Evaluation of early Parkinson’s Disease with $^{99m}\text{Tc}$TRODAT-1/SPECT Imaging

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一、計畫緣起與目的

The etiology of idiopathic Parkinson’s disease (PD) is unknown. There is no effective method to prevent the occurrence of this neurodegenerative disorder at the present time. The most important and practical approach to the management of these patients is to make the diagnosis at an early stage and introduce an intervention that protects the vulnerable neurons and slows or stops disease progression.

The major neuropathological feature in (PD) is severe degeneration of the dopamine (DA) neurons in the substantia nigra. Dopamine transporter (DAT) is an important protein in the presynaptic uptake sites which is important in terminating synaptic dopamine action and maintaining dopamine homeostasis. Specific binding to dopamine transporters may serve as a tool to detect early loss of nigrostriatal dopaminergic neurons in patients with Parkinson's disease.

Recently, single photon emission computed tomography (SPECT) imaging of the dopamine transporter with $^{123}\text{I}$-CIT is shown to be an alternative to positron emission tomography (PET) or postmortem studies for in vivo evaluation of presynaptic dopaminergic function. However, the production of $^{123}\text{I}$ is limited by the availability of cyclotron, which makes its routine clinical application difficult. The staff of INER (Institution of Nuclear Energy Research, Taiwan) has the honor to
work with Dr. Kung and is permitted to produce domestic $^{99m}$Tc TRODAT-1, a highly selective and safe dopamine transporter ligand.

The aim of this study is to investigate striatal DAT binding in early PD with $^{99m}$Tc TRODAT-1 and single photon emission computed tomography (SPECT).

二、執行方法

SUBJECTS

Twenty-six sequential patients with early untreated Parkinson's disease (Hoehn and Yahr stages I [n = 10] and II [n = 16] [symptom duration, <2 years]; mean age, 63.2 years; range, 36-83 years) who gave the informed consent were recruited. We followed the inclusion and exclusion criteria set by the CAPIT committee for clinical diagnosis of PD. Patients who had psychiatric disorders, alcohol or substance abuse, or unstable medical problems were also excluded. Written informed consent was obtained from each patient. This study was approved by the institutional review board of National Cheng Kung University Hospital.

The basic demographic data, mean duration of illness, co-morbid factors and Unified Parkinson’s Disease Rating Scale (UPDRS) motor subscore were collected in each patient. Every patient received $^{99m}$Tc TRODAT-1/SPECT and brain magnetic resonance imaging (MRI) at the beginning of this study and a second $^{99m}$Tc TRODAT-1/SPECT one year later after Selegiline 5 mg PO bid. Patients who suffered from severe resting tremor and had the potential for seriously degrading image quality were given midazolam 15 mg 30 min before MRI and $^{99m}$Tc TRODAT-1/SPECT study. The second UPDRS motor subscore will be done at the time of the second $^{99m}$Tc TRODAT-1/SPECT study.
The primary response variable is the averaged right and left striatum/occipital lobe ratios. The secondary response variables are the changes in UPDRS motor subscore and the time interval from randomization to the need of starting or adding other antiparkinsonian medications.

METHODS

Preparation of Tc-99m TRODAT-1 Injection

Description

Each 10 ml vial contained a pre-dispensed sterile, non-pyrogenic, lyophilized mixture of 126 μg TRODAT-1·3HCl (2-[[2-[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3.2.1]oct-2-yl]methyl](2-mercaptoethyl)amino-ethyl]amino]ethanethiolato(3-)N₂N₁₂S₂S₁₂ oxo-[1R-(exo-exo) hydrogen chloride], 320 μg sodium glucoheptonate, 930 μg Na₂·EDTA·2H₂O (disodium ethylenediamine tetraacetate dihydrate), 32 μg stannous chloride dihydrate, 20 mg mannitol, 4.1 mg anhydrous sodium phosphate dibasic and 460 μg sodium phosphate monobasic, sealed under nitrogen atmosphere with a rubber plug. No bacteriostatic preservative was present.

General Preparation Precautions:

1. A technetium Tc-99m generator was eluted within 24 hours prior to obtaining any eluate for reconstitution with the INER TRODAT-1 kit.
2. Only sterile 0.9% sodium chloride without bacteriostatic preservative was allowed to be used for dilution of Tc-99m before reconstitution.

Procedure for the Preparation of Technetium Tc-99m TRODAT-1 Injection

1. We placed one vial in a suitable shielding container and disinfected the rubber
plug with an alcoholic sterile swab.

2. Using a 10-mL syringe, we injected into the shielded vial 5 mL of sterile eluate (1.11~1.48 MBq) from a technetium Tc-99m generator. Before withdrawing the syringe from the vial, we drew 5 mL of gas from the space above the solution to normalize the pressure in the vial. The shielded vial was put upside down for 10 seconds to ensure complete dissolution of the contents.

