Neurosci Bull June 1, 2014, 30(3): 409–416. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1374-3

·Original Article·

Progressive loss of striatal dopamine terminals in MPTP-induced acute parkinsonism in cynomolgus monkeys using vesicular monoamine transporter type 2 PET imaging ([^{18}F]AV-133)

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ABSTRACT

The 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP)-induced parkinsonism model, particularly in non-human primates, remains the gold-standard for studying the pathogenesis and assessing novel therapies for Parkinson's disease. However, whether the loss of dopaminergic neurons in this model is progressive remains controversial, mostly due to the lack of objective *in vivo* assessment of changes in the integrity of these neurons. In the present study, parkinsonism was induced in cynomolgus monkeys by intravenous administration of MPTP (0.2 mg/kg) for 15 days; stable parkinsonism developed over 90 days, when the symptoms were stable. Noninvasive positron emission tomographic neuroimaging of vesicular monoamine transporter 2 with 9-[^{18}F] fluoropropyl-(+)-dihydrotetrabenazine ([^{18}F]AV-133) was used before, and 15 and 90 days after the beginning of acute MPTP treatment. The imaging showed evident progressive loss of striatal uptake of [^{18}F]AV-133. The dopaminergic denervation severity had a significant linear correlation with the clinical rating scores and the bradykinesia subscores. These findings demonstrated that [^{18}F]AV-133 PET imaging is a useful tool to noninvasively evaluate the evolution of monoaminergic terminal loss in a monkey model of MPTP-induced parkinsonism.

Keywords: Parkinson's disease; non-human primate; [^{18}F]AV-133; VMAT2; positron emission tomography

INTRODUCTION

Parkinson's disease (PD) is one of the most common neurodegenerative disorders and affects ~2% of the world's population aged over 65[1]. The cardinal clinical symptoms are bradykinesia, tremor, rigidity, and postural instability with the pathological characteristic of evolutional nigrostriatal dopaminergic neurodegeneration[2, 3]. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin that induces parkinsonism in both humans[4] and non-human primates[5] with the cognitive, biochemical, histological and classical behavioral changes that occur in PD[6]. Therefore, MPTP-lesioned monkeys have been used to evaluate the efficacy of anti-parkinsonian therapy. However, whether the acute MPTP-induced loss of dopaminergic neurons is a progressive process remains controversial, so a biomarker

that can non-invasively monitor this change longitudinally is critical.

Increasing evidence has suggested that single photon emission computed tomography (SPECT) and positron emission tomography (PET) imaging can be used to sensitively and objectively evaluate the integrity of the nigrostriatal dopaminergic system and may be useful tools for providing diagnostic information on PD[7-10]. There are numerous SPECT and PET imaging tracers for monitoring the integrity of dopaminergic neurons. 6-[^{18}F]fluoro-DOPA has been used to evaluate dopamine synthesis[11], ^{123}I-labeled 2β-carbomethoxy-3β-(4-[^{123}I]iodophenyl)tropane targets the membrane dopamine transporter[12, 13], [^{11}C]-raclopride[14] specifically binds to dopamine receptors, and [^{18}F]AV-133 targets the vesicular monoamine transporter type 2 (VMAT2). VMAT2, located on vesicle membranes in monoaminergic neurons, carries out the reuptake and packaging of monoamines (including dopamine, norepinephrine, and serotonin) into vesicles.

Increasing numbers of studies have found that the former three biomarkers are susceptible to disease-related compensation and dopaminergic drug-induced regulation, limiting their utility for accurately quantifying the lesion severity in PD. VMAT2-binding sites are not readily affected by dopaminergic regulation. Further, their density correlates well with the number of nigrostriatal dopaminergic neurons[15-17]. Thus, neuroimaging with markers targeting VMAT2 may be a more reliable and sensitive tool to monitor the progression of dopaminergic neuronal degeneration. Previous immunochemical analysis of VMAT2 showed that dopaminergic terminals are responsible for >95% of VMAT2 expression[18]. Multi-tracer PET imaging in a monkey model of PD induced by chronic MPTP treatment at a low dose shows progressive nigrostriatal dopaminergic neurodegeneration, and this results in a reduced storage capacity of VMAT2 and decreased uptake of [^{11}C]DTBZ in the nigrostriatal system. The level of VMAT2 staining with [^{3}H]DTBZ in MPTP-treated animals is reduced in tyrosine hydroxylase-positive neurons compared with that in controls[19], suggesting that VMAT2 binding sites are proportionally associated with the presence of functional dopaminergic neurons. Imaging VMAT2 is thus regarded as an effective tool to follow the degeneration of dopaminergic terminals. 9-[^{18}F]fluoropropyl-(+)-dihydrotetrabenazine ([^{18}F]AV-133) is a novel ^{18}F-labeled tetrabenazine derivative that selectively binds to VMAT2 with high affinity[20, 21]. [^{18}F]AV-133 PET studies in an MPTP-lesioned PD mouse model indicate that the specific uptake ratio (SUr) of [^{18}F]AV-133 declines significantly in the striatum. The imaging results correlate well with the results of immunohistochemical studies of tyrosine hydroxylase[22]. Moreover, preliminary clinical studies clearly demonstrate that [^{18}F]AV-133 sensitively detects VMAT2 reduction in PD patients, supporting [^{18}F]AV-133 as a potential tool to identify presymptomatic patients with nigrostriatal movement disorders[23].

