Original Article

In-house Preparation and Quality Control of Tc99m TRODAT 1 for Diagnostic Single-photon Emission Computed Tomography/Computed Tomography Imaging in Parkinson's Disease

Rakhee Vatsa, Jaya Shukla, Bhagwant Rai Mittal, Apurva Sood, Raman Kumar Joshi, Kanchan Palarwal, Priya Bhusari, Manish Modi[1]

Departments of Nuclear Medicine and [1]Neurology, Post Graduate Institute of Medical Education and Research, Chandigarh, India

Abstract

Purpose of Study: Loss of dopamine neurons in the brain is a characteristic feature of Parkinson's disease (PD). TRODAT-1 is a tropane derivative that binds to dopamine transporter (DAT) receptors. It can be used for noninvasive *in vivo* imaging of DAT receptors leading to the early detection of PD. The present study aims to optimize the in-house radiolabeling of TRODAT-1 with Tc-99 m in hospital radiopharmacy set up along with performing single-photon emission computed tomography/computed tomography imaging in patients with PD. **Materials and Methods:** Radiolabeling was performed through transchelation method. For optimization studies, varied amount of glucoheptonate (GHA) and stannous chloride was incubated with Tc-99 m for 10 min at room temperature. TRODAT-1 was added to the reaction mixture followed by incubation at 95ºC for various time intervals. Phosphate buffer saline was added to maintain the pH of the final product. After performing the quality checks, whole-body imaging was performed to check the biodistribution in 4 patients at 1 h postinjection of 20–25 mCi (740–925 MBq) of Tc-99 m-TRODAT-1. Regional brain imaging was performed at 3–4 h. Clinical evaluation was done in control (n = 5) and in patients with PD (n = 5). **Results:** Radiolabeling yield of 100% was achieved by incubating TRODAT-1 with Tc-99 m GHA. All the quality control indicated the suitability of radiopharmaceutical for the intravenous administration. Good uptake of Tc-99 m TRODAT-1 was observed in the striatum of normal patients. However, decreased uptake was seen in patients with PD. **Conclusion:** Tc-99 m TRODAT-1 is a potential radiopharmaceutical for the diagnosis and staging PD which can be radiolabeled in-house with good yield leading to its easy availability.

Keywords: *In-house radiolabeling Parkinson's disease, single-photon emission computed tomography/computed tomography, Tc-99 m TRODAT-1*

Introduction

Parkinson's disease (PD) is a commonly seen movement disorder and stands second only to Alzheimer's disease among the neurodegenerative disorders.[1] The disease prevalence of PD ranges from 7 to 53 per one lakh population in India.[2] Men are affected more than women with both the incidence and prevalence being 1.5–2.0 times higher.[3] Loss of dopamine neuron in substantia nigra pars compacta of mid-brain is the key pathological feature of PD.[4] The four cardinal clinical motor manifestations of patients of PD are rigidity, resting tremors, bradykinesia, and postural instability.[5]

PD is regarded to be a complex systemic disorder with both motor and nonmotor symptoms. The nonmotor symptoms precede the motor symptoms by many years, and it is during this early stage of the disease that the diagnosis of PD becomes challenging for a physician solely on the basis of signs and symptoms. The diagnosis is further complicated by the fact that the cardinal symptoms of PD are common to other disorders too. A definite diagnosis can only be made by demonstration of Lewy Bodies in the brain autopsy. Unfortunately, anatomic imaging modalities such as magnetic resonance imaging and computed tomography (CT), are neither helpful in diagnosing PD nor are they beneficial in monitoring the disease-related changes over the course of time.[6,7] Thus, there is a critical need for the development of a biomarker for the earlier and accurate diagnoses of PD.

