

Short communication

## Dopamine transporter loss and clinical changes in MPTP-lesioned primates

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### Abstract

Single photon emission computed tomography (SPECT) and the dopamine (DA) transporter tracer, 2 beta-carboxymethoxy-3 beta-(4-iodophenyl)tropane ( $[^{123}\text{I}]\beta\text{-CIT}$ ), were used to determine DA transporter density in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys with varying degrees of parkinsonism. The clinical stage of parkinsonism corresponded to SPECT measures of striatal DA transporter density suggesting that more severe parkinsonism was associated with a greater degree of dopaminergic terminal degeneration. These findings are similar to those reported earlier using positron emission tomography (PET) and the DA metabolism tracer, 6- $[^{18}\text{F}]$ fluoro-L-m-tyrosine (FMT), indicating that both are good methods for evaluating nigrostriatal degeneration in MPTP primate models. © 1999 Elsevier Science B.V. All rights reserved.

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2 $\beta$ -Carboxymethoxy-3 $\beta$ -(4-iodophenyl)tropane ( $[^{123}\text{I}]\beta\text{-CIT}$ ) has been used to measure dopamine (DA) transporter density in both human and nonhuman primates using single photon computed tomography (SPECT) [4,6,11,15,17,22,24]. SPECT studies in nonhuman primates have demonstrated that  $[^{123}\text{I}]\beta\text{-CIT}$  binding is highest in the striatum and hypothalamic/midbrain area. Accumulation of the tracer in the striatum was displaced by a selective DA uptake inhibitor (GBR 12909), but not by a selective serotonin (5-HT) uptake inhibitor (citalopram) [11]. The inverse was true in the hypothalamic/midbrain area, where the density of 5-HT transporters is greater than DA transporters. Thus, while  $\beta\text{-CIT}$  has a high affinity for both the DA and 5-HT transporters [3,19], the high density of DA transporters relative to 5-HT transporters in the striatum makes striatal tracer accumulation a specific indicator of striatal DA transporter density in this region [11]. The cerebellum, which has a low density of DA and 5-HT transporters [1,9,12], shows low levels of  $[^{123}\text{I}]\beta\text{-CIT}$  accu-

mulation and is often used as an indicator of nonspecific DA transporter binding. The ratio of striatal to cerebellar (or occipital)  $[^{123}\text{I}]\beta\text{-CIT}$  activity uptake can be used as a measure of specific to nonspecific binding and is an adequate method for determining the relative density of DA transporters in the striatum due to the stability of regional binding of  $[^{123}\text{I}]\beta\text{-CIT}$  18 to 24 h after bolus injection [13]. Indeed, SPECT  $[^{123}\text{I}]\beta\text{-CIT}$  studies have shown a reduction in striatal DA transporters (as indicated by reduced striatal to cerebellar binding ratios) in PD patients [4], as well as an association between clinical signs and striatal binding ratios [17,21].

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces a selective lesion of the DA nigrostriatal pathway and a parkinsonian condition. MPTP-lesioned primates have been used extensively as a model of PD. Recently, we used a new MPTP-lesion model to produce varying levels of parkinsonism and found an association between the clinical stage of parkinsonism and positron emission tomography (PET) measures of L-aromatic amino acid decarboxylase (L-AADC) activity [5]. Here, we used SPECT and DOPASCAN<sup>®</sup> Injection ( $[^{123}\text{I}]\beta\text{-CIT}$ ) to evaluate striatal DA transporter density in 16 MPTP-treated

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monkeys with varying degrees of parkinsonism. A SPECT marker of clinical severity in MPTP-lesioned monkeys would be more easily implemented than PET, has potentially wide clinical applicability, and could be useful for evaluating novel treatments for PD.

Sixteen *Macaca mulatta* received unilateral intracarotid artery (ICA) infusions (4 ml/min) of 60 ml of saline containing 2.5–4.0 mg of MPTP-HCl producing a near complete lesion on the side of infusion (ipsilateral), and mild to moderate damage in the other (contralateral) hemisphere. This is in contrast to earlier studies in which lower unilateral ICA doses produced damage that was limited to one hemisphere [2]. Three of the animals received 1–12 additional i.v. doses of MPTP (0.3 mg/kg over a 2–7 week period) in order to produce more severe damage in the contralateral hemisphere [5]. Animals were cared for in accordance with the guidelines of the Animal Welfare Committees at all participating Institutions.