So far, no study has used [^{18}F]AV-133 as a biomarker to evaluate parkinsonism in non-human primates, particularly in the model using the acute intravenous infusion of MPTP which has been extensively used for the preclinical evaluation of anti-parkinsonian drugs. The current study was designed to investigate the utility of [^{18}F]AV-133 as a biomarker for assessing the longitudinal loss of VMAT2 function and dopaminergic terminal degeneration in monkeys with acute MPTP-induced parkinsonism.

MATERIALS AND METHODS

Animals

Nine 10–15-year-old cynomolgus monkeys (5.2–8.0 kg, 2 females and 7 males) were purchased from Grandforest Co. (Nanning, China), a local primate-breeding company. All the animals were healthy, without any physical injury. For MPTP administration, the animals were anesthetized with a mixture of 3% isoflurane and 97% O_2 prior to treatment, and a low level of isoflurane (1%) was continued for maintenance. MPTP was injected intravenously as MPTP-HCl (Sigma Aldrich, St. Louis, MO) diluted in sterile saline at 0.2 mg/kg. Injection was performed daily for 15 days. No animal was euthanized in this study.

Ethics Statement

All animals were housed with a 12:12 h light/dark cycle at the facility of Wincon TheraCells Biotechnologies Co., Ltd., which is certified by and meets the guidelines of the Council on Accreditation of the Association for Assessment and Accreditation of Laboratory Animals Care (International). The ambient temperature was 24 ± 2 °C, and humidity was 65 ± 4%. RO (Reverses Osmosis) water was available

ad libitum. Fresh fruit and vegetables were supplied twice daily. This study was approved by the Institutional Animal Care and Utilization Committee of Wincon TheraCells Biotechnologies (Permit Number: W00019).

Radiosynthesis of [^{18}F]AV-133

Radiosynthesis of [^{18}F]AV-133 was carried out on a custom-made automated radiosynthesis apparatus according to a previously described method[24]. The mesylate precursor of [^{18}F]AV-133 was nucleophilic-substituted by [^{18}F]F$^-$ under the Kryptofix 222 catalyst. After [^{18}F] fluorination, the crude [^{18}F] AV-133 was purified *via* a solid-phase extraction column (Oasis HLB 3mL cartridge, Waters, Milford, CT). The radiosynthesis yield of [^{18}F]AV-133 was 45–50% (decay-corrected) at 350–500 mCi with radiochemical purity >95%. The mean specific radioactivity was 1000 Ci/mmol.

PET Image Acquisition and Analysis

Each monkey underwent three [^{18}F]AV-133 PET scans at different times. The first scan was prior to MPTP infusion, and the second and third scans were performed at 15 days (immediately after MPTP cessation; acute phase) and at 90 days (chronic parkinsonism phase) after MPTP injection. The PET system used was a Biograph Sensation 16HR (Siemens/CTI, Knoxville, TN). The PET images were attenuation-corrected using low-dose helical CT. The scan protocol for CT was as follows: peak voltage 120 kV, 420 mA, pitch 6 mm, and collimator 0.75 mm. Each monkey was anesthetized with ketamine (15 mg/kg) *via* intramuscular injection, and then injected with ketamine (7.5 mg/kg) every 30 min. [^{18}F]AV-133 in 1.0 mL sterile 10% ethanol saline was injected as a bolus into the femoral vein, and the cannula was immediately flushed with 10 mL saline. The total radioactivity injected was 0.2 mCi/kg. One 10-min frame of PET emission data from 30–40 min post-injection was used to estimate the [^{18}F]AV-133 binding. A transient equilibrium was achieved for [^{18}F]AV-133 from 30 min after intravenous injection according to our preliminary study[25].

