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Synthesis and Preliminary Biological Evaluation of ^{99m}Tc Tricarbonyl Ropinirole as a Potential Brain Imaging Agent

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Abstract—Ropinirole, a non-ergoline dopamine agonist, was labeled with ^{99m}Tc tricarbonyl {[^{99m}Tc(CO)$_3$·(H$_2$O)$_3$]$^+$} with the aim of obtaining a new brain imaging agent. For in vivo use, the radiosynthesis of ^{99m}Tc tricarbonyl ropinirole was performed by heating a solution containing ropinirole and the precursor, ^{99m}Tc tricarbonyl, on a boiling water bath for 30 min. The influence of the substrate amount and pH on the reaction was studied to optimize the synthesis. The biodistribution and scintigraphic studies demonstrate the suitability of ^{99m}Tc tricarbonyl ropinirole as a novel tracer for brain tumor imaging.

Keywords: technetium-99m tricarbonyl, ropinirole, brain imaging agents

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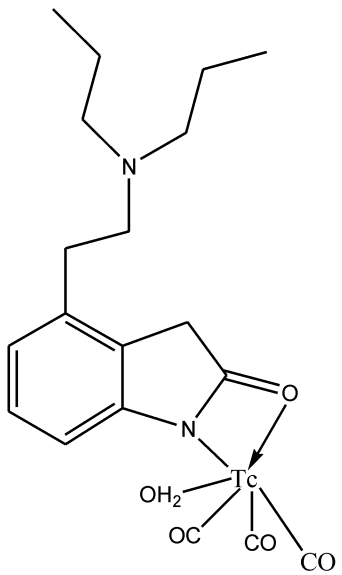
Ropinirole is a dopamine agonist of the non-ergoline class of medications. It is used in the treatment of Parkinson's disease (PD, a long-term degenerative disorder of the central nervous system that mainly affects the motor system) and restless legs syndrome (RLS). Ropinirole has high relative in vitro specificity and acts as a D_2, D_3, and D_4 dopamine receptor agonist with the highest affinity for D_2 [1–3]. The chemical name of ropinirole is 4-[2-(dipropylamino)ethyl]-2,3-dihydro-1*H*-indol-2-one. In addition, ropinirole is efficacious in the management of more advanced Parkinson's disease in patients experiencing motor complications after long-term use of levodopa [4] and is administered for the RLS treatment [5]. Therefore, it can be expected that ropinirole-based radiopharmaceuticals will be suitable for brain (or neuro) imaging using single photon emission computed tomography (SPECT) [6, 7]. The *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$)]$^+$ species is particularly attractive because of the ease of its preparation via an aqueous-based kit, versatile coordination chemistry, and formation of the kinetically inert complex [8]. The proposed structure of the complex, ^{99m}Tc tricarbonyl ropinirole, is shown below. The quality characteristics of the agent were determined, and the biodistribution was studied to evaluate the diagnostic potential of the new radiopharmaceutical.



^{99m}Tc tricarbonyl ropinirole (proposed structure).

EXPERIMENTAL

Reagents. Ropinirole was obtained as a gift from EVA Pharmaceutical Co. (Giza, Egypt). Ethanol and methanol were purchased from Sigma. Pertechnetate ($^{99m}TcO_4^-$) was eluted from a $^{99}Mo/^{99m}Tc$ generator (Elutec, Brussels, Belgium). All the other chemicals and solutions were purchased from Merck (the United States). All the reagents used in this study were of analytical grade and were used without additional purification. Whatman chromatographic paper no. 1 (PC-01) was supplied by Merck.

Apparatus. A well-type NaI scintillation γ-counter, Scalar Ratemeter SR-7 model (Nuclear Enterprises, the United States) was used for radioactivity measurement. An E.C. paper electrophoresis apparatus (3000 p-series programmable power and chamber supply units, Albany, OR, the United States) was used to study the charge nature of complex. The HPLC system (Shimadzu) consisting of LC-9A pumps, Rheodyne injector, UV spectrophotometer detector (SPD-6A) operating at a wavelength of 245 nm, and a reversed-phase column (RP-18, 250 × 4.6 mm, 5 μm, Lichrosorb) was used to check the purity of the radiopharmaceutical. HPLC analysis of the complex was done by injecting a 10-μL sample of the solution of the complex, prepared under optimum conditions. 1.0-mL fractions were collected separately up to a volume of 25 mL and counted with a γ-ray scintillation counter.