Various PET and single-photon emission computed tomography (SPECT) tracers are available for the imaging of the

How to cite this article: Vatsa R, Shukla J, Mittal BR, Sood A, Joshi RK, Palarwal K, *et al.* In-house preparation and quality control of Tc99m TRODAT 1 for diagnostic single-photon emission computed tomography/computed tomography imaging in Parkinson's disease. Indian J Nucl Med 2017;32:266-70.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Address for correspondence:
Dr. Jaya Shukla, Department of Nuclear Medicine, Post Graduate Institute of Medical Education and Research, Chandigarh - 160 012, India.
E-mail: shuklajaya@gmail.com

Access this article online

Website: www.ijnm.in

DOI: 10.4103/ijnm.IJNM_111_17

Quick Response Code:

© 2017 Indian Journal of Nuclear Medicine | Published by Wolters Kluwer - Medknow

dopaminergic system.[8] Several dopamine transporter (DAT) imaging agents based on cocaine or the closely related tropane derivatives have been reported. Tc-99 m TRODAT-1, ([2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3,2,1] oct-2-l] methyl](2 mercaptoethyl) amino] ethyl] amino] ethanethiolato (3)-*N*2,*N*2',*S*2,*S*2']oxo-[1*R*-(exo-exo)]-), is the first Tc-99 m-labeled tracer of DATs and was developed by Kung *et al*.[9] Tc-99 m-labeled radiotracer has many advantages, namely, it is less expensive, has optimal 6 h half-life, and 140 keV of gamma energy, making it suitable for detection by the conventional gamma camera. Imaging with Tc-99 m TRODAT-1 can help in the diagnosis of PD and differentiating PD from other movement disorders that do not affect the basal ganglia.

Various kit-based preparations have been reported earlier in the literature.[10-12] In the present study, the authors report the radiolabeling, characterization of TRODAT-1 in-house with Tc-99 m, and diagnostic SPECT-CT imaging in patients with PD.

Materials and Methods

Trodat-1 was procured from ABX (Advanced Biochemical Compounds), Germany, as a pale yellow liquid, which was reconstituted in the ethanolic hydrochloric acid. The 20 µl aliquots (5 µg/µl) were stored at −20°C till used further. Glucoheptonate (GHA) and anhydrous stannous chloride ($SnCl_2$) were purchased from Sigma. Supra pure HCl and high-performance liquid chromatography grade water were used. Tc-99 m was eluted from commercially available Mo-99/Tc-99 m generator. Instant thin-layer chromatography-Silica gel (ITLC-SG) paper (Merk) and thin-layer chromatography (TLC) scanner (EZ-Scan, USA) with multimode radiation detector (Omni Rad) were used for chromatography.

Radiolabeling

Radiolabeling of Trodat-1 with Tc-99 m was performed through transchelation. GHA was used as a transchelator and anhydrous stannous chloride as a reducing agent. Varied concentration of GHA and $SnCl_2$ were incubated with Tc-99 m at room temperature for 10 min. The pH of reaction mixture was kept acidic (3.0–3.5). Trodat-1 (100–200 µg) was added to the reaction mixture and incubated at 95°C for various time intervals (15 min, 30 min, 45 min, and 60 min). The total volume of the reaction mixture was restricted to maximum of 1.0 ml. After adding 50 µl of 0.05 M EDTA, the reaction mixture was again incubated for 10 min to bind free Tc-99 m if present in the solution. The pH of the final preparation was adjusted to the physiological range by adding phosphate buffer saline (PBS) to the reaction mixture.

Quality control

To ensure the safety of drugs before intravenous administration, Tc-99 m TRODAT-1 was subjected to various physiochemical, biological, and stability tests.[13,14]

Radiochemical purity

Radiochemical purity and labeling yield of Tc-99 m TRODAT-1 was determined by ITLC. Saline and acetone were used as mobile phase, and ITLC-SG was used as stationary phase. A volume of 3–4 µl drop of sample solution was applied to the ITLC strip. After running the strips in saline and acetone, the strips were read using radio TLC scanner. Preparations with <95% radiochemical purity were discarded.

Sterility

To test the sterility of Tc-99 m TRODAT-1 preparation, sterility testing procedures were followed as described earlier.[15] Briefly, the sample solution (1 ml) was incubated in tryptic soya broth at 37°C. Turbidity in the incubated samples was observed for up to 7 days to check the presence of any microorganism.

