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Simplified in-house kit formulation of ^{99m}Tc-TRODAT-1 with high radiolabeling efficiency for routine clinical use. Westmead Hospital Experience

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Abstract: **Objective**: The tropane derivative, TRODAT-1 when labelled with ^{99m}Tc has been shown to bind dopamine transporter (DAT) with high affinity and selectivity. In this study, we have modified the in-house formulation of TRODAT-1 cold kit. We investigated if the labeling procedure could be improved by using a block heater and thus be more efficient and consistent than using autoclaving or boiling water-bath methods described elsewhere.
Methods: Briefly, 1mg of TRODAT-1 was dissolved in 100µl of ethanolic-HCl. To this solution 500µg $SnCl_2$, 160mg sodium glucoheptonate, 19mg EDTA were added and mixed. The bulk solution containing TRODAT-1 was aseptically dispensed in 1.0mL aliquots and stored at -20°C. TRODAT-1 was radiolabelled by adding 1.4GBq of ^{99m}Tc-pertechneate in 3ml saline and heated for 30min at 100°C using a block heater enclosed with a lid. The radiochemical purity (RCP) was analysed using Instant Thin Layer Chromatography-Silica Gel (ITLC-SG) with 0.9% Sodium Chloride as the solvent and High-Performance Liquid Chromatography (HPLC) was performed using 0.1% Trifluoroacetic acid (TFA) / Water (H_2O) & Acetonitrile (ACN). As proof of concept, biodistribution of ^{99m}Tc-TRODAT-1 was studied in a rabbit model. Animals were injected with 30MBq of ^{99m}Tc-TRODAT-1 and images were acquired at 60 min post-injection with a Single-photon emission computed tomography (SPECT), Siemens Symbia camera.
Results: Previous studies have used autoclaving method or a boiling water bath for radiolabeling TRODAT-1 with ^{99m}Tc. These methods have some limitation including the evaporating of water during boiling, uncontrollable temperatures, variable yield and increased possibility of contamination. To ensure consistent temperature and eliminate the possibility of contamination we used a block heater. The percentage of RCP of ^{99m}Tc-TRODAT-1 by ITLC-SG was >99% and reproducible. The retention time by HPLC for labeled product was 16.18min and for free ^{99m}Tc was 2.5min. Animal biodistribution studies showed brain hippocampus uptake of 0.32±0.08 %ID/g at 60min post-injection.
Conclusions: This study describes a simple formulation and method of preparation for ^{99m}Tc-TRODAT-1, which is reproducible with radiochemical purity (RCP >99%). The pre-clinical results in rabbits indicated specific binding to dopamine receptors and therefore has the potential for use in DAT imaging in patients.

Key words: TRODAT-1, High Performance Liquid Chromatography (HPLC), Single-photon emission computed tomography (SPECT)

Introduction

^{99m}Tc labeled TRODAT-1 as a Single-photon emission computed tomography (SPECT) imaging agent for dopamine transporters (DAT) was first reported in 1997 by Kung *et al.*, to investigate Parkinson's disease (PD) (1, 2). ^{99m}Tc labeled TRODAT-1 binds to dopamine transporter which is located on the presynaptic nerve endings in striatum with high selectivity and with simple kinetics. Studies on PD have revealed that the degeneration of dopaminergic neurons in the substantia nigra leads to a decrease in the density of presynaptic dopaminergic nerve terminals and dopamine transporters (DAT) in the striatum (2). ^{99m}Tc-TRODAT-1 scans could also serve as an important biomarker for disease severity and may be able to provide prognostic information for individual patients (3). Evaluation of early-stage