The data were reconstructed using a 3D iterative algorithm after correction for randomness, scatter, and attenuation. All PET scans were analyzed simultaneously following the same protocol. Because of the negligible density of VMAT2 in the cerebellum, this region was used as a nonspecific reference. Two irregular regions of interest encompassing the whole right and left striatum were drawn on four consecutive slices through the striatum, and the background was readily identified and delineated on the cerebellum guided by detailed CT images and with reference to a stereotaxic brain atlas[26, 27]. The results are expressed as SUr, calculated as: (mean striatal uptake − mean cerebellar uptake)/mean cerebellar uptake[22], and dopaminergic denervation severity (DS) after MPTP treatment, calculated from SUr: ($SUr_{baseline}$ − $SUr_{lesioned}$)/ $SUr_{baseline}$[28, 29].

Clinical Rating Scores

The behavioral changes were observed and quantified twice a week on a previously-validated parkinsonian monkey clinical rating scale[30, 31]. Animal behaviors in testing cages were recorded by a videotape system. The scale rated nine items: bradykinesia (0–5), posture (0–2), rigidity (0–2), gait (0–5), balance (0–2), resting tremor on each side (0–3 for each side), gross motor skill (0–4) for each upper limb, gross motor skill (0–4) for each lower limb, and defense reaction (0–2). The minimum score 0 corresponds to normal, and the maximum total score 32 corresponds to severe disability. The scores were rated by both an experienced neurologist and a technician who were blinded to the study protocol.

Statistical Analysis

Comparisons of group characteristics were performed with paired-sample *t*-tests. Correlations between the clinical data and PET imaging results were evaluated using nonparametric Pearson analysis. P <0.05 was defined as statistically significant. Data are presented as mean ± SD.

RESULTS

PET Images

The images from PET scans at different time points (Fig. 1) revealed a progressive reduction of striatal [^{18}F]AV-133 uptake after MPTP lesioning. Before MPTP intoxication, [^{18}F]AV-133 had symmetrical uptake and retention in the bilateral striatum. The right and left striatal SUrs were 1.59 ± 0.48 and 1.63 ± 0.48 (n = 9), respectively (Fig. 2). At 15 days (cessation of MPTP infusion), the right and left striatal SUrs were decreased to 1.01 ± 0.35 (t = 2.930, P = 0.019) and 1.04 ± 0.33 (t = 2.985, P = 0.017) (n = 9) (Fig. 2) and the partial striatal DS values were 0.36 ± 0.30 (t = 3.475,

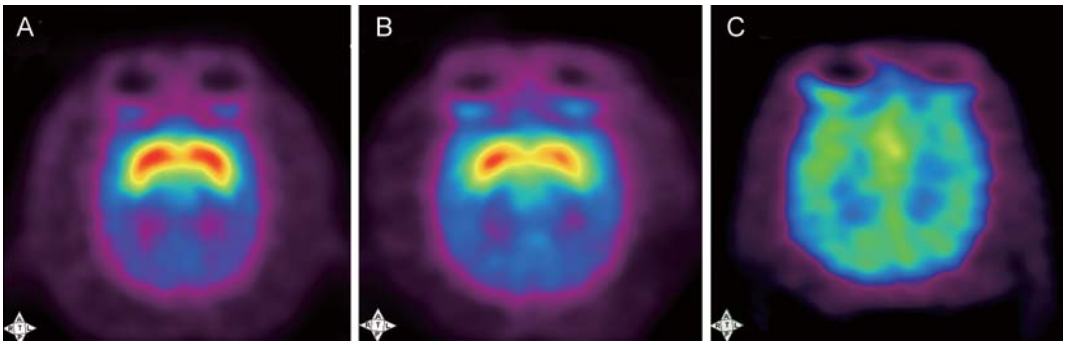

Fig. 1. Representative PET images of [^{18}F]AV-133 in an MPTP-lesioned monkey. A: Prior to MPTP treatment (baseline), B: 15 days after the first MPTP treatment (acute phase), C: 90 days after the first MPTP treatment (chronic parkinsonism phase). Images show transverse slices at the level of the striatum.