Pyrogenicity

Pyrogens are the fever causing substances if present in the preparations can cause adverse reaction. Depending on the amount of endotoxins present, the symptoms can be seen 30 min-2 h postadministration of the drug.[13] In the present study, pyrogenicity was checked by cartridge-based PTS (point-of-use, portable test system, Charles River, USA) which is based on the principle of kinetic chromogenic technique. The Limulus amebocyte lysate reagent on interacting with the endotoxins present in the Tc-99 m TRODAT-1 samples results in initiation of cascade of reactions and formation of the colored substrate.[16] Tc-99 m TRODAT-1 samples were selected randomly, and 25 µl from each preparation was added to each well in the PTS cassette. Incubation was done at 38°C for 15–20 min for the reaction to take place. All observations were noted with 50%–200% spike recovery. The test was sensitive for up to 0.01 Endotoxin Unit (EU)/ml.

Stability

Tc-99 m TRODAT-1 samples were incubated in PBS for up to 6 h at 37ºC. The radiochemical purity was assessed every hour for 6 h by ITLC-SG as illustrated earlier.[17]

Clinical evaluation

A total of 10 persons were enrolled in the study. Five of these participants (2 males and 3 females, mean age 61 years ± 11.9) were clinically diagnosed as idiopathic PD on the basis of the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria. Five were age- and gender-matched healthy volunteers (2 males and 3 females, mean age 62 years ± 11.3). Written informed consent was taken from all the participants before enrolling in the study. The study was duly approved by the Institute Ethics committee.

Imaging of the brain was performed 3–4 h after injecting 20–25 mCi (740–925 MBq) of Tc-99 mTRODAT-1, using a

dual-headed SPECT/CT (Symbia T-16; Siemens, Erlangen, Germany) equipped with LEHR (low energy high resolution) collimators. Data acquisition was performed in 128 × 128 matrix; 3° angular rotation, 180°/detector and 64 projections with each projection of 30 s. Reconstruction of data was done using OSEM (Ordered subset expectation maximization method) with CT-based attenuation.

In addition to the brain scan, 4 persons (2 from each group) underwent a whole-body scan 1-h postinjection to look for the biodistribution of the Tc-99 m TRODAT-1. The imaging was done in 256 × 1024 matrix with table speed of 13 cm/min.

Results

Radiolabeling

Various reaction parameters were optimized for obtaining good radiolabeling. Maximum radiolabeling of 100% (98%–100%) was achieved when 8 μg GHA/mCi Tc-99 m was used as shown in Figure 1a. However, for $SnCl_2$: GHA, 1:10 was observed as optimum ratio for obtaining good radiolabeling (~100%) [Figure 1b]. >95% radiolabeling yield was observed by incubating the reaction mixture at 95ºC for 30 min. However, maximum radiolabeling yield (100%) was achieved when the reaction mixture was incubated for 60 min at 95ºC as shown in Figure 1c. Under optimized condition, 100% radiolabeling yield was achieved by incubating 100 μg of TRODAT-1 with 70 mCi Tc-99 m, 560 μg GHA, and 56 μg $SnCl_2$ at 95ºC for 45–60 min.

Quality control

Radiochemical purity and radiochemical yield of Tc-99 m TRODAT-1 were 95%–100% using acetone and saline as a mobile phase for all radiolabeling preparations. All samples were found to be sterile on incubation for up to 7 days at 37°C, indicating that the samples were suitable for the intravenous administration. The endotoxin content was found to be well within the injectable limits (<175 EU/V, where V is the maximum injectable volume).[18] The maximum amount of endotoxin present in the samples was 3.1 EU/ml (1.02–3.1 EU/ml). The radiochemical purity was reduced to 95% when the radiopharmaceutical was incubated with PBS at room temperature for 3 h. Further drop in RCP (92%) was observed at 6 h (postlabeling) as shown in Figure 1d.

All the quality control parameters indicated suitability of the preparation for intravenous administration.

Patient imaging

Imaging was performed in total 10 candidates. Tracer uptake was noted in the brain, nasal mucosa, stomach, bilateral lungs, liver, and intestines at 1 h [Figure 2]. The brain scans done at 3–4 h showed symmetrical comma-shaped tracer uptake in the bilateral striatum (caudate and putamen) in the control group. This uptake was intense with less background uptake in rest of the brain [Figure 3a]. However, the cases of PD showed asymmetrical tracer uptake in the bilateral striatum region with more profound loss in the putamen region, contralateral to the side of dominant symptoms. The background tracer activity was higher in comparison to the control group [Figure 3b].

