行政院原子能委員會
委託研究計畫研究報告

以 $[^{99mTc}]$TRODAT-1 單光子腦部斷層掃描評估 selegiline 對
巴金森氏病的神經保護作用

Evaluating the Neuroprotective Effect of Selegiline on
Parkinson’s Disease Patients with $[^{99mTc}]$TRODAT-1/SPECT Imaging

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（二）中文摘要

由動物實驗及組織細胞培養的研究發現 selegiline 這種單胺氧化酵素抑制劑可以保護分泌多巴胺的神經細胞免於MPTP毒素，缺氧，及興奮性神傳導物質的傷害。對於吸入不純的毒品導致 MPTP-induced parkinsonism 的研究也發現單胺氧化酵素在整個致病機轉中扮演很重要的角色。

$[^{99m}Tc]$TRODAT-1/SPECT 影像學檢查被發現是一種安全、簡易可行且能反映黑質細胞中分泌多巴胺神經細胞的機能。本研究即利用一系列的$[^{99m}Tc]$TRODAT-1/SPECT 影像學檢查來評估 selegiline 對早期的巴金森氏病是否具有神經保護作用。

我們一共收錄 18 位未曾服藥之早期巴金森氏病人，依隨機分配分成治療組及對照組。治療組給予 selegiline 5mg 一天兩次，對照組則無。每位病人都接受靜脈注射 25 mCi $[^{99m}Tc]$TRODAT-1，四個小時後再利用 SPECT 取像。我們以紋狀體/枕葉的比值作為半定量分析。病人同時接受磁振造影檢查以作為對位使用。另外，每個病人也都用 UPDRS 作臨床症狀嚴重度評估。一年後，每個病人再作一次$[^{99m}Tc]$TRODAT-1/SPECT 影像學檢查及 UPDRS。

初步結果發現 16 位(89%)病人臨床症狀較嚴重的對側的紋狀體有比較低的$[^{99m}Tc]$TRODAT-1 uptake (S/O ratio: 1.67 ± 0.19 vs. 1.74 ± 0.19, p<0.001)。另外我們發現利用 UPDRS 評分所得的臨床嚴重度與 Corpus striatum/Occipital lobe $[^{99m}Tc]$TRODAT-1 uptake ratio 並沒有很好的相關性。至於 selegiline 對早期巴金森氏病患者是否有神經保護作用因為追蹤的時間還不夠長，目前還沒有辦法作出結論。截至目前為止，接受這項檢查的病人都沒有產生任何副作用。
（三）英文摘要

In tissue culture and animal models, selegiline was proved to have protective effect on dopaminergic neurons from MPTP toxicity, hypoxia, and excitotoxicity. Clinical investigation on illicit drug abuser has shown that MAO-B plays a key role in the pathogenesis of MPTP-induced parkinsonism. Recently, \([^{99m}\text{Tc}]\) TRODAT-1/SPECT imaging has been found to be a safe and easily applicable tool for in vivo evaluation of presynaptic dopaminergic function. The purpose of study is to evaluate the potential neuroprotective effect of selegiline on PD patients by serial \([^{99m}\text{Tc}]\) TRODAT-1/SPECT imaging.

Eighteen patients with early PD were enrolled by the inclusion and exclusion criteria set by the CAPIT committee. They were randomly assigned to either treatment or placebo group. Patients in the treatment group took selegiline 5mg bid from the beginning of the study and continued for one year. The placebo group did not take selegiline. Every patient received 25 mCi \([^{99m}\text{Tc}]\) TRODAT-1 intravenously and the SPECT examination was done 4 hours later. Corpus striatum/Occipital lobe ratio was used for semiquantitative analysis. In addition, every patient had a co-registered MRI study and an evaluation of clinical severity by UPDRS motor subscore. The second \([^{99m}\text{Tc}]\) TRODAT-1/SPECT imaging and UPDRS motor subscore were repeated one year later.

Our preliminary results show that 16/18 (89%) of the patients have a decreased corpus striatum/occipital lobe \([^{99m}\text{Tc}]\) TRODAT-1 uptake ratio contralateral to the clinically worse side (S/O ratio: 1.67 ± 0.19 vs. 1.74 ± 0.19, p<0.0001). We also find that there is no correlation between UPDRS motor subscore and the averaged right and left corpus striatum/occipital lobe \([^{99m}\text{Tc}]\) TRODAT-1 uptake ratio. It is hard to determine the neuroprotective effect of selegiline on PD patients at present time because the follow-up period is not long enough. There are no side effects reported from this SPECT study.
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. Clinical investigation on illicit drug abuser and animal experiments have shown that some environmental toxins are metabolized by monoamine oxidase B (MAO-B), and transported intracellularly by dopamine transporter to produce nigral cell damage. Selegiline, one kind of MAO-B inhibitor, has been shown to protect dopaminergic neurons from aging, excitotoxicity, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity, and hypoxia in both tissue culture and animal models.