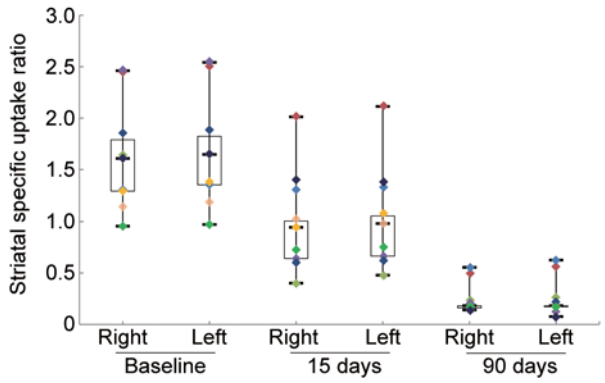

Fig. 2. Striatal specific uptake ratio (SUr) of [^{18}F]AV-133 prior to MPTP treatment (baseline), 15 days after the first MPTP injection (acute phase), and 90 days after the first MPTP injection (chronic parkinsonism phase). The values of striatal SUr (mean ± SD) were 1.59 ± 0.48 (right side) and 1.63 ± 0.48 (left side) for baseline, 1.01 ± 0.35 (right side) and 1.04 ± 0.33 (left side) for day 15, and 0.26 ± 0.13 (right side) and 0.24 ± 0.16 (left side) for day 90 (n = 9 monkeys, colored symbols in the boxplot).

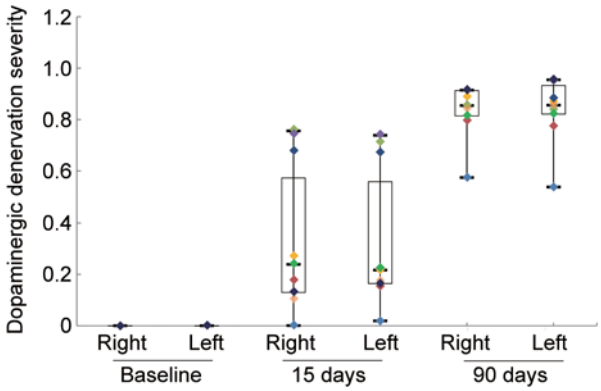

Fig. 3. Dopaminergic denervation severity (DS) of [^{18}F]AV-133 prior to MPTP treatment (baseline), 15 days after the first MPTP injection (acute phase), and 90 days after the first MPTP injection (chronic parkinsonism phase). The DS values (mean ± SD) were 0.00 ± 0.00 for baseline, 0.36 ± 0.30 (right side) and 0.34 ± 0.28 (left side) for day 15, and 0.84 ± 0.11 (right side) and 0.84 ± 0.12 (left side) for day 90 (n = 9 monkeys, colored symbols in the boxplot).

P = 0.008) and 0.34 ± 0.28 (t = 3.662, P = 0.006) for the right and left sides, respectively (n = 9) (Fig. 3). At 90 days, when the parkinsonian symptoms were stable, a further reduction in striatal uptake was found. The right and left striatal SUrs were 0.26 ± 0.13 (t = 8.179, P = 0.000) and 0.24 ± 0.16 (t = 7.828, P = 0.000) (n = 9) (Fig. 2), and the right and left DSs were 0.84 ± 0.11 (t = 20.534, P = 0.000) and 0.84 ± 0.12 (t = 20.773, P = 0.000) (n = 9), respectively (Fig. 3). The values of [^{18}F]AV-133 uptake in the striatum did not overlap between baseline and 90 days (Fig. 2).

Correlation between [^{18}F]AV-133 Uptake and Clinical Rating Scores

At 15 days, the mean total clinical rating score was 14.56 ± 6.76. The increased DSs in the right side striatum were accompanied by significantly increased total clinical rating scores and bradykinesia subscores. Also, significant correlations were found between SUrs of right side striatum and clinical rating scores and bradykinesia subscores. However, no significant correlation was found between the rigidity subscores and SUrs or DSs of the right side