Discussion

Loss of DAT is the hallmark of PD. TRODAT-1 is a tropane derivative which specifically binds to DATs and can be used for the early diagnosis of PD. We have demonstrated successful radiolabeling of TRODAT-1 with Tc-99 m, in-house in a hospital radiopharmacy set up. In the present study, GHA was used as transchelator. Only microgram quantity of GHA is sufficient for

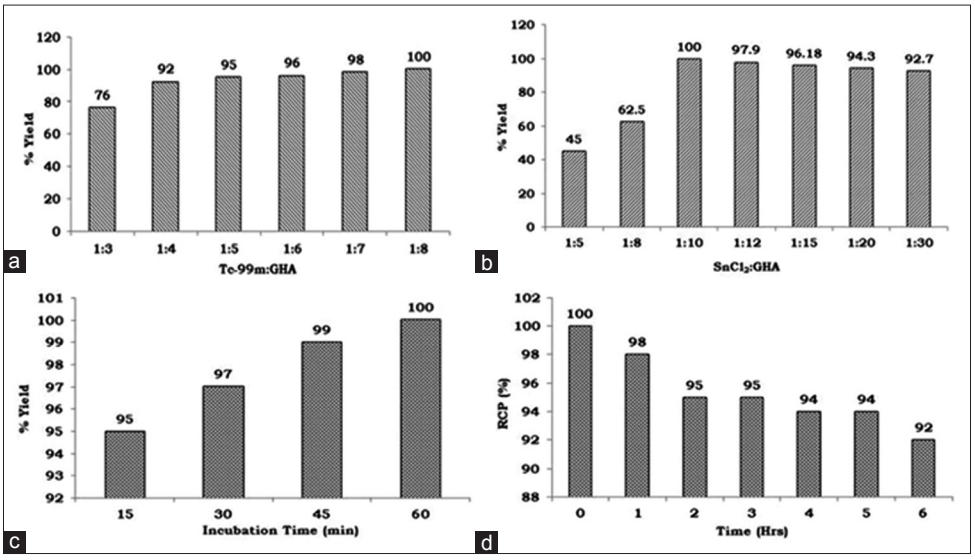

Figure 1: Effect of (a) amount of glucoheptonate with respect to Tc-99 m radioactivity, (b) $SnCl_2$ with respect to glucoheptonate, (c) incubation time on radiolabeling yield, and (d) stability of Tc-99 m TRODAT-1 at room temperature with time

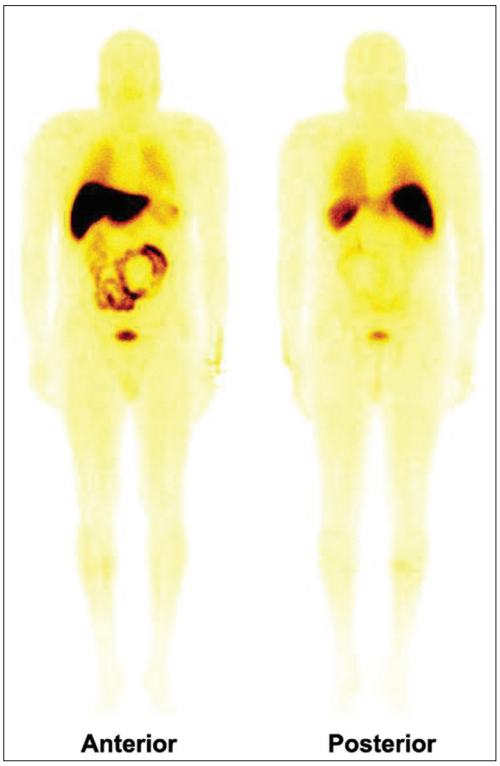

Figure 2: Whole-body imaging performed at 1 h showing physiological tracer distribution in the brain, nasal mucosa, stomach, bilateral lungs, liver, and intestines