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Parkinson's disease with ^{99m}Tc-TRODAT-1 imaging was studied by Wen-Sheng Huang *et al.,* (4). Imaging with ^{99m}Tc-TRODAT-1 can help in the diagnosis of PD and differentiating PD from other movement disorders that do not affect the basal ganglia (5). ^{99m}Tc-TRODAT-1 has potential clinical use for neurological investigation of a number of other diseases besides Parkinson's (6). The preclinical pharmacologic study of dopamine transporter imaging agent ^{99m}Tc-TRODAT-1 was studied by Fang *et al.,* (7). Dopamine transporter imaging with tropane derivative such as Iodine-123-beta-carbomethoxy-3beta-(4-iodophenyltropane) (^{123}I-β-CIT) was previously developed to measure degeneration of dopamine presynaptic nerve terminal (8) which has several limitations. Firstly, ^{123}I is a high-energy cyclotron produced radionuclide, as such it is not readily available in Australia and is expensive to import. Secondly, once administered there is a 48 hr waiting prior to image acquisition. Finally, free ^{123}I from ^{123}I-β-CIT may accumulate in the thyroid and therefore it warrants blocking the thyroid with lugol solution. These limitations can be overcome with the use of other radionuclides Positron Emission Tomography (PET) tracers based on ^{11}C and ^{18}F. Carbon-11-2β-carbomethoxy-3β-(4-fluorophenyl tropane (^{11}C-CFT), has been used in PET imaging and shown to have high affinity and favourable imaging features for dopamine transporters in the basal ganglia (9). 9-[(^{18})F] fluoropropyl-(+)-dihydrotetrabenazine (^{18}F-AV-133) targeting the vesicular monoamine transporter type 2 (VMAT2) to detect monoaminergic terminal reduction in PD patients has also been reported (10). The major limitations associated with ^{11}C is its short half-life (20.33min) and therefore availability is an issue. A cyclotron needs to be available within the facility for ^{11}C studies to occur.

^{99m}Tc is generator produced within most hospital facilities and thus readily available for the preparation of ^{99m}Tc-TRODAT-1. It has the advantage of no thyroid uptake and shows good pharmacokinetics. Furthermore, images can be acquired within hours of tracer administration, but has the limitation of SPECT tracer such as compromised resolution. The in-house preparation of TRODAT-1 cold kit is cost-effective and is readily available. This also negates any logistical issues that may arise from purchasing through an overseas manufacturer. We undertook this study to standardise the in-house formulation of TRODAT-1 radiopharmaceutical cold kit and modified the radiolabeling method over the published methods.

Materials and Methods

All chemicals were obtained from Sigma, Australia. TRODAT-1 was purchased as colourless liquid from ABX (define ABX) Chemicals, Germany with purity $\geq$95% and stored at -20 $\pm$ 5°C while protected from light.

Preparation of bulk solution of TRODAT-1

Briefly, 1.0mg of TRODAT-1 was dissolved in 100µl of ethanolic HCl. (1.0mL Ethanol in 50µl HCl (2.0M)). To this solution 500µg of stannous chloride ($SnCl_2$), 10mL of sodium glucoheptonate (160mg) and 1.65mL of 0.05 M disodium Ethylenediaminetetraacetic acid (EDTA) were added and mixed. The pH of the mixture was adjusted to pH 5.0. Aliquots of 1.0mL of TRODAT-1 solution were aseptically dispensed into sterile nitrogen filled vials and stored at -20 $\pm$ 5°C while protected from light for future labeling with ^{99m}Tc pertechenate. Random samples were tested for sterility and endotoxins.

Radiolabeling of ^{99m}Tc-TRODAT-1

TRODAT-1 was radiolabeled by adding 1.5GBq of sterile pyrogen free ^{99m}Tc-pertechenate in 5.0mL of physiological saline and heated for 30min at 100°C in a block heater and allowed to cool for 10min.

Quality control (QC)

The Radiochemical purity (RCP) was measured using Instant Thin Layer Chromatography-Silica Gel (ITLC-SG) and High-Performance Liquid Chromatography (HPLC) methods. ITLC-SG was performed using ITLC-SG paper and 0.9% sodium chloride as the solvent. HPLC was performed using gradient method- solvent: 0.1 % TFA/ H_2O at 0 min (95%) at 20min (100%) & ACN, column: Kinetex C18, 250 x 4mm 5 µm, flow rate: 1.0 mL /min.