Synthesis of ^{99m}Tc tricarbonyl precursor. The ^{99m}Tc-tricarbonyl precursor, *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$, was prepared by adding 1 mL of pertechnetate solution (184–370 MBq $^{99m}TcO_4^-$) to a solution containing 4.5 mg of sodium boranocarbonate $[Na_2(H_3BCO_2)]$, 7.15 mg of Na_2CO_3, 8.5 mg of sodium tartrate, and 2.85 mg of sodium tetraborate in a penicillin vial [8]. The total volume of the solution was 2 mL. The solution was heated for 30 min on a boiling water bath, cooled, and alkalized to pH 10–11 with 0.1 N NaOH. The radiochemical yield of ^{99m}Tc tricarbonyl precursor was determined after filtration through an 0.22 μm Millipore filter, followed by RP-HPLC. The HPLC was performed at a flow rate of 0.6 mL min^{-1}; the retention time (R_t) was 4.7 min for ^{99m}Tc tricarbonyl and 11.65 min for free pertechnetate (Fig. 1). The ^{99m}Tc tricarbonyl precursor was successfully prepared with a high radiochemical yield (96%).

Radiosynthesis of ^{99m}Tc tricarbonyl ropinirole was performed using by adding 500 μg of ropinirole dissolved in 0.5 mL of ethanol to 1 mL of the already prepared solution of the ^{99m}Tc tricarbonyl precursor in a reaction vial at room temperature. The final solution volume was ~1.5 mL. The reaction mixture was heated at 100°C for 30 min. After cooling to 37°C, the radiolabeling yields were determined and checked by paper electrophoresis and radio-HPLC analysis.

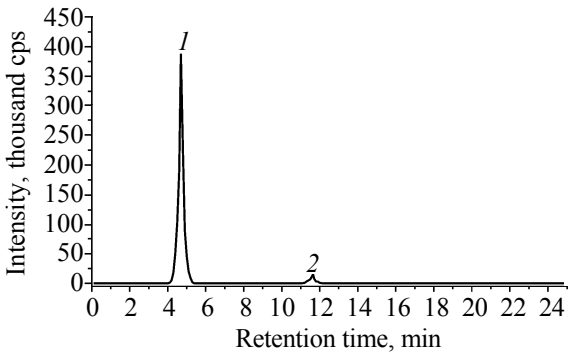


Fig. 1. HPLC radiochromatogram of (*1*) ^{99m}Tc tricarbonyl precursor (4.7 min) and (*2*) free ^{99m}Tc pertechnetate (11.65 min).

Radiochemical analysis of ^{99m}Tc tricarbonyl ropinirole. The radiochemical purity was further confirmed by paper electrophoresis (PE) using Whatman paper no. 1 (2 cm width, 30 cm length). 2 μL of the reaction mixture was placed at a distance of 15 cm (center point) from the negative electrode edge of the paper sheet. Electrophoresis was carried out for 1.5 h at a voltage of 300 V using phosphate buffer solution (0.05 M, pH 7.2 ± 0.2) as electrolyte. On completion of the experiment, the paper was removed, dried, cut into 1-cm-wide segments, and counted in a γ-counter.

Radio-HPLC of ^{99m}Tc-tricarbonyl ropinirole. A 10 μL aliquot of the ^{99m}Tc tricarbonyl ropinirole reaction mixture was injected into an RP-18 column. The mobile phase consisted of 0.05 M TEAP (triethylammonium phosphate, pH 2.8, solvent A) and methanol (solvent B); the flow rate was 0.6 mL min^{-1}. The following gradient system was used: isocratic elution (100% A), 0–5 min; linear gradient from 100% A/0% B to 75% A/25% B, 5–8 min; linear gradient from 75% A/25% B to 66% A/34% B), 8–11 min; linear gradient from 66% A/34% B to 0% A/100% B, 11–22 min; and isocratic elution (100% B), 22–25 min.

Biodistribution study in normal animal models. The animal (Swiss Albino mice, 25–35 g) experiments

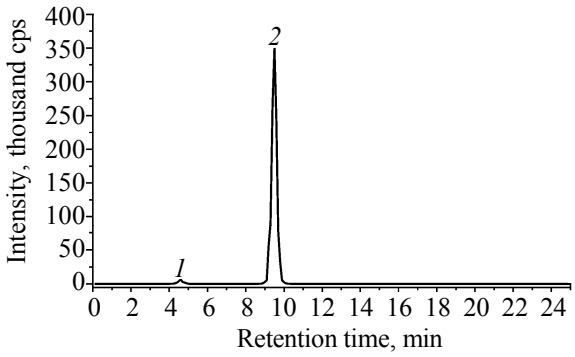


Fig. 2. HPLC radiochromatogram of (*1*) free ^{99m}Tc tricarbonyl (4.6 min) and (*2*) ^{99m}Tc tricarbonyl ropinirole (9.5 min)

were approved by the Ethical Committee of the Labeled Compounds Department. Five mice were taken for each experimental point (a total of 25 mice). After intravenous administration of 0.2 mL of ^{99m}Tc tricarbonyl ropinirole at physiological pH via tail vein, the mice were sacrificed at various time points for quantitative determination of the organ distribution of the complex. All the organs were separated, and the accumulated dose was measured by comparison with a standard solution of the labeled substrate (^{99m}Tc tricarbonyl ropinirole). Fresh blood, bone, and muscle samples were also collected and measured. The mean percentage of the administrated dose per gram of the organ was calculated. The weights of blood, bones, and muscles were assumed to be 7, 10, and 40% of the total body weight, respectively. Corrections were made for background radiation and decay during the experiments. The data were estimated with one-way ANOVA test. The results for *P*-value are reported, and all the outcomes are given as mean ± SD. The level of significance was set at $P < 0.05$ [9–12].

