Development of a freeze-dried radiopharmaceutical kit for dopamine transporters imaging

Original Article

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ABSTRACT

Introduction: ^{99m}Tc-TRODAT-1 is a promising new radiopharmaceutical with the potential for routine use as the radiopharmaceutical for dopamine transporters scintigraphy as far as its image quality and daily availability are concerned. Here we describe the development of a freeze-dried kit formulation based on the tricine exchange labeling approach for the preparation of this radiopharmaceutical in a clinical setting.

Methods: A freeze-dried formulation contained of TRODAT-1, tricine, $SnCl_2$ and manitol was prepared. Labeling was performed by addition of 1480 MBq ^{99m}Tc sodium pertechnetate in a total volume of 2 mL and incubation for 15 min in a boiling water bath. Radiochemical analysis involved ITLC and HPLC methods. The stability of radioconjugate was checked in the presence of human serum at 37 °C up to 24 h.

Results: ^{99m}Tc-TRODAT-1 was prepared with a radiochemical purity of >95% and a high stability up to 24 h of the final preparation, and retained biological activity.

Conclusion: The developed kit formulation forms the basis for further clinical evaluation of this promising new radiopharmaceutical.

Key words: Radiopharmaceutical; Trodat-1; Kit; Dopamine transporters

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INTRODUCTION

Dopamine is a neuroendocrine transmitter in the catecholamine and phenethylamine families that plays a number of important roles in the brain and other organs of human. The brain includes several specific dopamine systems, one plays a major role in motivated behavior. Several important diseases of the nervous system are associated with dysfunctions of the dopamine system. One of the regulatory mechanisms being pumping of the dopamine back to the presynaptic neurons through the dopamine transporters (DAT). In patients with Parkinson and Alzheimer diseases decline in density of these transporters have been accessed [1, 2].

Ligands such as [^{11}C]CFT for PET imaging [3], and [^{123}I]β-CIT [4] for SPECT imaging, have shown high affinity and imaging features for dopamine transporters in nervous system. Despite all these useful results, radioisotopes of ^{11}C or ^{123}I are produced by the cyclotron is necessary to use the imaging technique. Access to a cyclotron radioisotope can cause serious limitations in using this method in routine clinical diagnostic procedures. The radionuclide of choice would be ^{99m}Tc, produced by a radionuclide generator and therefore early daily available, with 6 h half-life and 140-keV monoenergetic gamma-ray emission ideal for conventional nuclear medicine imaging procedures [5]. So preparing technetium-based radiopharmaceutical for dopamine transporters with same characteristics can be ideal for clinical application. In reaching this goal, recent success in preparation and development of ^{99m}Tc-TRODAT-1 could give researchers a new prospect for further research in this area [6, 7]. Technepine is another thecnetium-99m based compound that can be useful for research in this field [8].

^{99m}Tc-TRODAT-1 prepared by a multistep procedure is previously reported [9] as well as all the components in a single vial [10]. To achieve the desired purity of the product, solvent extraction and liquid chromatography have been proposed [11]. In all these methods, need to autoclave for 30 min to achieve high purity, is one of the difficulties of the method. It is highly desirable to develop a simplified radiopharmaceutical kit formulation with no need to autoclave the final product during the labeling step. We recently reported a new formulation for preparation of ^{99m}Tc-TRODAT-1 with high labeling yield in boiling water bath [12]. However, since this is a wet formulation, we attempted to develop a clinically suitable freeze-dried kit preparation. The aim of this study was to develop a freeze-dried kit formulation with good labeling yields for routine preparation of ^{99m}Tc-TRODAT-1 in clinical situations.

METHODS

All reagents were obtained from commercial sources and used without further purification. TRODAT-1 was commercially available from ABX advanced biochemical compounds. $^{99m}TcO_4^-$ was eluted from an in-house ^{99}Mo/^{99m}Tc column generator using 0.9% saline. Radiolabeling was always performed using an eluate with not more than 4 h of age from a generator eluted 24 h previously.

A JASCO 880-PU intelligent pump HPLC system equipped with a multiwavelength detector and a flow-through Raytest-Gabi γ-detector was used for analytical reverse phase high performance liquid chromatography (RP-HPLC). A CC 250/4.6 Nucleosil 120-5 C-18 column from Teknokroma, flow rates of 1 mL/min and UV detection at 280 nm were employed with the following gradient.