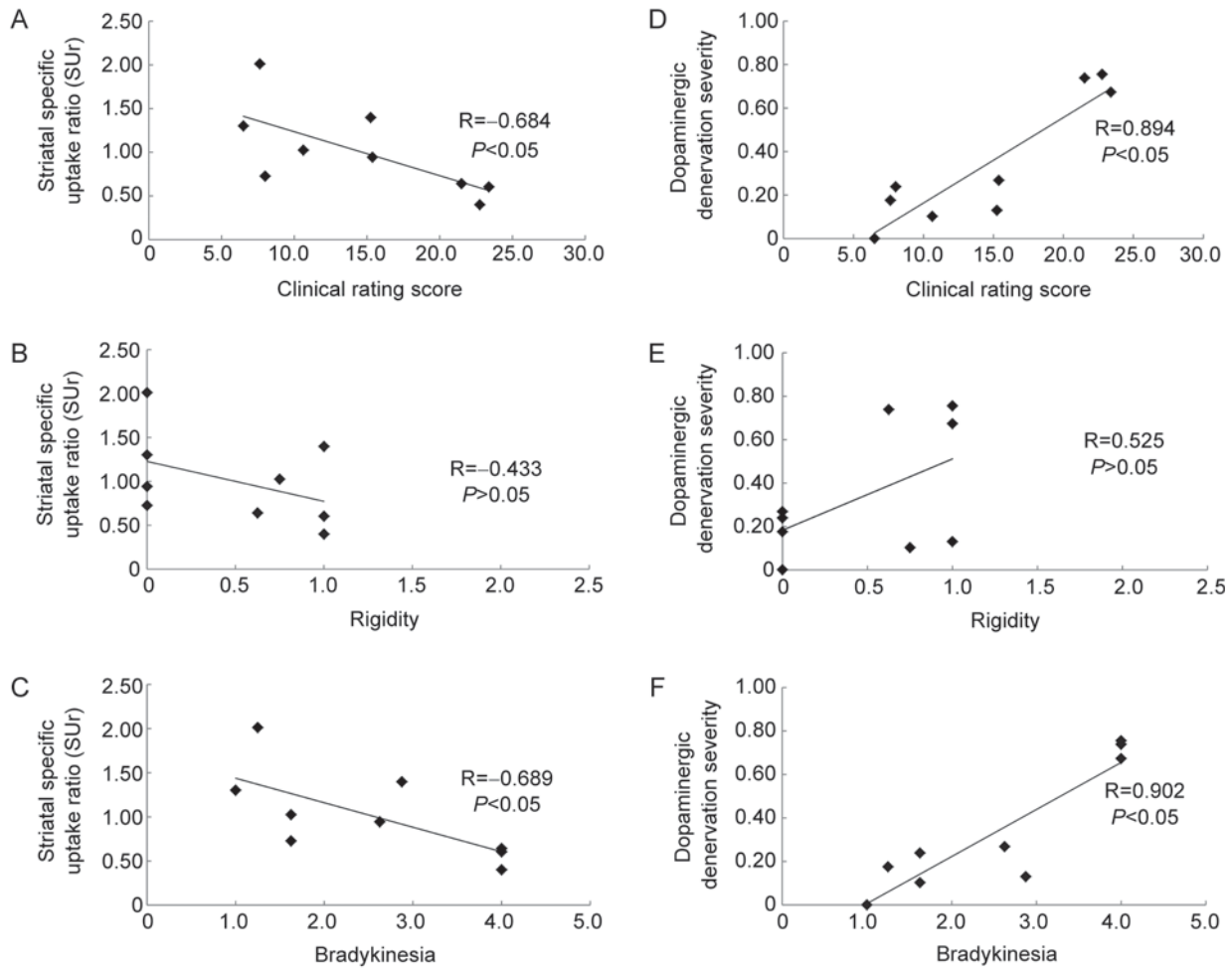

Fig. 4. Correlation of clinical rating results with [^{18}F]AV-133 PET imaging results at day 15 after MPTP injection. Significant negative correlations were found between SUrs and clinical rating scores (A) and bradykinesia (C) (*n* = 9). Significant positive correlations were also found between DSs and clinical rating scores (D) and bradykinesia (F) (*n* = 9). There was no correlation between rigidity and SUr (B)/DS (E) (*n* = 9).

striatum (Fig. 4).

At 90 days, when stable experimental parkinsonism was developed, the mean total clinical rating score reached 22.19 ± 5.34. The clinical rating scores and subscores for rigidity and bradykinesia were all positively correlated with DSs (right side), and significant negative trends were found for SUrs (right side) (Fig. 5).

