Binding of [^{99m}Tc]TRODAT-1 to Dopamine Transporters in Patients with Parkinson's Disease and in Healthy Volunteers

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[^{99m}Tc]TRODAT-1 is a radiolabeled tropane that binds dopamine transporters. The primary goal of this study was to determine whether its regional cerebral distribution could differentiate between patients with Parkinson's disease and healthy human volunteers. **Methods:** The sample consisted of 42 patients with Parkinson's disease, 23 age-matched controls, and 38 healthy adults younger than 40 y old. SPECT scans of the brain were acquired on a triple-head γ camera 3–4 h after the intravenous injection of 740 MBq (20 mCi) [^{99m}Tc]TRODAT-1. Mean counts per pixel were measured manually in subregions of the basal ganglia and normalized to the mean background counts to give specific uptake values ([SUVs] ≈k3/k4). Patient and control groups were also compared with automated statistical parametric mapping techniques. Logistic discriminant analyses were performed to determine the optimum uptake values for differentiating patients from age-matched controls. **Results:** Quantitative image analysis showed that the group mean SUVs in patients were less than the mean values in controls for all regions (all *P*s < 0.000001). There was overlap in the caudate as well as in the anterior-most portion of the putamen, but not in the posterior putamen, even when the asymptomatic sides of 5 patients with clinically defined hemi-Parkinson's disease were factored in. **Conclusion:** The findings indicate that Parkinson's disease can be detected with [^{99m}Tc]TRODAT by simply inspecting the images for uptake in the posterior putamen. Appropriate asymmetries seem to be visible with quantification in patients with clinically defined hemi-Parkinson's disease, even though changes in the putamen contralateral to the clinically unaffected side in these patients appear to precede the development of symptoms.

Key Words: normal; movement disorders; SPECT; Parkinson's disease

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Dopamine transporters mediate the reuptake of free dopamine from the intrasynaptic cleft (*1*). The regional concentration of dopamine transporters may reflect the tone of the dopaminergic system in that area (*2*).

[^{99m}Tc]TRODAT-1 is a radiolabeled tropane that binds dopamine transporters (*3–8*). It could be an effective probe for investigating the dopaminergic system for several reasons. ^{99m}Tc is relatively safe, inexpensive, and widely available (*9*). Its decay photon has highly favorable imaging characteristics. This radiopharmaceutical is technically easy to prepare from lyophilized kits with long shelf lives. It has already been shown to be biologically sensitive to the effects of normal aging in healthy human volunteers (*10*). Its specific uptake values [SUVs] correlate with the ability to perform several motor tasks that are known to be at least partially mediated by the dopaminergic system (unpublished data). It follows that its uptake values should be decreased in patients whose diseases affect the dopaminergic system.

Neuroimaging studies with other radiopharmaceuticals have consistently corroborated postmortem findings of decreased transporter concentrations in patients with Parkinson's disease (*11–15*). Parkinson's disease is one of several progressive degenerative disorders that affect dopaminergic neurons in the brain (*16,17*) and alimentary tract (*18*). It follows that the uptake of TRODAT-1 should aid in differentiating between patients with symptomatic Parkinson's disease and healthy individuals. The primary goal of this study was to test this hypothesis.

MATERIALS AND METHODS

All procedures were approved by the institutional review boards of all the participating clinics as well as the U.S. Food and Drug Administration.

Patients

The sample included 42 patients with clinically diagnosed Parkinson's disease (27 men, 15 women; mean age, 65.4 ± 10.4 y; age range, 38.3–82.2 y). The time interval between symptom onset and participation in this study ranged from <1 mo to >25 y (mean, 7.4 ± 5.2 y; median, 7.0 y). The time since diagnosis was <1 mo in 4 patients.

The subjects were referred from 2 of the larger movement disorders clinics in the greater Philadelphia metropolitan area and a

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third research clinic at another nearby university medical center. As a result, all diagnoses were confirmed by specialists in the field. All patients suffered from at least 2 of the 4 cardinal features of Parkinson's disease, verified at the time of scanning. The selection criteria for this study also included a history of having a favorable response to dopamine replacement therapy, although dyskinesias had developed in 5 patients by the time of study. The exclusion criteria were designed to prevent patients with a history of any other co-morbid neuropsychiatric disease from participating. Patients with only 1 of the 4 cardinal features of the disease who did not have a favorable response to dopamine replacement therapy at some time during their illness were excluded from this analysis.