Animals received SPECT scans 6–8 weeks after MPTP at a time when they were clinically stable. [ $^{123}\text{I}$ ] $\beta$ -CIT (DOPASCAN<sup>®</sup>, Guilford Pharmaceuticals) was prepared by MDA Nordion in a manner similar to that described by Neumeier et al. [19]. All SPECT studies were performed on the Strichman Neuro 900, a single slice tomograph with a resolution of 6 mm in-plane and a slice thickness of 12 mm. Consecutive slices were obtained by translating the scanner bed in the  $z$ -axis to image sequential coronal sections of the monkey brain. All animals underwent magnetic resonance (MR) imaging one or more months prior to SPECT scanning in order to aid in the preselection of slices and region drawing. Monkeys were pre-treated with 15 mg/kg of potassium iodide via a gastric tube 5–7 h prior to the injection of [ $^{123}\text{I}$ ] $\beta$ -CIT in order to block thyroid uptake of free radioactive iodine. SPECT studies were performed 18–21 h after the i.v. injection of 5 mCi of [ $^{123}\text{I}$ ] $\beta$ -CIT, a time when specific and non-specific tracer uptake is at equilibrium [13]. Animals were anesthetized with a 70/30 mixture of ketamine/rompun (5–10 mg/kg), placed in the stereotaxic frame, and positioned in the scanner using a laser light aligned with the predetermined coordinates. For the purposes of this report, we analyzed data acquired at two levels, one passing through the striatum and one passing through the cerebellum.

A parkinsonian rating scale (PD scale) was utilized to quantify the clinical status of the monkeys [5]. The monkeys were rated at least once a week. The scale includes ratings of 10 parkinsonian features (tremor, posture, locomotion, hypokinesia, bradykinesia, balance, fine and gross motor skills, startle response, and freezing) and, drug-related side effects (hyperkinesia, psychological disturbance, vomiting, and diarrhea). Scores on a 40 point scale were used to classify the monkeys as stage 1 to stage 4, with stage 1 representing mild parkinsonism, and stage 4 severe bilateral parkinsonism.

Data were reconstructed in the coronal plane, as acquired, with attenuation correction using an ellipse with

uniform attenuation equivalent to that of water. Regions of interest were drawn in the contralateral hemisphere in the striatum and the cerebellum with reference to the MRs and a stereotaxic atlas of the monkey brain [23]. Mirror images of the regions were created in the ipsilateral hemisphere. Striatal to cerebellar radioactivity count ratios were constructed for each hemisphere, representing specific (DA transporter binding) to nondisplaceable binding. Linear regressions were performed to evaluate the relationship between PD scores and SPECT measures of DA transporter binding.

Scores on the PD scale ranged from 14 to 27 and included two stage 2 animals, 11 stage 3 animals, and three stage 4 animals. All animals showed severe parkinsonian signs on the side of the body opposite ICA MPTP administration and mild to severe parkinsonian signs on the other side of the body. [ $^{123}\text{I}$ ] $\beta$ -CIT accumulation was most highly concentrated in the contralateral striatum for the stage 2 and stage 3 animals with no appreciable activity accumulation in the ipsilateral hemisphere, while the three stage 4 animals showed a less distinct difference