3. Autoclave the shielded vial at 121 ℃ for 30 min to complete the labeling.

4. Following cooling to room temperature, we assayed the total radioactivity and calculated the volume to be injected.

5. The pH of the prepared injection was 6.5~7.5.

6. The radiochemical purity was over 90%

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**Tc-99m TRODAT-1/SPECT Imaging**

A dose of 25 mCi of [99mTc] TRODAT-1 was injected intravenously into each patient. The binding to dopamine transporter was assessed 4 hours after injection with SPECT. A rotating three-headed gamma camera with fan-beam collimator (Multi SPECT 3, Siemens, Germany) and a commercially available computer system were used for data acquisition and processing. Data were collected for 120 projections (360° rotation) in a 128 × 128 matrix. The acquisition time was 40 seconds per projection. Attenuation correction was performed in selected transverse slices according to a modified Chang’s method. In-plane resolution of the reconstructed images was 8.5 mm FWHM, and slice thickness was approximately 6 mm.
**MRI Image**

1. T1-weighted axial and sagittal, proton density axial, and T2-weighted axial and coronal MRI images were obtained by a Magneton 1.5 Tesla scanner (Siemens, Iselin, NJ). The slice thickness was 3 mm at the level of basal ganglia.

2. Regions of interest (ROI) template derived from co-registered MRI image were used for analyzing [99mTc]TRODAT-1 activity within the neostriatum.

**SPECT Quantitative**

**Conventional semiquantitation**

The right and left striatum activity in all reconstructed transaxial slices was summed as total volume activity, and then was divided by total areas as average activity. The striatum activity was further divided into caudate, and lenticular nucleus according to the finely adjusted ROI on MRI image registration. The occipital lobe was chosen for comparison. An elliptical ROI was drawn on occipital lobe in the transverse slice with striatum activity. The ratio of striatum to occipital lobe was then calculated.

**New automated quantitative method**

A new quantitative method developed by our group in the first year project was applied to this study (See Quantification of D2-receptor imaging with [123I] IBZM and Single Photon Emission Tomography; N87-2218-E-006-065-NU.

**Statistical Analysis**

Both the primary and secondary response variables in either group were expressed as mean ± S.D.. Two-tailed Student’s t-test was applied for comparison and values of p<0.05 were considered significant.
三、結果

In patients with early PD, the striatum/occipital ratio was reduced as compared with normal controls. The decrease was more prominent in the tail of putamen compared with caudate nucleus. (Figure 1). Striatal TRODAT-1 binding was significantly reduced in patients with early Parkinson's disease not only contralaterally to the more severely affected side, but also in the normal or mildly disturbed side (Figure 2). The striatum/occipital ratio in patients with early PD was significantly reduced in contralateral striatum (1.73 ± 0.23; range, 1.34-2.10) than in ipsilateral striatum (1.80 ± 0.25; range, 1.35-2.19; p = 0.003) (Figure 3). Striatum/occipital ratios were lower in patients with Hoehn and Yahr stage II (contralateral striatum: 1.68 ± 0.23; ipsilateral striatum: 1.76 ± 0.25) than in Hoehn and Yahr stage I (contralateral striatum: 1.80 ± 0.23; ipsilateral striatum: 1.86 ± 0.24). However, the difference is not statistically significant (p = 0.324 and 0.185, respectively) (Figure 4). Twenty-one patients (patient 1-21) showed a more decreased striatum/occipital ratio on the side contralateral to clinically worse side. One case (patient 22) showed equal activity bilaterally. Only 4 patients (patient 23-26) showed a more decreased activity on the ipsilateral side (Figure 5). There is no significant decline of striatum/occipital ratio with age in early PD, both in ipsilateral striatum (Figure 6) and contralateral striatum (Figure 7). Striatum/occipital ratios were not significantly correlated with the severity of clinical symptoms-UPDRS motor subscore (Figure 8).