Because studies in rats and baboons showed that antiparkinsonian drug regimens that act on the postsynaptic receptors do not affect the specific uptake of TRODAT-1 (*19*), the design allowed patients to be studied while taking most of their usual medications. Specifically, all patients were on dopamine replacement therapy. Drugs that act, or could act, on presynaptic transporters were discontinued for 7 half-lives before study. This feature affected 5 of the 42 patients, all of whom were taking tricyclic antidepressants, which were stopped at least 1 wk before the study. Prospective patients taking selective serotonin reuptake inhibitors were excluded because the half-lives of these medications are too long.

Healthy Volunteers

Most of the control population has been described previously (*3*). The total sample included 61 consenting healthy volunteers whose demographic characteristics reflected those of the local community. Of these, 23 were chosen to match the ages of the patients. This subgroup contained 13 men and 10 women (mean age, 58.4 ± 11.0 y; age range, 41.1–73.8 y). The other 38 healthy volunteers had a mean age of 26.6 ± 6.0 y (age range, 18.2–39.7 y). In healthy volunteers who were studied more than once, only the data from the first scan were used in the analyses.

Imaging Acquisition Protocol

Individuals were placed at rest on the imaging table. A catheter was placed in an antecubital vein and capped with a well containing normal saline. Vital signs and electrocardiography were then recorded continuously for at least 20 min before a dose of 740 MBq (20 mCi) [^{99m}Tc]TRODAT-1 was injected. Individuals remained at rest for the next 25 min, while dynamic images of the brain were acquired. The subjects then underwent neuropsychologic testing for about 90 min before having lunch. SPECT scans of the brain were obtained again from 3 to 4 or more h after administration at a framing rate of 5 min per scan to minimize the effects of physical decay (≈11%/h) and biologic redistribution on the images. All scans were obtained on the same triple-head γ camera equipped with ultra-high-resolution fanbeam collimators (Picker 3000; Picker International, Cleveland, OH). The acquisition parameters included a continuous mode with 40 projection angles over a 120° arc to obtain data in a 128 × 128 matrix with a pixel width of 2.11 mm and a slice thickness of 3.56 mm. The center of rotation was always 14.0 cm, regardless of head size.

Image Processing

All images were reconstructed with the same procedure based on simulation studies of the data from the first 20 subjects. After backprojection, a Butterworth low-pass filter with an order of 4.0 and a cutoff of 0.351/cm was applied. Chang's first order correction method was used to compensate for photon attenuation (*20*). The images were then reinterpolated into 2 × 2 × 4 mm voxels in planes parallel to the line connecting the frontal and occipital poles on another platform (Sun Microsystems, Mt. Pleasant, CA).

Image Analysis

The frames that were acquired from 180 to 240 min after administration of [^{99m}Tc]TRODAT-1 were summed and imported into a locally developed image analysis package (PETVU; UGM Inc., Philadelphia, PA). A manual analysis began by fitting a set of standardized templates representing the basal ganglia and the whole supratentorial brain on the summed images. The template has been described previously (*15,21*). Within the x–y plane, each region of interest (ROI) in the template was smaller than the actual structure it represented, to minimize resolution-induced problems with ill-defined edges. To reduce the effects of volume averaging in the axial direction, the ROIs were only placed on the 2 slices that contained the most intense activity. This tended to limit the ROIs to the central aspect of structures they represented. Boundaries for the whole brain were drawn beginning 12 mm above the uppermost slice containing basal ganglia activity. The cerebellum and occipital cortex were not used to model nonspecific binding. Previous work with this acquisition and processing protocol has shown that these relatively small regions sometimes have low counting rates. This tends to destabilize some kinetic analyses, particularly at fast framing rates. As a consequence, the supratentorial tissue that was used to model nonspecific binding may have contained dopaminergic nerve terminals in some pixels.

