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Positron emission tomography with 4-[^{18}F]fluoro-L-*m*-tyrosine in MPTP-induced hemiparkinsonian monkeys

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PET imaging studies with 4-[^{18}F]fluoro-L-*m*-tyrosine (FMT) in normal macaca monkeys showed selective accumulations of radioactivity in the striatum with time. In monkeys rendered hemiparkinsonian by intracarotid infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), FMT uptake was eliminated in the lesioned striatum. FMT-PET studies were able to detect dopaminergic terminals in both normal and hemiparkinsonian monkeys, and clearly showed a reduction in aromatic L-amino acid decarboxylase (AAAD) activities in the MPTP-lesioned striatum. These results show that FMT is promising as a PET tracer for the evaluation of central dopaminergic systems in parkinsonism.

Key words: PET, Parkinson's disease, fluoro-L-*m*-tyrosine, MPTP, primates

INTRODUCTION

THE MOST PROMINENT NEUROCHEMICAL FEATURE of Parkinson's disease is the dopaminergic depletion in the nigrostriatal pathway. Approximately an 80% reduction in nigrostriatal dopamine content is thought to be necessary before the symptoms arise.[1]

PET scan with a dopaminergic probe such as 6-[^{18}F]fluoro-L-DOPA (FDOPA) is an useful technique to use in estimating nigrostriatal dopaminergic function in living brains. Several studies have been performed in normal human subjects,[2,3] patients suffering from idiopathic parkinsonism,[3–5] and non-human primates.[6,7] Furthermore, FDOPA-PET study has been reported as a method for detecting preclinical Parkinson's disease.[8]

However, recent lines of evidence strongly indicate disadvantages in the FDOPA-PET study. FDOPA is extensively metabolized in the periphery not only to [^{18}F]fluoro-dopamine by AAAD, but also to 3-*o*-methyl-6-fluoro-L-DOPA (3-OMFD)[9,10] by catechol-*o*-methyl transferase (COMT). Significant formation of 3-OMFD, which crosses the blood-brain-barrier bidirectionally, makes image contrast and biochemical interpretation of a FDOPA-PET study ambiguous and complicated.

To circumvent this problem, L-DOPA analogs that are not substrates for COMT but still maintain specificity toward AAAD[11] were sought. A fluorinated analog of *m*-tyrosine is proposed as such a substance.[11–15] 4-[^{18}F]Fluoro-L-*m*-tyrosine is a L-DOPA analog that is essentially involved in L-DOPA metabolic pathways but without 3-*o*-methylation or extensive peripheral metabolism. Recent FMT-PET studies reported improvement in the image contrast compared to that obtained with FDOPA.[12,15]

Discovery of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) provides a valuable tool to use in investigating the effects of nigrostiatal denervation and its relationship to parkinsonian symptoms in human subjects[16] and primates.[17] The infusion of MPTP into one internal carotid artery of primates damages dopaminergic cells of the substantia nigra pars compacta (SNpc) ipsilateral to the side of infusion with little or no apparent damage to the contralateral side, thus producing a unilateral parkinsonian or hemiparkinsonian syndrome.[18–20]

We demonstrate imaging PET studies with a new dopaminergic PET tracer, 4-[^{18}F]fluoro-L-*m*-tyrosine in normal and hemiparkinsonian model monkeys.

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MATERIALS AND METHODS

4-[^{18}F]Fluoro-L-*m*-tyrosine (FMT) was synthesized by an acetylhypofluorite method as described earlier.[21,22] The HPLC-purified product was determinded as more than 98% chemical pure.

Scanning was performed with a SHR2000 positron tomograph (Hamamatsu Photonics, Co. Ltd., Japan),[23] which has a reconstructed spatial resolution of 3.5 mm full width at half-maximum (FWHM) at the center of the image. The slice thickness is 4.5 mm FWHM, and seven equally spaced parallel planes with a 6.5 mm interval from center to center are obtained simultaneously. Attenuation correction was performed by means of a transmission scan with a ^{68}Ga source. Four monkeys (*Macaca fuscata*) weighing 6.3–9.0 kg were anesthetized with ketamine (20 mg/kg, i.m.) and catheterized in a femoral artery for blood samplings and in a vein for an injection of FMT. General anesthesia was maintained throughout the study with pentobarbital (10 mg/kg, i.v. every hour). For reproducible positioning, we developed a special animal chair equipped with ear bars and a head fixation device referred to as a stereotaxic device. The animal was fixed on the chair to slice parallel to the canthomeatal line. The laser beam on the PET scanner projected an external landmark of the monkey's head to confirm the place to slice.