Stability Studies

The shelf life of the prepared TRODAT-1 cold kit was determined by radiolabeling with ^{99m}Tc and QC was performed at different time intervals from day 1 to 6 months. At each occasion, stability of ^{99m}Tc-TRODAT-1 was evaluated at different time points 0, 30, 60 and up to 180 minutes by ITLC-SG and HPLC. Human serum stability study was performed by mixing 0.3 ml of ^{99m}Tc-TRODAT-1 solution with 1.5ml of human serum and incubating at 37°C. Aliquots were removed and the RCP was estimated at different time points: 0min, 30min and 60min. Percentage of RCP was estimated at the end of each incubation period.

Animal Imaging

Biodistribution of ^{99m}Tc-TRODAT-1 was studied in a rabbit model. Healthy rabbits (n=2) were anesthetized by intra-peritoneal injection of the solution containing ketamine/xylazine (2:1 v/v). Animals were injected

intravenously with 30MBq of ^{99m}Tc-TRODAT-1. Images were acquired on a single head SPECT/CT gamma camera (Siemens Symbia). Whole body images of each animal were acquired for 600sec each at 2.5 zoom with a 256 x 256 matrix. Images were taken at 30 and 60min intervals post injection. Animals were euthanised according to Westmead hospital animal ethics guidelines, the animals were then dissected and the organs of interest were removed for analysis. The percentage activity of the injected dose per gram of tissue (% injected dose (ID) /g) was calculated by dividing the %ID by the actual weight of each individual tissue (g).

Patient Imaging

Patients presenting to the Movement Disorder Clinic for assessment of tremor were studied with in-house labeled ^{99m}Tc-TRODAT-1 kit. Twenty-two patients have undergone with ^{99m}Tc-TRODAT-1 scans who were aged between 35 and 74 years (mean age 54 years). Patients were injected with 800MBq of ^{99m}Tc-TRODAT-1 and early whole-body images were taken at 10 min post injection and delayed images were taken at 3-hour post injection. Images were acquired using SPECT/CT, Siemens Symbia T16 SPECT CT camera using parallel, low energy high resolution collimators OSEM reconstruction (4 iterations, 8 subsets).

Results

Quality Control

When ^{99m}Tc-TRODAT-1 was prepared as described above the RCP was high and consistent (>99%). ITLC-SG procedure took less than 2min to complete, the labeled product remained at the bottom of the strip (Rf=0.2) (Figure 1a) and free ^{99m}Tc migrated to the top (Rf=0.8) ((Figure 1b). HPLC of ^{99m}Tc-TRODAT-1 showed retention time for free ^{99m}Tc was 2.5min and for labeled product at 16.18min (Figure 2). Some minor peaks were detected earlier at 13-16min.

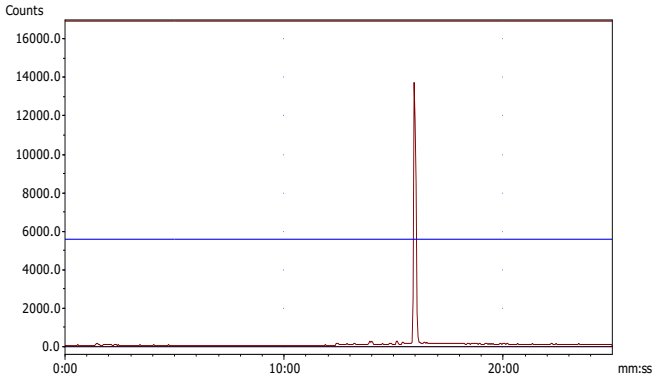


Figure 2. HPLC of ^{99m}Tc-TRODAT-1. The elution time for free ^{99m}Tc was 2.5 min and for labeled product was 16.18 min with labeling yield of > 99%.