Determination of the partition coefficient of ^{99m}Tc tricarbonyl ropinirole. The octanol/water partition coefficient of ^{99m}Tc tricarbonyl ropinirole was determined at pH 7.4 by measuring its distribution between *n*-octanol and phosphate buffer saline (PBS). A 100-μL solution sample was added to the system consisting of 900 μL of the aqueous phase PBS (pH 7.4) and 1 mL of *n*-octanol. The mixture was vigorously vortexed for 5 min and then allowed to stand for 30 min at room temperature. Centrifugation at 5000 rpm for 5 min ensured complete separation of the organic and aqueous layers. An aliquot (100 μL) was withdrawn from each layer, and the activity was measured with a γ-counter. The partition coefficient, expressed as log*P*, was averaged over five measurements.

Blocking study of dopamine receptor. Different amounts of unlabeled ropinirole in the range 10–1000 μg were injected into the animals 15 min prior to the injection of ^{99m}Tc tricarbonyl ropinirole, and the percent of brain uptake was evaluated at 15 min post injection of ^{99m}Tc tricarbonyl ropinirole ($n = 5$).

In vitro stability study. In vitro stability of ^{99m}Tc tricarbonyl ropinirole was evaluated in two different media: human serum and saline. In serum, an 0.2-mL sample of ^{99m}Tc tricarbonyl ropinirole solution was mixed with 1.8 mL of serum and allowed to stand at ambient temperature. In addition, a 10-μL sample of ^{99m}Tc tricarbonyl ropinirole was added to normal saline and allowed to stand at room temperature. The relative content of ^{99m}Tc tricarbonyl ropinirole complex in the two media was determined by HPLC at various time intervals.

RESULTS AND DISCUSSION

Evaluation of radiochemical yield. The radiochemical yield was assessed by paper electrophoresis (PE) and radio-HPLC. In paper electrophoresis, ^{99m}Tc tricarbonyl ropinirole remained at the point of spotting (neutral labeled compound). Under similar conditions, free ^{99m}Tc-pertechnetate moved toward the anode to a distance of 12 cm from the spotting point, and another species (probably the ^{99m}Tc tricarbonyl precursor, about 0.1%) moved toward the cathode to a distance of 4 cm. The radiochemical yield of ^{99m}Tc tricarbonyl ropinirole under optimum conditions was 98.0%.

Radio-HPLC analysis of ^{99m}Tc tricarbonyl ropinirole. The radio-HPLC analysis gave 98% purity for ^{99m}Tc tricarbonyl ropinirole. The retention times of free ^{99m}Tc tricarbonyl and ^{99m}Tc tricarbonyl ropinirole were 4.6 and 9.5 min, respectively (Fig. 2).

Reaction optimization. The pH of the reaction mixture and amount of the substrate were optimized. The maximal conversion to ^{99m}Tc tricarbonyl ropinirole, 98.0%, with 185 MBq of ^{99m}Tc-tricarbonyl was reached with 500 μg of the substrate at pH and temperature kept constant (37°C, pH 6; Table 1). pH is a critical factor in conversion to ^{99m}Tc-tricarbonyl ropinirole; it should be adjusted to 6 (optimum value) to achieve 98.0% purity of the complex, which may

Table 1. Influence of ropinirole amount on the radiochemical yield of ^{99m}Tc tricarbonyl ropinirole (mean ± SEM, $n = 3$)

Ropinirole, mg	^{99m}Tc tricabonyl ropinirole, %	Free $^{99m}TcO_4^-$, %	$[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor, %
0.25	90.0 ± 0.8	6.0 ± 0.1	4.0 ± 0.9
0.50	98.0 ± 0.4	0.8 ± 0.2	1.2 ± 0.8
1.0	98.1 ± 0.5	1.0 ± 0.4	0.9 ± 0.7
2.0	98.2 ± 0.2	1.1 ± 0.2	0.7 ± 0.5
3.0	97.8 ± 0.9	0.8 ± 0.4	1.4 ± 0.7
4.0	97.6 ± 0.9	0.5 ± 0.6	1.9 ± 0.3
5.0	97.5 ± 0.7	1.5 ± 0.1	1.0 ± 0.1

reflect in part the stability of ^{99m}Tc tricarbonyl ropinirole. In acid medium (pH 2), the radiolabeling yield was relatively low (56%), and alkalization also led to a decrease in the radiochemical yield of ^{99m}Tc tricarbonyl ropinirole (to 61% at pH 12).