FMT was administered intravenously (5–6 mCi; specific activity, 1–2 Ci/nmol) following carbidopa pretreatment (5 mg/kg, i.v., 60 min before FMT administration). Dynamic image aquisitions were performed for 2 hours immediately after tracer administration. The full study consisted of a sequence of twelve 2-min frames, nine 4-min frames and six 10-min frames. This procedure yielded dynamic information on tracer accumulations in brain tissues. Following the last dynamic frame, a static image acquisition was performed for 30 min. Changes in regional radioactivity with time were calculated for the striatum and cerebellum.

Arterial blood samples were collected as follows: every 15 sec for the 3rd min, then 5, 7, 10, 15, 20, 30, 40, 60, 100 and 160 min after the tracer injection. Plasma samples (300 μl) were separated by centrifugation at 1000 × G. The [^{18}F]radioactivity of each sample was measured with a well counter (Aloka Universal Scaler, model TDC-501).

After baseline FMT-PET studies, two monkeys received MPTP infusions and were scanned after MPTP treatment (between 2 and 5 months). MPTP administration was performed according to the method described in a previous report.[24] Under general anesthesia with ketamine (20 mg/kg, i.m.) and pentobarbital (10 mg/kg, i.v.), a dose of 0.2 mg/kg MPTP in 50 m*l* heparinized saline was infused directly into the right internal carotid artery over 20 min. The animals displayed persistent signs of left-sided flexed posture, bradykinesia, and no tremor, and developed a preference to use the right limbs and to turn spontaneously toward the right side. Apomorphine (0.1 mg/kg, i.m.) reversed these hemiparkinsonian syndromes and produced rotation in the direction contralateral to the side of infusion of MPTP. The parkinsonian signs and symptoms remained for at least 6 months. No behavior recovery was observed.

RESULTS

PET imaging studies in normal monkeys displayed selective accumulations of ^{18}F after FMT administration with time in the striatum. Bilateral striatums were clearly demarcated on the PET images (Fig. 1, left). After MPTP treatment, accumulation of ^{18}F in the infused side of the striatum was eliminated, compared with the uninfused side (Fig. 1, right). Low nonspecific binding and little apparent defluorination of FMT were found, as there was little accumulation of radioactivity in bone structures.

The decay-corrected accumulation time course in normal monkeys showed rapid accumulation at the striatal structure (Fig. 2A). The ratio of radioactivity in the striatum for FMT to that in the cerebellum increased with time. After MPTP infusion, uptake of radioactivity in the MPTP-lesioned striatum was reduced to the level of non-specific distribution in the cerebellum (Fig. 2B). The amount of ^{18}F in each region of interest was expressed as the average cpm/pixel within the region normalized to the number of mCi of FMT injected. The time course of radioactivity in the arterial plasma showed fast clearance of tracers from circulating plasma (Fig. 3).

Figure 4 shows the striatum/cerebellum accumulation ratio; the striatal activity over the period 120–150 min after administration of FMT divided by corresponding cerebellar activity. The mean striatum/cerebellum ratio in normal monkeys reached 3.2. In hemiparkinsonian monkeys, the lesioned-striatum to cerebellum ratio was 1.3; but on the other hand, the contralateral striatum to cerebellum ratio reached 3.9. MPTP infusion significantly reduced the accumulation of FMT in the striatum.