0.1% trifluoroacetic acid/water (Solvent A) and acetonitrile (Solvent B) as a mobile phase: 0 min 95% A (5% B), 5 min 95% A (5% B), 20 min 0% A (100% B), 25 min 0% A (100% B), 30 min 95% A (5% B).

Instant thin layer chromatography on silica gel (ITLC-SG) was performed using 1M ammonium acetate/acetone 1/1 for ^{99m}Tc colloid (Rf=0). The radioactivity was quantified by cutting the strip (1.5 × 10 cm^2) into 1 cm pieces and counting in a well type gamma counter. Quantitative gamma counting was performed on an ORTEC Model 4001M γ-system well counter.

Kit formulation and freeze drying

A stock solution was prepared by dissolving tricine and mannitol in phosphate buffer (20 mg/mL). TRODAT-1 was dissolved in ethanol containing 10% 1N HCl to a concentration of 500 µg/mL, $SnCl_2$. $2H_2O$ in nitrogen purged 0.1 N HCl (1 mg/mL) immediately before use.

The TRODAT-1 and $SnCl_2$. $2H_2O$ were added to the stock solution and final solution dispensed in 1 mL containing 20 mg tricine, 20 mg mannitol, 40 µg $SnCl_2$. $2H_2O$ and 10 µg TRODAT-1. Dispending was performed done in sterile type I glass vials after passing the solution through 0.22 µ sterile filter.

Vial was immediately frozen and loaded into freeze dryer with a shelf temperature of -40°C and freeze drying was started. Primary drying was performed for 20 h with a shelf temperature of -10°C and 0.630 mbar pressure for the first 4 h and 0.310 mbar for 16 h, secondary drying increasing the shelf temperature up to 10°C and reducing the pressure to 0.050 mbar for 4 h. Vial was capped under vacuum and stored at 2-8°C.

Radiolabeling with ^{99m}Tc

Radiolabeling was performed by adding 0.5 mL saline to the freeze dried kit formulation in the evacuated vial and immediately afterwards 1480 MBq of $^{99m}TcO_4^-$ in 1 mL saline was added. Labeling was completed by incubation of vial in boiling water bath for 15 min and subsequently cooling down to room temperature for 15 min.

Quality control

The kit quality was evaluated studying the appearance of the pellet, the dissolution time when the pellet disappeared by addition of saline to the vial and its radiochemical purity. ^{99m}Tc-labeled TRODAT-1 was characterized by analytical RP-HPLC and ITLC-SG immediately and up to 24 h after radiolabeling with the above mentioned methods.

The stability was evaluated in saline and in human serum. Aliquots were taken out at different time point post reconstitution at room temperature and analyzed by HPLC and ITLC. An aliquot of labeled formulation (100 µl) was added to freshly prepared human serum (1 mL) and the mixture was incubated in a 37 °C environment. 100 µl aliquots were removed at the different time points and treated with 100 µl of alcohol. Samples were centrifuged for 5 min at 3000 rpm to precipitate serum proteins and for supernatants ITLC and HPLC were performed.

Biodistribution

Animal experiments were performed in compliance with the regulations of our institution and with generally accepted guidelines governing such work. A group of three rats received 20 MBq of high specific activity radiotracer in 0.15 ml of saline via a tail vein. The rats were sacrificed at different post injection times and the tissues and organs of interest were collected, wet weighed and counted in a NaI well-type γ-counter. The percentage of injected dose per gram (%ID/g) for each sample was calculated by comparing its activity with appropriate standard of injected dose (ID). The values are expressed as mean ± SD. At 4 h after injection, accumulation of the radioligand was also assessed by planar scintigraphy under ether anesthesia.

RESULTS AND DISCUSSION

Freeze dried formulation

The radiopharmaceutical development of a freeze dried kit formulation for TRODAT-1 (Figure 1) was based on an exchange labeling protocol with optimized labeling conditions. To develop a one vial kit of ^{99m}Tc-TRODAT-1, ingredients including TRODAT-1, tricine, mannitol and $SnCl_2$. $2H_2O$ were combined in the adequate quantity. A patient dose of 10 µg was considered as an optimal amount to give the maximum target uptake in vivo. Although this amount was very low, but sufficient amount of ligand in formulation was presented yet to reach a high labeling yield complex formation.