In vitro stability and lipophilicity. In vitro stability of the complex in saline was studied in order to determine the suitable time for injection. ^{99m}Tc tricarbonyl ropinirole complex remained nearly stable in saline, as well as in serum, for more than 6 h after labeling. The logarithm of the partition coefficient ($\log P$) of ^{99m}Tc-tricarbonyl ropinirole was found to be 2.77 ± 0.15.

Biodistribution study. The biodistribution study of ^{99m}Tc tricarbonyl ropinirole in different body organs and fluids is shown in Table 2. All radioactivity levels are expressed as average percent of the injected dose per gram tissue (%ID/g tissue ± SEM). ^{99m}Tc tricarbonyl ropinirole is distributed rapidly in most organs at 5 min post injection (p.i). The liver uptake increased to 25.5 ± 0.9% at 60 min p.i. and decreased to 13.3 ± 0.9% at 2 h p.i. The intestine uptake increased to 27.4 ± 1.1% at 60 min p.i. and decreased to 20.7 ± 1.2% at 2 h p.i. This means that the tracer is excreted through the hepatobiliary pathway. After 60 min, the ^{99m}Tc tricarbonyl ropinirole uptake appreciably decreased in most of the organs. The brain uptake of ^{99m}Tc-tricarbonyl ropinirole significantly increased to 7.8 ± 0.5% ID/g at 30 min p.i. and decreased to 2.4 ± 0.3% at 2 h p.i. The brain to blood ratios of ^{99m}Tc-tricarbonyl ropinirole were high: 0.24, 0.61, 1.45, 1.47, and 1.04 at 5, 15, 30, 60, and 120 min, respectively. These values exceed the levels reached with many other radiotracers [13–16], in particular, with ^{99m}Tc-HMPAO (3.5% ID/g at 30 min p.i. in rats) [15] and ^{99m}Tc-ECD (4.70% ID/g at 24 h post injection in monkeys) [16].

Blocking study of dopamine receptor. Administration of unlabeled ropinirole 15 min before the injection of ^{99m}Tc tricarbonyl ropinirole decreased the brain uptake at 30 min p.i. from 7.8 to 0.60% ID/g. This means that ^{99m}Tc tricarbonyl ropinirole binds selectively to the dopamine receptor in the brain.

Thus, we have developed an optimized protocol for the synthesis of ^{99m}Tc tricarbonyl ropinirole in high yield. ^{99m}Tc tricarbonyl ropinirole shows high brain

Table 2. Biodistribution of ^{99m}Tc-tricarbonyl ropinirole in normal mice (% ID/g) at different time intervals post injection (mean ± SEM, $n = 5$)

Organs and body fluids	5 min	15 min	30 min	60 min	120 min
Blood	15.2 ± 0.9	8.9 ± 0.2	5.4 ± 0.3	3.2 ± 0.2	2.3 ± 0.3
Bones	9.4 ± 0.1	7.1 ± 0.2	4.5 ± 0.2	3.4 ± 0.2	2.2 ± 0.2
Muscles	3.3 ± 0.6	3.4 ± 0.9	2.9 ± 0.3	1.9 ± 0.6	1.3 ± 0.2
Brain	3.7 ± 0.2	5.4 ± 0.9	7.8 ± 0.5	4.7 ± 0.1	2.4 ± 0.3
Lungs	2.5 ± 0.2	3.3 ± 0.4	3.5 ± 0.3	4.2 ± 0.6	3.6 ± 0.2
Heart	1.4 ± 0.3	1.3 ± 0.1	1.44 ± 0.24	1.5 ± 0.2	1.11 ± 0.21
Liver	9.17 ± 0.17	10.47 ± 0.25	16.21 ± 0.12	25.5 ± 0.9	13.3 ± 0.9
Kidneys	2.1 ± 0.1	3.2 ± 0.5	4.1 ± 0.8	6.1 ± 0.9	4.22 ± 0.11
Spleen	1.15 ± 0.3	1.2 ± 0.8	1.35 ± 0.12	1.22 ± 0.19	1.4 ± 0.3
Intestine	7.5 ± 0.5	9.1 ± 0.14	15.2 ± 0.9	27.4 ± 1.1	20.7 ± 1.2
Stomach	11.0 ± 1.3	12.3 ± 0.9	15.7 ± 1.2	13.3 ± 1.0	14.3 ± 1.1
Brain/blood	0.24	0.61	1.45	1.47	1.04

uptake, 7.8% ID/g at 30 min, and can be considered as a radiopharmaceutical for brain imaging.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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