DISCUSSION

The cerebral uptake of ^{18}F after FMT administration was stereoselective and showed a persistent regional accumulation in the striatum. This selective retention of radioactivity is in agreement with the high concentration of dopaminergic terminals in the striatal region.

m-Tyrosine is thought to cross the blood brain barrier, not to be the substrate for COMT,[25] and to be decarboxylated[26] to give *m*-tyramine which could act as a false dopamine neurotransmitter.[27] FMT was probably decarboxylated to 4-[^{18}F]fluoro-L-*m*-tyramine by AAAD, similar to that reported in the case of *m*-tyrosine.[12,28] HPLC analysis of rat striatal radioactivity revealed that at 30 min after FMT injection, most of the activity was associated with 4-fluoro-3-hydroxyphenylacetic acid (FPAC).[12] As the transformation of FMT to FPAC essentially depends

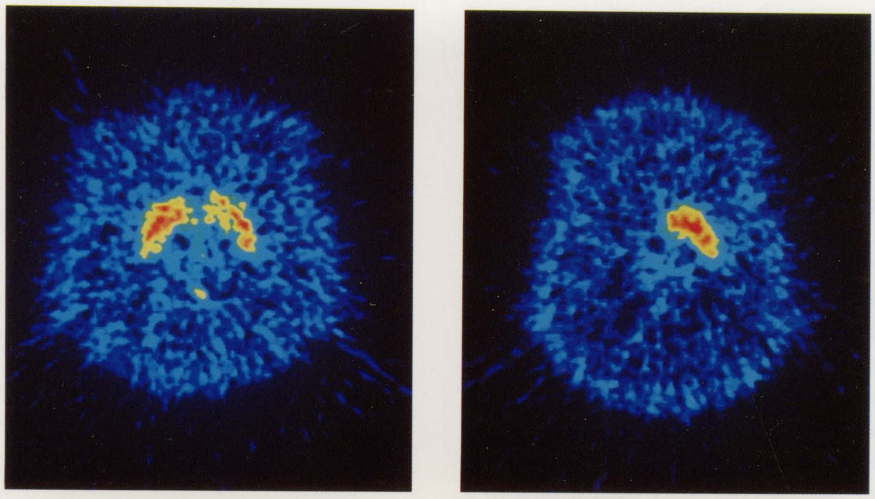

Fig. 1 Characteristic PET images showing uptakes of FMT in the striatum of macaca monkey brain. The left and right is the scan before and after intracarotid MPTP infusion, respectively. The images were obtained from 120 to 150 min after FMT injection.

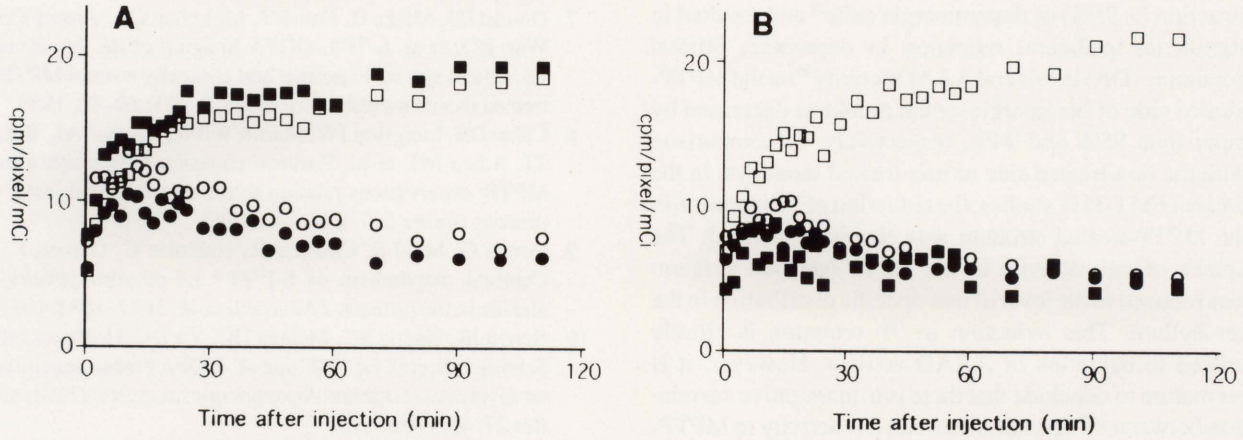

Fig. 2 Decay-corrected accumulation time course in normal monkey (A); □: Lt. striatum, ■: Rt. striatum, ○: Lt. cerebellum, ●: Rt. cerebellum, and in MPTP-induced hemiparkinson monkey (B); □: Lt. striatum, ■: Rt. striatum (treated side), ○: Lt. cerebellum, ●: Rt. cerebellum.