The analyses were performed independently with statistical parametric mapping. The images were coregistered to each other using a count difference algorithm (*22*) and affine (linear) transformations, with a randomly selected image as the baseline template. All the coregistered images were then added together, scaling each by their mean background counts, into a single average image. The coregistration was then repeated, this time registering each image to the average template. The registration procedure was repeated 2 additional times to reduce any residual bias. Each registered image was scaled by its mean background counts, effectively giving parametric images of the SUVs (*23,24*). Pixel-by-pixel statistical tests were applied to the images using statistical parametric mapping (SPM96) (*25*), correcting the resulting statistical images for nonindependent multiple comparisons using the theory of random Gaussian fields (*26,27*). Pixel-level thresholds on the statistical maps were set to $P < 0.001$, and surviving clusters of pixels were thresholded at $P < 0.05$.

Statistical Analyses

The mean activity per pixel in a structure was calculated across the 2 slices containing the maximum concentration of radioactivity in the basal ganglia by adding up the total number of counts in each ROI and dividing by the total number of corresponding pixels. Homotopic regions of the right and left hemisphere were averaged together for some analyses. Analyses of covariance were used to compare the 2 subject groups, controlling for age as a potentially confounding covariate. Logistic discriminant analysis (LDA) was applied with a commercial software package (JMP; SAS Institute, Cary, NC) to determine the optimum discriminatory factor between the patient and control groups, under the a priori assumption that only 2 groups made up the entire dataset (*28*). Cutoff SUVs were

calculated and represented the probability levels at which a subject was more likely to be a patient than a healthy volunteer.

RESULTS

Visual inspection frequently revealed pronounced differences between the uptake of TRODAT-1 in patients versus healthy volunteers, as shown in Figure 1A. It was frequently difficult to distinguish the posterior putamen from the surrounding background noise in patients.