100% radiolabeling yield. Anhydrous $SnCl_2$ was used as a reducing agent. For GHA: $SnCl_2$, 1:10 ratio was found to be optimum. Further increasing the amount of $SnCl_2$ resulted in colloid formation leading to decreased radiolabeling yield as shown in Figure 1b. However, in single- and multiple-vial kit-based preparation higher amounts (in mg) of GHA and $SnCl_2$ are needed for radiolabeling of TRODAT-1.[10,11] In the another study, tricine was used in lieu of GHA for radiolabeling of TRODAT-1 with Tc-99 m.[18]

In the present study, the reaction was carried out in boiling water bath; however, autoclaving of reaction mixture for radiolabeling has been reported in the literature.[9,10] Similar results were obtained (95%) with 30-min heating at 95°C as shown in Figure 1c. However, 99%–100% radiolabeling could be achieved by hearing at 95°C for 45–60 min. Various authors have reported the use of 200 μg of peptide for up to 30 mCi of Tc-99 m.[9,10] However, in our study, up to 140 mCi of Tc-99 m was used for radiolabeling of 200 μg peptide. Hence, in-house preparation gives an advantage of using peptide more judiciously according to the number of patients and makes the tracer cost-effective.

As the preparation was proposed for human use, strict quality control procedures were followed. RCP of 95%–100% was achieved for all preparations. All the samples passed sterility and endotoxins test indicating the suitability of the radiopharmaceutical for intravenous

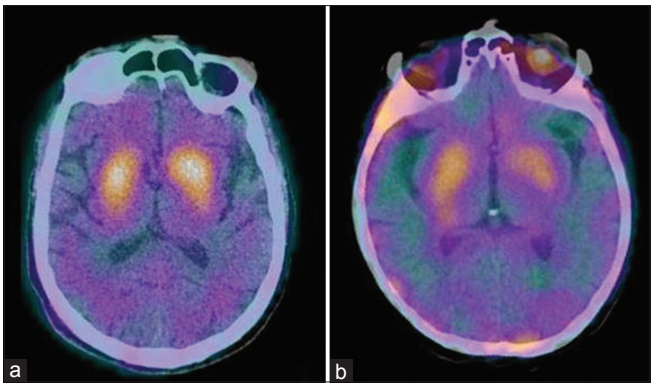

Figure 3: Single-photon emission computed tomography/computed tomography tranxial image (a) of 70-year-old healthy volunteer showing bilaterally symmetrical comma-shaped normal uptake in striatum, (b) of a 68-year-old patient diagnosed to have idiopathic Parkinson's disease with predominant right-sided symptoms showing reduced tracer uptake in the left putamen

administration. The preparations with <95% RCP were not used for patients administration.

Similar to the study conducted by Mozley *et al.*, SPECT/CT of the brain and whole -body images demonstrated that the TRODAT-1 binds to the dopaminergic transporters in the bilateral striatum.[19] Tracer activity was seen in the brain, nasal mucosa, and stomach. Tracer excretion through the hepatobiliary excretion with uptake seen in the liver and the intestines was noted. Diffuse tracer activity in the bilateral lung fields was seen, which has been attributed to the prolonged plasma activity. The lack of the tracer uptake in the thyroid and bilateral salivary glands up to 1 h, points toward the absence of the free pertechnetate which is consistent with the quality control procedure showing 95%–100% labeling efficiency.

The control group showed lesser background tracer activity, and more intense symmetrical tracer uptake in bilateral striatum suggesting the absence of dopaminergic dysfunction in this group. While the patients of PD showed asymmetrical tracer uptake in the bilateral striatum with more profound loss in the putamen contralateral to the dominant symptom side. The background activity was higher in the cases compared to the control group. Therefore, the presence of asymmetry in the striatal uptake is helpful in differentiating cases of PD from healthy population.[15]

Conclusion

TRODAT-1 can be successfully radiolabeled with Tc-99 m in-house with high radiolabeling and radiochemical yield. The clinical images demonstrate the usefulness of Tc-99 m TRODAT-1 for DAT imaging.