Recently, single photon emission computed tomography (SPECT) imaging of the dopamine transporter with \(^{[123]}\text{I}\) \(\beta\)-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 \(^{99m}\text{Tc}\) TRODAT-1, a highly selective and safe dopamine transporter ligand.

The objective of this study is to evaluate the potential neuroprotective effect of selegiline (parkryl\textsuperscript{®}) on PD patients with serial \(^{99m}\text{Tc}\) TRODAT-1/SPECT imaging. A positive result from this study can be used to improve both patients’ and social welfare.
( 五 ) 計畫緣起

Parkinson’s disease (PD) is an important neurodegenerative disorder because it is common and treatable. The primary deficit of PD lies in the presynaptic dopaminergic neurons in the substantia nigra. Dopamine transporters are presynaptic uptake sites which are important in terminating synaptic dopamine action and maintaining dopamine homeostasis (1,2). In tissue culture models, selegiline protects dopaminergic neurons from aging, excitotoxicity, 1-methyl-4-phenyl-pyridinium (MPP⁺) toxicity, glutathione deficiency (3-6). In animal models, selegiline has been shown to protect against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity, and ischemia/hypoxia (7,8). These laboratory findings and the clinical investigation on MPTP-induced parkinsonism have shown that some environmental toxins are metabolized by monoamine oxidase B (MAO-B), and transported intracellularly by dopamine transporter to produce nigral cell damage. The possibility that selegiline may act as a neuroprotective agent in PD was based on the ability of MAO-B inhibition.

A neuroprotective therapy can be defined as an intervention that protects vulnerable neurons and slows or stops disease progression (9). [¹⁸F] DOPA/PET is the gold standard for in vivo measurement of the loss of dopamine neurons in PD (10,11). However, the radiochemical demands of [¹⁸F] DOPA synthesis and the availability of PET scanner greatly limited its clinical use. Measures of disease progression can target neurons (PET, SPECT, magnetic resonance spectroscopy [MRS]), motor function (Unified Parkinson’s Disease Rating Scale [UPDRS]), or more general functions such as activities of daily living and quality of life (12). Recently, SPECT
imaging of the dopamine transporter with $[^{123}\text{I}]\beta$-CIT is shown to be an alternative to $[^{18}\text{F}]$ DOPA/PET or postmortem studies for in vivo evaluation of presynaptic dopaminergic function (12-19). However, the availability of $[^{123}\text{I}]$ produced by cyclotron makes its routine application difficult. The development of a highly specific $^{99m}\text{Tc}$-labeled ligand for dopamine transporter, $[^{99m}\text{Tc}]$ TRODAT-1 (technetium, 2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3,2,1.]oct-2-yl]-methyl](2-mercaptoethyl)amino]ethanethiolato(3-)]-oxo-[1R-(exo-exo)]-], by Kung HF et al, greatly improved the ease of use of SPECT imaging procedure for routine clinical studies of presynaptic dopaminergic function (15,20-22).

The staff of INER has the honor to work with Dr. Kung and is permitted to produce domestic $[^{99m}\text{Tc}]$ TRODAT-1. It is supplied in kit form and thus convenient for routine daily practice.

The purpose of this study is to evaluate the potential neuroprotective effect of selegiline (parkryl®, LOTUS PHARMACEUTICAL CO., LTD, TAIWAN) on PD patients with serial $[^{99m}\text{Tc}]$ TRODAT-1/SPECT imaging.
SUBJECTS

Sixty sequential patients of early stage (Hoehn and Yahr stage I & II) untreated PD patients who gave the informed consent were recruited. We followed the inclusion and exclusion criteria set by the CAPIT committee for clinical diagnosis of PD (23). 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. Random assignment was made before the enrollment of patients. To make the random assignment, we selected a two-digit random number from random number table for 60 participants. Any duplicate numbers were thrown out, so each participant had a unique random number. We then assigned the participants with the 30 largest numbers to the treatment group, and the participants with the 30 smallest numbers to placebo group. Patients were then assigned to either group according to the sequence of enrollment. The placebo group received no antiparkinsonian medications at the beginning. The treatment group was given selegiline (parkryl®) one tablet (5 mg) bid orally from the beginning and continued for 1 year. The selegiline treatment will be discontinued when patients developed cardiac arrhythmia, elevated serum aminotransferase levels, or mental changes. The major considerations for
starting levodopa therapy or initiating other antiparkinsonian medications in either group were: (a) the threat to the subject’s employability; (b) the threat to the subject’s ability to management of domestic or financial affairs; (c) an appreciable decline in the subject’s handling of the activities of daily living; and (d) an appreciable worsening of gait or balance (24).