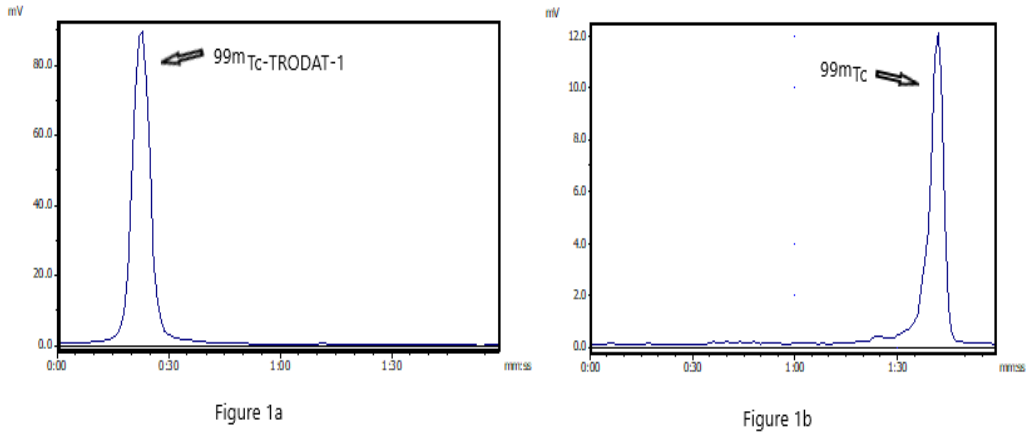


Figure 1. Comparison of ^{99m}Tc-TRODAT-1 (Figure-1a) against free ^{99m}Tc (Figure-1b) by ITLC-SG paper /0.9% sodium chloride chromatogram. Labeled product ^{99m}Tc-TRODAT-1 remained at the bottom (Rf=0.2) and free ^{99m}Tc migrated to top (Rf=0.8).

Stability

The TRODAT-1 cold kit was stable with RCP of 99% for up-to 6 months when stored at -20 $\pm$ 5°C. The results of radiolabeled ^{99m}Tc-TRODAT-1 showed stability during the period of study (0 – 180min), without any significant change in the RCP values (>99%). ^{99m}Tc-TRODAT-1 complex was stable in human serum as shown by RCP >99% when studied for up to 1h at 37°C.

Animal study

When ^{99m}Tc-TRODAT-1 (30MBq in 0.2 ml saline) was injected into healthy rabbits they showed brain hippocampus uptake of 0.32±0.08 %ID/g (Figure 3). Whole body images of the rabbits show that a small percentage (0.01%) of activity was seen in lungs and considerable uptake of the activity was seen in cardiac, blood-pool, liver and stomach (Figure 4).

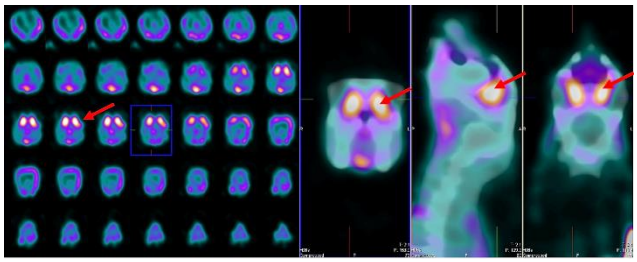


Figure 3. ^{99m}Tc-TRODAT-1 (30MBq in 0.2 ml saline) was injected into healthy rabbits they showed brain hippocampus uptake of 0.32±0.08 %ID/g

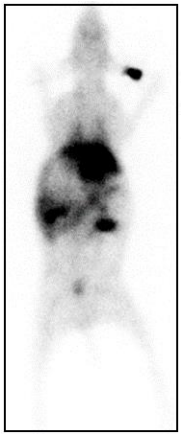
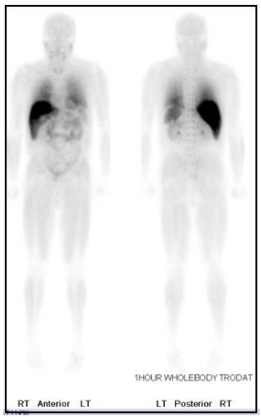


Figure 4. Rabbit whole body image. Rabbit injected with 30MBq ^{99m}Tc-TRODAT-1. The image was acquired 60 min post- injection.