Acknowledgment

This work is a part of the research project grant sanctioned by the Indian Council of Medical Research, New Delhi.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Muangpaisan W, Hori H, Brayne C. Systematic review of the prevalence and incidence of Parkinson's disease in Asia. J Epidemiol 2009;19:281-93.
2. Gourie-Devi M. Epidemiology of neurological disorders in India: Review of background, prevalence and incidence of epilepsy, stroke, Parkinson's disease and tremors. Neurol India 2014;62:588-98.
3. Haaxma CA, Bloem BR, Borm GF, Oyen WJ, Leenders KL, Eshuis S, *et al.* Gender differences in parkinson's disease. J Neurol Neurosurg Psychiatry 2007;78:819-24.
4. Pavese N, Brooks DJ. Imaging neurodegeneration in Parkinson's disease. Biochim Biophys Acta 2009;1792:722-9.
5. Williams DR, Litvan I. Parkinsonian syndromes. Continuum (Minneap Minn) 2013;19:1189-212.
6. Koller WC, Langston JW, Hubble JP, Irwin I, Zack M, Golbe L, *et al.* Does a long preclinical period occur in Parkinson's disease? Geriatrics 1991;46 Suppl 1:8-15.
7. O'Sullivan SS, Massey LA, Williams DR, Silveira-Moriyama L, Kempster PA, Holton JL, *et al.* Clinical outcomes of progressive supranuclear palsy and multiple system atrophy. Brain 2008;131:1362-72.
8. Stockner H, Sojer M, K KS, Mueller J, Wenning GK, Schmidauer C, *et al.* Midbrain sonography in patients with essential tremor. Mov Disord 2007;22:414-7.
9. Kung MP, Stevenson DA, Plössl K, Meegalla SK, Beckwith A, Essman WD, *et al.* [99mTc] TRODAT-1: A novel technetium-99m complex as a dopamine transporter imaging agent. Eur J Nucl Med 1997;24:372-80.
10. Choi SR, Kung MP, Plössl K, Meegalla S, Kung HF. An improved kit formulation of a dopamine transporter imaging agent: [Tc-99m] TRODAT-1. Nucl Med Biol 1999;26:461-6.
11. Shinto AS, Antony J, Kamaleshwaran K, Vijayan K, Selvan A, Korde A, *et al.* Correlative Tc-99m labeled tropane derivative single photon emission computer tomography and clinical assessment in the staging of Parkinson disease. World J Nucl Med 2014;13:178-83.
12. Yeh CB, Chou YH, Cheng CY, Lee MS, Wang JJ, Lee CH, *et al.* Reproducibility of brain dopamine transporter binding with tc-99m TRODAT-1 SPECT in healthy young men. Psychiatry Res 2012;201:222-5.
13. Saha GB. Fundamentals of Nuclear Pharmacy. 5th ed. New York: Springer-Verlag; 2004.
14. Rajan MG. PET radiochemistry and PET radiopharmaceuticals. In: Pant GS, editor. Advances in Diagnostic Medical Physics. 1st ed. Gurgaon: Himalaya Publishing House; 2006. p. 382-402.
15. Shukla J, Vatsa R, Garg N, Bhusari P, Watts A, Mittal BR, *et al.* Quality control of positron emission tomography radiopharmaceuticals: An institutional experience. Indian J Nucl Med 2013;28:200-6.
16. Joiner TJ, Kraus PF, Kupiec TC. Comparison of endotoxin testing methods for pharmaceutical products. Int J Pharm Compd 2002;6:408-9.
17. Erfani M, Shafiei M. Preparation of 99mTc-TRODAT-1 with high labeling yield in boiling water bath: A new formulation. Nucl Med Biol 2014;41:317-21.
18. Mozley PD, Stubbs JB, Plössl K, Dresel SH, Barraclough ED, Alavi A, *et al.* Biodistribution and dosimetry of TRODAT-1: A technetium-99m tropane for imaging dopamine transporters. J Nucl Med 1998;39:2069-76.
19. Weng YH, Yen TC, Chen MC, Kao PF, Tzen KY, Chen RS, *et al.* Sensitivity and specificity of 99mTc-TRODAT-1 SPECT imaging in differentiating patients with idiopathic Parkinson's disease from healthy subjects. J Nucl Med 2004;45:393-401.