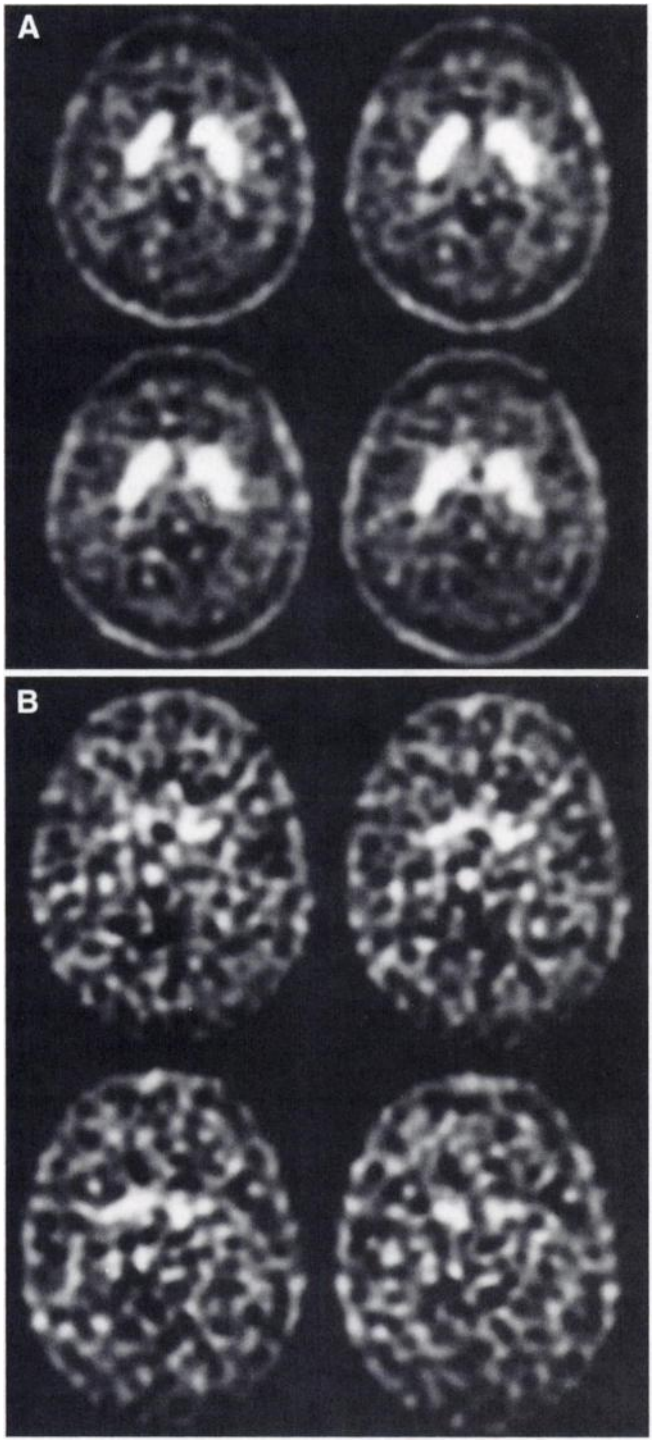

FIGURE 1. (A) A 57-y-old right-handed healthy volunteer. (B) A 56-y-old patient with bilateral Parkinson's disease. Images were acquired and processed with the same protocol.

TABLE 1
Mean SUVs in Patients and Age-Matched Volunteers

Region	Patients (n = 42)	Age-matched volunteers (n = 23)	Young adult volunteers (n = 38)
Caudate	0.85 ± 0.16	1.31 ± 0.15	1.58 ± 0.19
Anterior putamen	0.60 ± 0.21	1.11 ± 0.17	1.39 ± 0.18
Posterior putamen	0.37 ± 0.17	1.04 ± 0.24	1.32 ± 0.20
Whole striatum	0.65 ± 16	1.26 ± 0.09	1.54 ± 0.16

Values are group means ± SD.

The group mean SUVs in patients were less than the group mean values in healthy volunteers for all regions (all $P < 0.000001$), as shown in Table 1. The differences could not be accounted for by age, as shown in Figure 2. However, some overlap was noted in the caudate and the anterior-most portion of the putamen, most of which could be explained by preserved anterior striatal activity in some, but not all, patients with early disease. There was little overlap in the ROIs representing the posterior putamen. Quantification showed that the mean uptake values in patients were only about a third of the values in the corresponding ROIs of age-matched volunteers.

SPM also showed significant differences between patients and volunteers. As shown in Figure 3, almost all subregions of the basal ganglia were significantly affected.

LDA showed that the SUVs in the posterior putamen were the most sensitive indicator of Parkinson's disease. However, as listed in Table 2, the SUVs in this subregion were not the most specific, at only 97%. Most of the inaccuracy could be accounted for by 2 volunteers whose uptake values as well as abilities to perform dopaminergically mediated neuropsychologic tasks were the extreme outliers for the sample of volunteers. The most specific discriminator was the mean value for the ROI representing the whole striatum. Uptake in the whole striatum had a sensitivity of only 97%, however. Most of the inaccuracy could be accounted for by 2 patients with clinically defined hemi-Parkinson's disease, who had normal uptake values in their caudates as well as the anterior-most portions of their putamina, and 2 elderly volunteers, who had the 2 lowest values in the sample.

The uptake values in all 5 patients with clinically defined hemi-Parkinson's disease were abnormally decreased in the posterior striatal regions of both hemispheres, but in an appropriately asymmetric manner. The uptake values in the ROI representing the whole putamen averaged 0.87 on the asymptomatic side compared with a mean of 1.20 ± 0.24 in healthy volunteers. The SUVs for the same ROI on the symptomatic sides ranged from 0.43 to 0.64. The asymmetries in the remaining ROIs were also consistent with the clinical presentation and ranged from a low of 8% in the central caudate to 33.7% ± 10.8% in the affected versus the