四、結論與建議
We found a bilateral decrease of striatum activity in early PD patients, even in stage 1 PD patients whose clinical symptoms were unilateral. This finding indicates that $[^{99m}Tc]$TRODAT-1/SPECT imaging is able to detect early subclinical PD patients. With its clinical availability, $[^{99m}Tc]$TRODAT-1/SPECT imaging is suitable for clinical screening of high risk patients. In addition, we found that $[^{99m}Tc]$TRODAT-1/SPECT imaging showed a more decreased or equal striatum/occipital lobe uptake ratio contralateral to clinically worse side in 85% of the studied patients. These findings are consistent with previous $[^{18}F]$DOPA/PET and autopsy reports. Our preliminary results suggest that $[^{99m}Tc]$TRODAT-1/SPECT imaging is a useful tool for detecting presynaptic dopaminergic dysfunction. There is a great overlap of striatum activity between stage I and stage II PD. It is not surprise to expect this finding, since the clinical differentiation is not clear. We are planning to follow up these patients longitudinally with both $[^{99m}Tc]$ TRODAT-1 and $[^{123}I]$ IBZM/SPECT to observe the sequential changes of dopamine transporter and receptor in striatum. This may detect underlying the rate of progression of PD and provide the scientific explanation for the development of motor fluctuation in late stage PD patients.

There is no correlation between UPDRS motor subscore and the striatum/occipital ratio. This might imply that clinical evaluation with UPDRS may not reflect the true status of dopaminergic neurons in the substantia nigra. The items for tremor evaluation account for more than one-fifth of the total motor subscore (24/108). As we know, the severity of tremor shows marked fluctuation in a short period of time and is aggravated by anxiety, nervousness, or any factors increasing sympathetic activity. Another explanation for the discrepancy between UPDRS motor subscore and the striatum/occipital ratio is the labeling efficiencies of $[^{99m}Tc]$ TRODAT-1 are different among patients. This hypothesis has been tested and we do not find significant influence of labeling efficiency on the striatum/occipital ratio.
In early PD, there is apparent reduction of activity in bilateral putamen, while the activity in caudate nucleus is preserved. For those patients at stage I, who have unilateral symptoms the striatum uptake of $[^{99m}\text{Tc}]$ TRODAT-1 was decreased bilaterally, which means $[^{99m}\text{Tc}]$ TRODAT-1 SPECT is capable to detect pre-clinical Parkinson’s disease, and has the potential for screening of pre-clinical Parkinson’s patients. We are planning to collect more data of normal controls as comparable age groups. This is important for the interpretation of subtle change for any age-matched individual. We will continue on the protocol to work out if selegiline has neuroprotective effect after treatment for 1 year. The use of $[^{99m}\text{Tc}]$ TRODAT-1/SPECT imaging for differentiating different categories of parkinsonism will also be tested in the near future. Finally, the TRODAT-1 kit produced by INER is safe and easy for clinical use. None of the patients reports side effects in this study.

In conclusion, $[^{99m}\text{Tc}]$ TRODAT-1/SPECT demonstrates a reduction of dopamine transporter binding in patients with early Parkinson’s disease. Significantly reduced TRODAT-1 binding already observed in the ipsilateral striatum of patients with Hoehn and Yahr stage I and II demonstrates the potential of this method to detect preclinical disease.
Fig 1. The striatum uptake of $[^{99m}Tc]$ TRODAT-1 in normal control and early PD. There is decreased striatum uptake in early PD with more prominent in the tail of putamen than in caudate nucleus.
Fig 2. The striatum uptake of $[^{99m}Tc]$ TRODAT-1 in stage I and stage II PD. The patient with stage I PD had unilateral right side symptoms, while $[^{99m}Tc]$ TRODAT-1 SPECT shows a slight decrease of striatum uptake on contralateral side. The patient with stage II PD had mild left side symptoms and worse on right side. $[^{99m}Tc]$ TRODAT-1 SPECT shows a marked decrease of striatum uptake on contralateral side and a slight decrease on ipsilateral side.
Fig 3. The striatum/occipital ratio in patients with early PD was significantly reduced in contralateral striatum (1.73 ± 0.23; range, 1.34-2.10) than in ipsilateral striatum (1.80 ± 0.25; range, 1.35-2.19).

P-value = 0.003*

* Paired samples T-test
Fig 4. Striatum/occipital ratios in contralateral striatum and ipsilateral striatum in patients with Hoehn and Yahr stage II and Hoehn and Yahr stage I. The difference is not statistically significant (p = 0.324 and 0.185, respectively)
Fig 5. The $^{99m}$Tc TRODAT-1 SPECT findings show an excellent correlation with the asymmetry of clinical symptoms, i.e. 21/26 of patients (81%) show a more decreased striatum / occipital ratio on the side contralateral to the clinically worse side, 1/26 showed equal uptake bilaterally. Only 4/26 patients showed a more decreased striatum / occipital ratio on the ipsilateral side.
Fig 6. Striatum/occipital ratio in ipsilateral striatum in early PD. There is no significant decline of striatum uptake with age.
Fig 7. Striatum/occipital ratio in contralateral striatum in early PD. There is no significant decline of striatum uptake with age.
Fig 8. Correlation between striatum/occipital ratios and the severity of clinical symptoms (UPDRS-motor subscore)
五、參考文獻