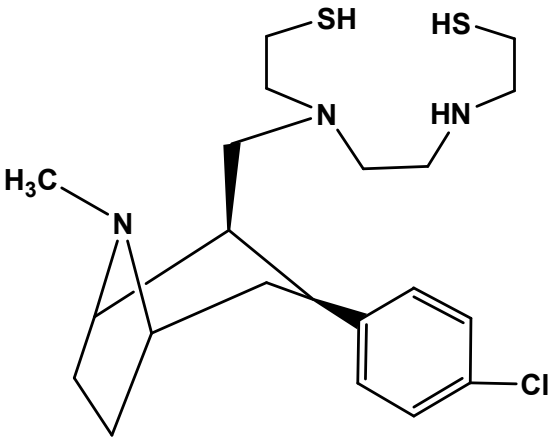

Fig 1. Chemical structure of TRODAT-1.

Tricine has been used as a ligand exchange in formulation which its optimal required amount was found to be 20 mg. Although tricine previously has been used as a coligand, but it has been showed that tricine could also had an exchange labeling function when it has been used in combination with EDDA as a coligand in labeling of peptides with HYNIC moiety [13].

Stannous chloride was used to reduce ^{99m}Tc to lower stage which could be able to react with ligand. A stable labeling with high radiochemical purity (>95%) can be acquired with 40 µg $SnCl_2$. This low amount is still sufficient to reduce technetium in the labeling process.

The pH in the freeze drying solution was of great importance to achieve high labeling yields. The optimal pH range to produce a high labeling yield of ^{99m}Tc-TRODAT-1 was found to be neutral pH. In the previous reported formulation ^{99m}Tc-TRODAT was formed in an acidic medium (in the presence of HCl) which was adjusted to 5-6 by adding 0.5 mL of phosphate buffer before injection into humans [9, 10]. Our previous studies showed, while tricine is used with EDDA as a coligand the highest labeling yield would be obtained when pH is in neutral range [14-18].

A bulking agent such as mannitol also was used as an excipient in formulation to provide a sufficient mass of substances to achieve a well shaped pellet of the freeze dried product.

Labeling and quality control

The lyophilized vial contain 10 µg TRODAT-1, 20 mg tricine, 40 µg $SnCl_2$, 20 mg mannitol was labeled with radioactivity of 1480 MBq of ^{99m}Tc (specific activity of 64 MBq/nmol). Labeling completed over a short period of time (15 min) and in a lower temperature (95 °C) compared with previous formulations which a time of 30 min autoclaving is necessary to reach ≥90% radiochemical purity [9-11].

A typical radiochromatogram of ^{99m}Tc-TRODAT-1 prepared via this kit formulation has been shown in Figure 2.

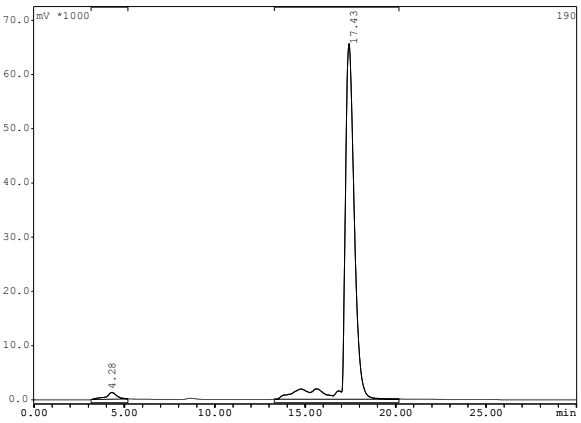

Fig 2. HPLC profile of the radiopharmaceutical kit after labeling with ^{99m}Tc.

The radiochemical purity of the radiolabeled preparation was clearly above 95%. A single peak is shown at a retention time of 17.67 min corresponding to the radiolabeled TRODAT-1, additionally minor peaks corresponding to ^{99m}Tc-tricine and free pertechnetate are seen at early retention time (4.28 min). These results confirm the exchange reaction of tricine with TRODAT-1. The results from ITLC analysis showed reduced hydrolysed technetium which remained at origin, was less than 1%.