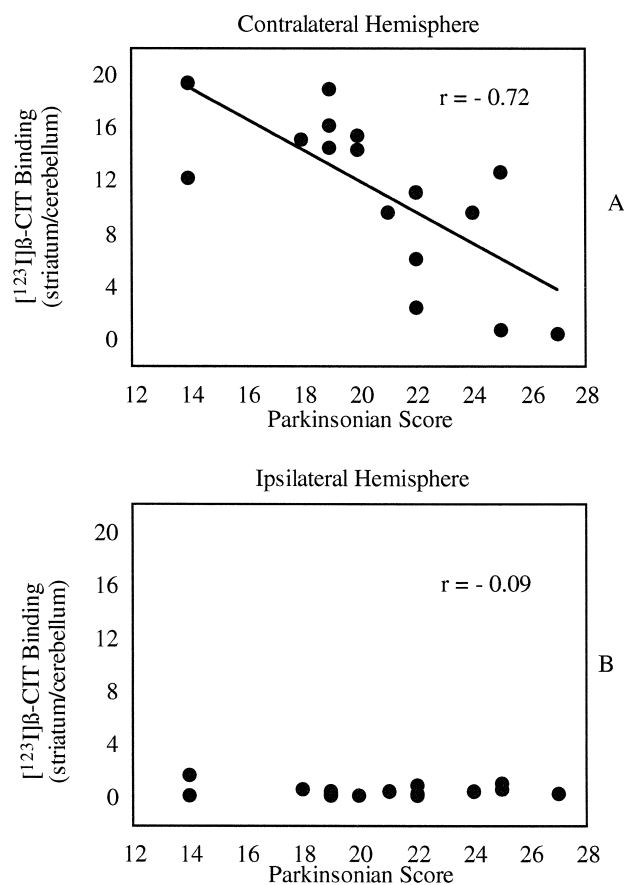


Fig. 1. Striatal [ $^{123}\text{I}$ ] $\beta$ -CIT binding and clinical rating scores. Relationship between striatal [ $^{123}\text{I}$ ] $\beta$ -CIT binding (striatum/cerebellum) for the hemisphere contralateral (A) and ipsilateral (B) to intracarotid artery infusion of MPTP and scores on the parkinsonian rating scale. A significant correlation ( $r = -0.72$ ,  $p = 0.002$ ) was observed in the contralateral hemisphere, with less [ $^{123}\text{I}$ ] $\beta$ -CIT binding in more severely parkinsonian monkeys.

between contralateral and ipsilateral hemispheres. The variability in contralateral [ $^{123}\text{I}$ ] $\beta$ -CIT binding was associated with the variability in clinical signs on the opposite side of the body. As shown in Fig. 1, contralateral striatal [ $^{123}\text{I}$ ] $\beta$ -CIT binding (striatal to cerebellar ratio) was significantly correlated with the PD scores ( $r = -0.72$ ,  $p = 0.002$ ), while no relationship was observed in the ipsilateral hemisphere ( $r = -0.09$ ,  $p = 0.74$ ). The lack of correlation in the ipsilateral hemisphere is not surprising since all animals had near complete ipsilateral striatal lesions and therefore showed very little [ $^{123}\text{I}$ ] $\beta$ -CIT binding.

DA transporter density appears to be reduced in parallel with DA levels in PD [10], suggesting that it is a good marker of DA terminal degeneration and disease severity. SPECT studies using [ $^{123}\text{I}$ ] $\beta$ -CIT have shown reduced striatal DA transporter density in PD patients relative to controls, and correlations between clinical features and presynaptic DA transporter binding [4,17,20,21,25]. Here, we report that striatal [ $^{123}\text{I}$ ] $\beta$ -CIT binding was decreased in association with clinical signs of parkinsonism in MPTP-lesioned monkeys. These results extend earlier findings to show that SPECT [ $^{123}\text{I}$ ] $\beta$ -CIT measures of MPTP-induced changes in DA transporter density are clinically relevant in the MPTP primate model. While the reduction in striatal DA transporter density produced by MPTP may reflect downregulation rather than DA terminal degeneration, SPECT [ $^{123}\text{I}$ ] $\beta$ -CIT measures provide a window into the functional status of the nigrostriatal DA pathway.

PET has been used extensively to image L-AADC activity using [ $^{18}\text{F}$ ]6-fluoro-L-dopa (FDOPA) and 6-[ $^{18}\text{F}$ ]-fluoro-L-*m*-tyrosine (FMT) [8,14,16,18]. Comparative studies in PD patients have shown correlations between clinical severity and both PET FDOPA and SPECT [ $^{123}\text{I}$ ]FP-CIT measures [7], similar to those reported here using SPECT and previously with PET in MPTP-lesioned monkeys [5]. The SPECT studies did not require the collection of arterial blood samples and were completed within 1 h, while the PET studies required multiple arterial blood draws and an imaging time of at least 90 min. Whereas the PET studies provided quantitative kinetic data with superior spatial resolution (2.6 mm vs. 6 mm in-plane) and thus the ability to resolve small structures including striatal subnuclei and the substantia nigra, SPECT was able to accurately discriminate different clinical severities in parkinsonian monkeys. The close correspondence between clinical features and both PET and SPECT measures of DA function indicate that both are good methods for evaluating the severity of nigrostriatal degeneration in MPTP-lesioned primates. While PET has several advantages over SPECT, including higher spatial resolution and more quantifiable results, SPECT is more easily implemented and more widely available making it more practical for longitudinal studies and for clinical use. SPECT [ $^{123}\text{I}$ ] $\beta$ -CIT studies may be useful for monitoring changes in DA transporter density in response to novel PD treatments, and the relationship of such changes to parkinsonian signs.

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