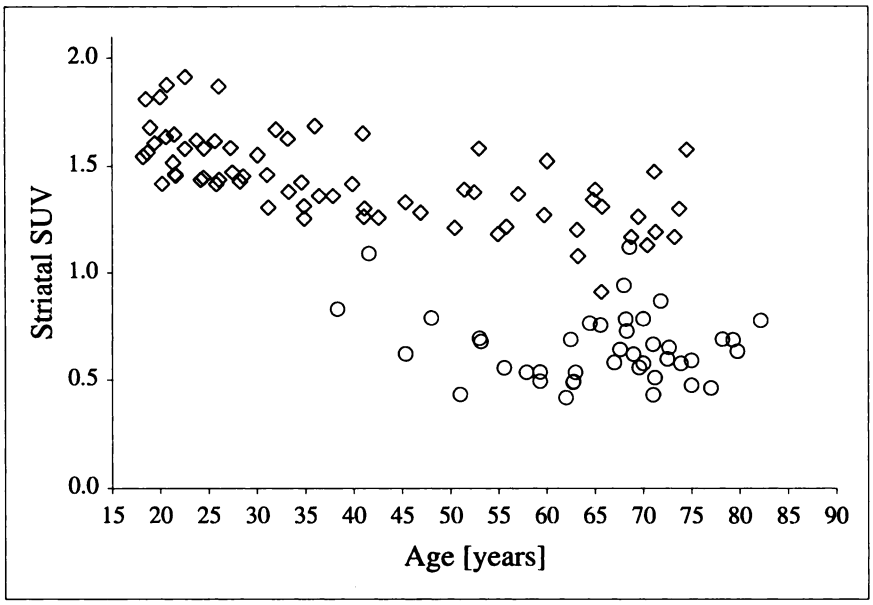

FIGURE 2. SUVs in patients and healthy volunteers as function of age.

unaffected whole hemi-striatum ($P = 0.0009$). A problem in the caudate or the anterior-most aspect of the putamen could not be detected at all in 3 of the 5 subjects with hemi-Parkinson's disease (2 of the 3 were in their early 70s), whereas an abnormality was detected in the other 2, who were only 38 and 41 y old (Fig. 4A). Both patients in their early 70s with hemi-Parkinson's symptoms had completely normal uptake values on the unaffected side of the brain. One of these patients had been symptomatic for <1 y, but the other's disease was initially diagnosed 8 y before the study and the patient had been symptomatic for 9 y.

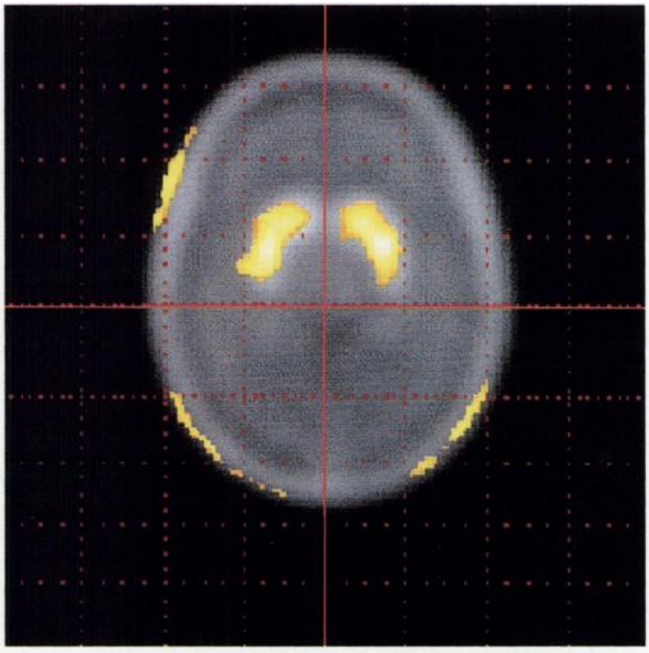

FIGURE 3. Statistical parametric image shows differences between patients and healthy volunteers in almost all subregions of basal ganglia. Pixels that seem significant outside of head reflect dependence of coregistration procedures on pixels with high counting rates.

DISCUSSION

The results support previous findings that have suggested that TRODAT-1 uptake values are clinically meaningful (*10*, unpublished data). The data show that a standard, static outcome measure based on 1 h of imaging can successfully discriminate, without kinetic modeling, between patients with Parkinson's disease and healthy volunteers.

The findings of appropriate asymmetries in patients with clinically defined hemi-Parkinson's disease indicate that TRODAT-1 may provide enough dynamic range within the pathologic spectrum to detect differences within patient groups, as well as distinguish patients from healthy volunteers.