Every patient received \textsuperscript{99m}Tc TRODAT-1/SPECT and brain magnetic resonance imaging (MRI) at the beginning of this study and a second \textsuperscript{99m}Tc TRODAT-1/SPECT one year later. 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 \textsuperscript{99m}Tc TRODAT-1/SPECT study. The second UPDRS motor subscore will be done at the time of the second \textsuperscript{99m}Tc TRODAT-1/SPECT study.

The primary response variable is the averaged right and left lenticular nucleus/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-dispersed 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-mercaptopethyl]amino]-ethyl]-amino]ethane-thiolato(3-)·N2,N2′,S2,S2′ oxo-
[1R-(exo-exo) hydrogen chloride], 320 µg sodium glucoheptonate, 930 µg Na₂EDTA-2H₂O (disodium ethylenediaminetetraacetate 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 closure. 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°C 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%

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

A dose of 25 mCi of $[^{99m}Tc]$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 $[^{99m}Tc]$TRODAT-1 activity within caudate and lenticular nucleus.
**SPECT Quantitative**

**Conventional semiquantitation**

The right and left striatal activity in all reconstructed transaxial slices was summed as total volume activity, and then was divided by total areas as average activity. The striatal 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 striatal activity. The ratio of striatum to occipital lobe was then calculated.

**New automated quantitative method**

A new quantitative method developed by ourselves in the first year project was applied to this study (See [123I] IBZM SPECT imaging of striatal dopamine D2 receptor in normal controls, idiopathic Parkinson’s disease patients of variable severity and patients with “parkinson-plus” syndromes; N3105).

**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.
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We found a bilateral decrease of striatal activity in early PD patients, even in stage 1 PD patients whose clinical symptoms were unilateral (Fig 3). This finding indicates that $[^{99m}\text{Tc}]$TRODAT-1/SPECT imaging is able to detect as early as subclinical PD patients. In addition, we found that $[^{99m}\text{Tc}]$TRODAT-1/SPECT imaging showed a more decreased corpus striatum/occipital lobe uptake ratio on the side contralateral to clinically worse side in 89% of the studied patients (Fig. 1) (S/O ratio: 1.67 $\pm$ 0.19 vs. 1.74 $\pm$ 0.19, p<0.001). These findings are consistent with previous $[^{18}\text{F}]$DOPA/PET and autopsy reports. Our preliminary results suggest that $[^{99m}\text{Tc}]$TRODAT-1/SPECT imaging is a useful tool for detecting presynaptic dopaminergic dysfunction. We are planning to extend the examination to different stages of PD patients and follow up early stage PD patients longitudinally with both $[^{99m}\text{Tc}]$TRODAT-1 and $[^{123}\text{I}]$IBZM/SPECT to determine the sequential changes of dopamine transporter and receptor in striatum. This may provide some knowledge about the rate of progression of PD and the scientific explanation for the development of motor fluctuation in late stage PD patients.

There is no correlation ($r = 0.02$) between UPDRS motor subscore and the corpus striatum/occipital lobe uptake ratio (Fig. 2). 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 corpus striatum/occipital lobe uptake ratio is the labeling efficiencies of $[^{99m}\text{Tc}]$TRODAT-1 are different among patients. This hypothesis has been tested and we do not find significant influence of labeling efficiency on the corpus striatum/occipital lobe uptake ratio.

Fig. 3 shows the striatal uptake of $[^{99m}\text{Tc}]$TRODAT-1 in normal controls and early PD. In early PD, there is apparent reduction of activity in bilateral putamen, while the activity in caudate nucleus is preserved. Clinically, this patient had worse movement disorders on the left side. However, the striatal 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 early screening of pre-clinical Parkinson's patients. We are planning to collect more data of normal controls at different age groups. This is important for the interpretation of results 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.

The TRODAT-1 kit produced by INER is safe and easy for clinical use. None of the patients reports side effects in this study.
Fig 1. The $[^{99m}Tc]$ TRODAT-1 SPECT findings show an excellent correlation with the asymmetry of clinical symptoms, i.e. 16/18 of patients (89%) show a more decreased corpus striatum / occipital lobe ratio on the side contralateral to the clinically worse side. (corpus striatum = neostriatum + paleostriatum) (S/O ratio: 1.67 ± 0.19 vs. 1.74 ± 0.19, p<0.001)
Fig 2. There is no correlation ($r=0.02$) between decreased single-photon emission computed tomographic [¹⁹⁵Te] TRODAT-1 striatal uptake and symptom severity in Parkinson's disease. (corpus striatum = neostriatum + paleostriatum; UPDRS=Unified Parkinson's Disease Rating Scale)
Fig 3. The striatal uptake of $[^{99m}Tc]$ TRODAT-1 in normal control and early PD


17. Innis RB: Single-photon emission tomography imaging of dopamine


