore, correlations were observed between MC-I activity and DAT availability and also between MC-I activity and dopamine synthesis in the striatum. We previously confirmed that the degeneration of nigrostriatal dopamine neurons was induced by the intravenous administration of MPTP to monkeys over a period of several months and resulted in impaired presynaptic dopamine parameters, as determined by DAT availability, dopamine synthesis, and tyrosine hydroxylase immunoreactivity in the brain (9,10). A previous study reported that although plotting the striatal MTGA Ki values of 11C-6MeTyr, β-11C-L-DOPA, and 18F-FDOPA against the BPND of 11C-6MeTyr provided greater correlation coefficients than those assessed using 18F-FDOPA and 11C-L-DOPA (10). The present study also demonstrated that the sensitive PET probe for the detection of impaired MC-I activity was 11C-6MeTyr. The different abilities of β-11C-L-DOPA, 18F-FDOPA, and 11C-L-DOPA to detect presynaptic dopamine damage may be attributed to their different metabolic profiles on the basis of catechol-β-11C-L-DOPA and 18F-FDOPA and noncatechol-type (11C-6MeTyr) structures (10).

In the nigrostriatal regions containing high dopaminergic neurons, PET imaging using dopamine-specific probes is useful for diagnostic and treatment efficacy assessment of PD patients (5). In
contrast, several recent studies suggested that other monoaminergic neurons, such as serotonin and noradrenaline, may be affected in non-dopaminergic brain regions of PD patients (22,23), indicating that multiparametric PET imaging with PET probes for other kinds of monoamines or with PET probes for a more general neurophysiological index is required. Our preliminary PET study using 18F-BCPP-EF has revealed that MC-I activity outside nigrostriatal regions is impaired in the brain of MPTP-treated monkey (H. Tsukada and M. Kanazawa, unpublished data, March 2012).

CONCLUSION

The preliminary results of the present study suggest that 18F-BCPP-EF has potential as a PET probe for the quantitative imaging of neuronal degeneration as impaired MC-I activity in the living brains of PD model monkeys.

DISCLOSURE

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 USC section 1734. No potential conflict of interest relevant to this article was reported.

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REFERENCES

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