The findings also suggest that it may be possible eventually to establish a threshold uptake value for the develop-

TABLE 2
Logistic Discriminant Analyses

Region	Cutoff*	% Sensitivity†	% Specificity‡
Whole striatum	1.09	97	91
Whole caudate	1.18	87	83
Anterior putamen	0.82	92	91
Posterior putamen	0.72	100	96
Maximum striatum	1.48	90	87

*SUV at which to apply the cutoff for each region, which maximizes probabilities of correctly assigning patients and healthy volunteers to their respective groups.

†True-positive rate (i.e., percentage of Parkinson's patients correctly identified using discriminant cutoff shown).

‡One minus the false-positive rate (i.e., percentage of volunteers correctly assigned as healthy using discriminant cutoff shown).

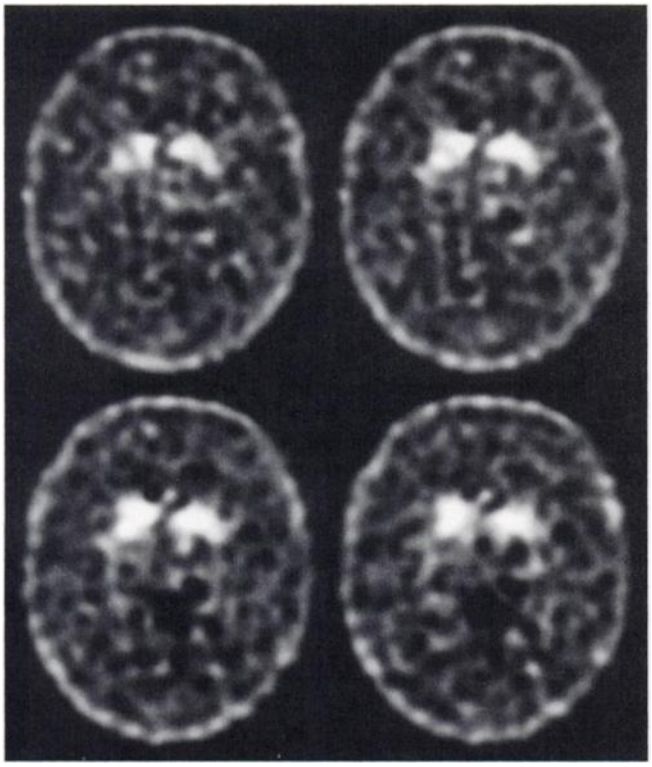

FIGURE 4. A 41-y-old patient with hemi-Parkinson's disease affecting right side of body. Left brain is on right side of image.

ment of symptoms that will be well below the average uptake value in age-matched controls. This speculation is supported by the observation that patients with clinically defined hemi-Parkinson's disease had abnormally low uptake values in some regions of the contralateral side that were symptomatically silent. This observation has been noted consistently by other investigators who used different radiopharmaceuticals (*11,14,29,30*). It suggests that successful interventions may be manifested by something significantly less than a restoration of normal uptake values.

Although it is clear from the data that the posterior striatum was more severely affected than the anterior, some patients, including 2 with hemi-Parkinson's disease, had abnormally decreased SUVs in their caudates. This finding, particularly on the unaffected side of 2 relatively young patients with hemi-Parkinson's disease, is difficult to reconcile with hypotheses that suggest that the caudate is only affected late in the course of illness. On the other hand, there was not much more support for models of more uniform disease progression throughout the dopaminergic system, because some patients with relatively long-standing disease had patches of relatively preserved transporter concentrations. The conclusion that seems most defensible with this dataset suggests that regional transporter concentrations in Parkinson's disease can be highly variable within the abnormal range among patients.

It follows from all these observations that a radiopharmaceutical like TRODAT-1 could eventually become clinically useful. Visual inspection of the images was frequently impressive. Given the ease of manufacture with ordinary pertechnetate and the fact that only 1 h of scanning time seems essential, the results suggest that tracers like TRODAT-1 could be used efficiently as well as economically in conventional medical settings.

CONCLUSION

The findings indicate that patients with Parkinson's disease can be accurately distinguished from age-matched controls with [^{99m}Tc]TRODAT-1. Appropriate asymmetries seem to be visible in patients with hemi-Parkinson's disease. Simple uptake values seem to have enough dynamic range within the pathophysiologic spectrum to detect differences that may be clinically meaningful.

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