DISCUSSION

The MPTP-lesioned monkeys showed degeneration of nigrostriatal dopaminergic neurons, as seen in PD patients. The present data confirmed that [^{18}F]AV-133 PET imaging is a useful tool for assessing parkinsonism in a monkey model of PD, and demonstrated a progressive reduction of VMAT2 expression in the MPTP-treated monkeys. Our PET studies showed that [^{18}F]AV-133 had high specific uptake in the bilateral striatum, and the PET images provided significant signal-to-noise ratios. The striatal [^{18}F] AV-133 uptake assessed here is consistent with previous studies of the primate brain[21, 25, 32]. MPTP administration induced striatal dopaminergic denervation, and resulted in a decrease in VMAT2 expression (Fig. 2). After 15 days of MPTP exposure, the SUrs of right and left side striatum

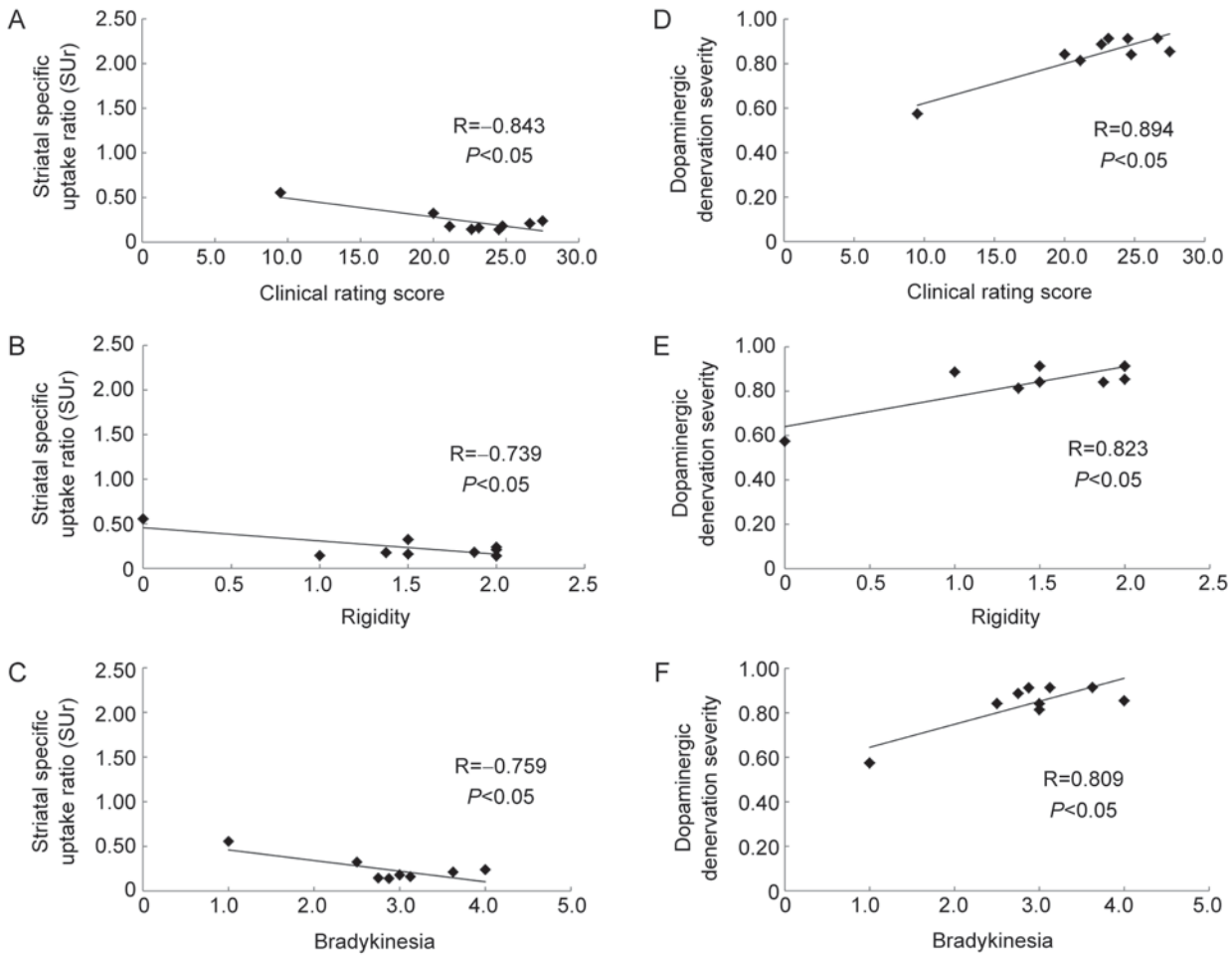

Fig. 5. Correlation of clinical rating results with [^{18}F]AV-133 PET imaging results at day 90 after MPTP injection. Significant negative correlations were found between SUrs and clinical rating scores (A), rigidity (B), and bradykinesia (C) (*n* = 9). Significant positive correlations were found between DSs and clinical rating scores (D), rigidity (E), and bradykinesia (F) (*n* = 9).