The stability of the radiolabeled kit was very high (>90%) and it did not drop below this level even after 24 h which generally can be considered suitable for clinical applications. It is extremely important for radiodiagnostic agents that isotope chelation remain stable with the passing of time. The high labeling yield and stability could be attributed to the amounts of materials which were used and also in our labeling method.

Biodistribution

The results of the biodistribution studies revealed high uptake in kidney, liver, muscle, skin and lung (Table 1).

Table 1: Biodistribution of radiopharmaceutical labeled kit in rat (%ID/g ± SD, n=3).

Organs	Time (min)			
	2	30	60	120
Blood	1.21 ± 0.12	0.31 ± 0.04	0.15 ± 0.01	0.17 ± 0.21
Spleen	1.48 ± 0.21	3.57 ± 0.41	1.43 ± 0.10	1.57 ± 0.14
Kidney	5.21 ± 0.52	3.34 ± 0.55	2.54 ± 0.20	1.89 ± 0.35
Intestines	1.35 ± 0.41	6.84 ± 1.01	5.12 ± 0.98	10.61 ± 1.53
Liver	2.58 ± 0.54	5.01 ± 0.68	4.52 ± 0.32	3.98 ± 0.64
Lung	17.98 ± 1.66	15.21 ± 1.34	3.92 ± 0.10	4.01 ± 0.68
Heart	5.03 ± 0.96	1.46 ± 0.35	0.50 ± 0.08	0.40 ± 0.06
Brain	0.49 ± 0.05	0.38 ± 0.08	0.22 ± 0.04	0.17 ± 0.05

The brain uptake was 0.49 ± 0.05% ID/g at 2 min. The striatal region (ST) exhibited the highest uptake within the brain, where the dopamine transporters are located, in contrast to a region with no dopamine neurons like cerebellar region (CB). At 4 h after injection the ratio of ST/CB was found to be 4.16. All of the uptakes by the different organs in rats were in agreement with the published data [19] which this confirming the similarity of its biological characteristics.

Scintigraphy study showed early uptake 1 h post injection of radioligand in liver, intestine, kidneys and brain (Figure 3).

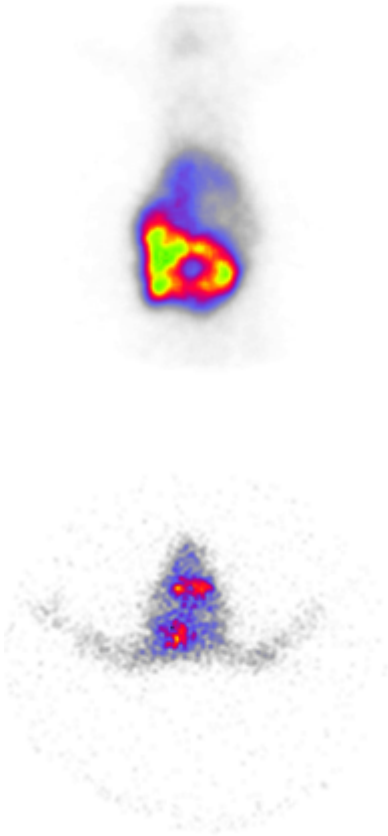

Fig 3. Sintigraphy image of labeled radiopharmaceutical kit in rat at 1 h post injection of 18.5 MBq of radiotracer in 100 µL of saline via tail vein. Whole body scan (above) and brain site after masking of abdominal region (below).

The brain uptake could be visualized through scintigraphy which confirms the specific uptake of radioligand. The behavior like liver uptake and intestinal excretion is a typical pharmacokinetic characteristic for a lipophilic diagnostic radiopharmaceutical.

CONCLUSION

Our results show that ^{99m}Tc-TRODAT-1 can be prepared using the described freeze-dried kit formulation conveniently applicable at clinical levels. Biodistribution studies and scintigraphic images confirmed the suitable properties of this preparation. Therefore, our results presented by this work suggest that this kit is a promising formulation for in vivo imaging studies.

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