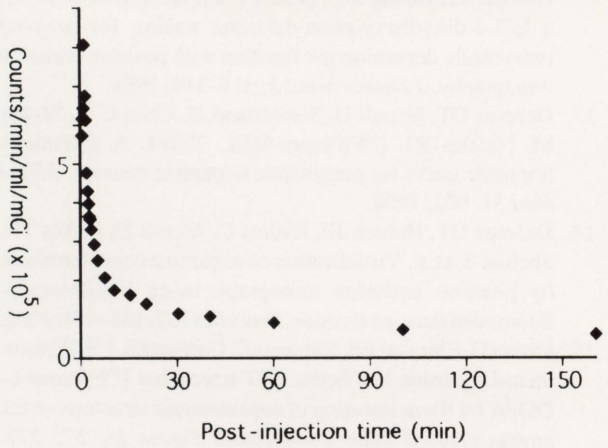

Fig. 3 Plasma time activity curve for [^{18}F]radioactivity in normal monkey.

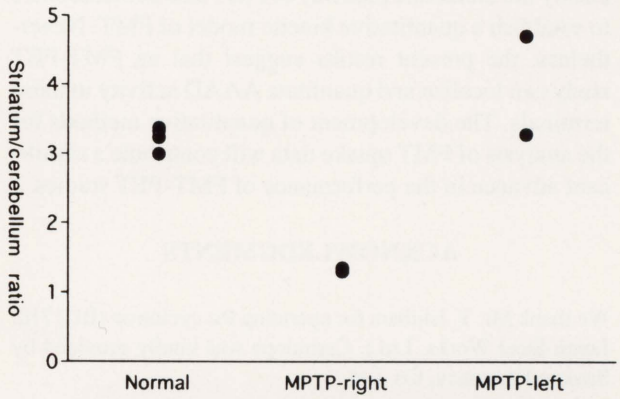

Fig. 4 Striatal activity determinded from FMT-PET scans. Each point represents a value for striatal activity divided by background (cerebellar) activity. Results for MPTP-lesioned striatum are significantly less than contralateral side.

on AAAD at nerve terminals, specific localization of ^{18}F in FMT-PET closely reflects AAAD activity in the striatum.

The decay-corrected accumulation time course curves showed differences between the pharmacokinetic behavior in the striatum and the rest of the brain. The striatal distribution of FMT in normal monkeys was relatively rapid, and increased lineally over time at 2 hours, compared to the gradual decrease in non-specific accumulation in the cerebellum. Despite the fact that the clearance of tracers from circulating plasma was fast, the accumulation of ^{18}F in the striatum gradually rose. As sulfo-conjugated 4-fluoro-3-hydroxyphenyl-ethylamine (FMA), which is major peripheral metabolite of FMT,[12] is not likely to cross the blood-brain-barrier, the specific-to-nonspecific accumulation ratio increases. The striatum/cerebellum ratio of radioactivity more than tripled in 2 hours. In contrast to FDOPA-PET,[7,8,10] significant and specific time dependent accumulation of ^{18}F activity occurred after FMT administration.

MPTP treatment produced almost complete destruction (> 95%) of dopaminergic cells[20] and resulted in significant ipsilateral reduction in dopamine. Striatal dopamine (DA) levels and AAAD activity[30] on the MPTP-treated side of hemiparkinsonian monkeys decreased by more than 95% and 94%, respectively, in comparison with the non-treated side or non-treated monkeys. In the present FMT-PET studies, the reduction of ^{18}F retention in the MPTP-treated striatum was clearly visualized. The uptake of radioactivity in the MPTP-lesioned striatum was reduced to the level of non-specific distribution in the cerebellum. This reduction in ^{18}F retention is closely related to depletion of AAAD activity. However, it is premature to conclude that there is a quantitative correlation between ^{18}F retention and AAAD activity in MPTP-hemiparkinsonian monkey without metabolic analysis.

We showed the capability of PET employing FMT to visualize nigrostriatal dopaminergic functions *in vivo*, and also revealed a significant reduction in ^{18}F accumulation in the MPTP-lesioned striatum. It is mandatory to clarify the metabolic pathway of FMT and its metabolites to establish a quantitative kinetic model of FMT. Nevertheless, the present results suggest that an FMT-PET study can localize and quantitate AAAD activity in these terminals. The development of quantitative methods for the analysis of FMT uptake data will contribute a significant advance in the performance of FMT-PET studies.

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