were decreased by 36% and 34% compared to the baseline respectively. When stable parkinsonism developed, as demonstrated by high clinical rating scale scores, the PET imaging by [^{18}F]AV-133 in the striatum showed significantly lower VMAT2 density; bilateral striatal SUrs for [^{18}F]AV-133 were only 16% of baseline. Okamura *et al.* found the greatest VMAT2 reduction in the posterior putamen (−81%) of PD patients, next in the anterior putamen (−70%), and then in the caudate nucleus (−48%)[23], which is consistent with our results. In 2010, [^{11}C]DTBZ PET imaging was used to evaluate the model of chronic MPTP-induced parkinsonism in monkeys induced by a low dose of MPTP administered over several months[33]. Different from the previous study, we used a model of acute infusion of MPTP (0.2 mg/kg/day for 15 days), which is the standard model for anti-parkinsonian drug studies. In the Obeso study, the comparisons were made among four groups, controls, asymptomatic, recovered, and stable parkinsonism[34]. In the present study, changes of [^{18}F]AV-133 uptake in the striatum between three stages were analyzed based on self-comparison. Because of individual differences in striatal uptake of [^{18}F]AV-133 in the healthy state, the self-comparison of SUrs was more reliable for evaluating nigrostriatal dopaminergic neuronal degeneration.

Bezard *et al.* reported that after receiving daily injections of MPTP (0.2 mg/kg, i.v.) for 15.5 ± 1.1 days,

monkeys appeared to have parkinsonian symptoms, and reached a score >8 on their clinical rating scale[6]. Because of the different clinical rating scales used, in our study the clinical rating score was 14.56 ± 6.76 after 15 doses of MPTP. Compared with the striatal SUrs for [^{18}F]AV-133, the DSs in the striatum showed a strong linear correlation with the clinical rating scores on days 15 and 90 (Fig. 4A, D; Fig. 5A, D). This indicated that the DS is a better index to evaluate VMAT2 loss than striatal SUr. Our PET results also showed that the subscores for bradykinesia were negatively correlated with SUrs and positively correlated with DSs in the MPTP-lesioned monkeys at days 15 and 90 (Fig. 4C, F; Fig. 5C, F). This is consistent with previous studies on PD patients[23]. On day 15, no significant correlation was found between the subscores for rigidity and SUrs or DSs, suggesting a weak association between this symptom and [^{18}F]AV-133 uptake in monkeys with mild parkinsonism. However, on day 90, when the parkinsonian signs were marked, there was a significant correlation between the rigidity subscores and the PET imaging results. Additional studies with a larger sample size and a high PET scan frequency are warranted to fully determine the usefulness and application of [^{18}F]AV-133 PET imaging.

In conclusion, a rapid and progressive VMAT2 reduction was detected in the striatum with [^{18}F]AV-133 PET imaging in acute MPTP-lesioned monkeys. These findings indicated that PET with [^{18}F]AV-133 is a sensitive marker to follow the progress of dopamine depletion in MPTP-induced acute parkinsonism in non-human primates.

ACKNOWLEDGEMENTS

This work was supported by grants from the Ministry of Science and Technology of China (2012AA02A514, 2011CB504101), a Science Research and Technology Development Project from the Bureau of Science and Technology of Nanning, China (201001010A), the China Postdoctoral Science Foundation (20110490452), the Beijing Postdoctoral Research Activity Foundation (2011ZZ-07), and the Open Fund for the Key Laboratory on Neurodegenerative Disease of the Ministry of Education (2012SJBX01). We thank Avid Radiopharmaceuticals, Inc. for kindly providing the mesylate precursor. We thank C. Qin, S. Huang and Y. Qi for technical assistance with the PET scans, and Q Li, Y Gan and M Yang for their valuable assistance in the monkey experiments.

Received date: 2013-04-09; Accepted date: 2013-07-01

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