Patient Imaging

Physiological biodistribution of the tracer was observed in lungs, liver and intestinal gut (Figure 5a). Uptake of the tracer was observed in the brain (striatum, putamen, and caudate nucleus) (Figure 5b).



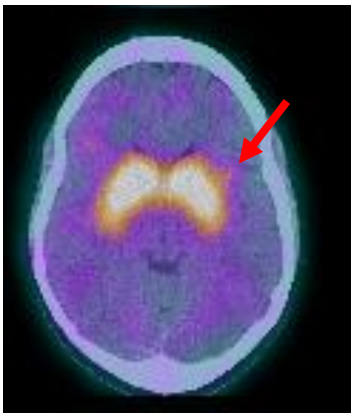


Figure 5a Figure 5b

Figure 5. Patients were injected with 800MBq of ^{99m}Tc-TRODAT-1 and the whole body and brain images were acquired at 60 min post-injection. Right Anterior and Left Posterior patient images are shown in (Figure 5a). Striatal uptake (red arrow) was observed (Figure 5b).

Discussion

^{99m}Tc-TRODAT-1 was first developed by Kung *et al.*, (1997) and subsequently several methods were published by different researchers (2, 10). Based on these published methods, we standardised the formulation of in-house TRODAT-1 cold kit.

Radiolabeling of TRODAT-1 with ^{99m}Tc using an autoclave was previously reported (2) and some groups published the use of a boiling water bath (11, 12). The water bath method described above has the limitation of water evaporating during boiling, variable yield, radiation exposure to the operator and possibility of kit contamination. With the autoclave method the temperature is uncontrollable which may result in inconsistent data. To overcome these limitations, we used a Thermoline scientific Dry Block Heater (Model TDB-1) in our study to radiolabel TRODAT-1 with ^{99m}Tc. Thermoline scientific Dry Block Heater is designed to provide uniform dry heating with accurate control stability of ±0.2°C. It also has an auto over temperature safety feature which controls the temperature. For radiation safety aspect the Dry block heater holds a 10mL vial housed inside a lead shielded pot with a lid.

This method guaranteed controlled temperature of 100°C for 30min. The RCP results using HPLC of ^{99m}Tc-TRODAT-1 clearly showed a distinct single peak. Some minor peaks at 13-16 min were detected but they are insignificant. It is likely that these peaks were due to unreacted ligand, sodium glucoheptonate, and other substrates generated used during radiolabeling process (13).

Previous formulation of TRODAT-1 cold kit used tricine as a ligand exchange agent (11). In our formulation we have used sodium glucoheptonate (16 mg) as the ligand exchange agent (2) and stannous chloride ($SnCl_2$) as a reducing agent. The advantage of excess glucoheptonate over tricine may facilitate in better solubility of TRODAT-1 ligand (2). The published methods suggested optimal amounts of $SnCl_2$ required for the reaction were found to be 32–64µg (2). We determined that 50µg of $SnCl_2$ was required per vial to ensure stability of the cold kit for up to 6 months and when stored at -20°C at pH 5.0.

Animal imaging results indicated that this radiopharmaceutical specifically bound the dopamine receptors and presented suitable characteristic for its use as an imaging agent of the basal ganglia. Cardiac blood-pool, liver and lung uptake were persistently high with very little renal excretion, which was consistent with minimal bladder activity. ^{99m}Tc-TRODAT-1 is excreted primarily by the hepatobiliary system (14).

Conclusion

In conclusion, a simple method was described to prepare ^{99m}Tc-TRODAT-1 with and high radiochemical purity (>99%), which is ideal for routine clinical preparation. This improved method can be adopted conveniently for routine productions in nuclear medicine departments for routine clinical study of dopamine transporters in humans.

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