Synthesis and biological evaluation of $^{99m}Tc(CO)_3$-OH-PP-CS_2 for brain receptor imaging

Original Article

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ABSTRACT

Introduction: 5-HT_{1A} receptor is related with a variety of neuropsychiatric disorders. . In this study a phenolic analogue derived from DWAY [Desmethyl WAY-100635 (N-(2-(1-(4-(2-methoxyphenyl)piperazinyl)-ethyl))-N-(2-pyridinyl) cyclohexanecarboxamide)] is used to design the desired structure of 5-HT_{1A} receptor imaging agents after labeling with $[^{99m}Tc\ (CO)_3(H_2O)_3]^+$ core via dithiocarbamate moiety.

Methods: 2-(piperazin-1-yl) phenol Dithiocarbamate was synthesized by the reaction of 2-(piperazin-1-yl) phenol with an equivalent amount of carbon disulfide in KOH solution then radiolabeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core. Radioligand chemical analysis involved high-performance liquid chromatography methods. Radioconjugate stability and lipophilicity were determined. Biodistribution of labeled compound was studied in rats.

Results: The final complex was characterized by HPLC and its radiochemical purity was more than 90%. In vitro stability studies have shown the complex was stable at least 6-hrs after labeling at room temperature. The n-octanol/water partition coefficient experiment demonstrated Log P = 0.74 for $^{99m}Tc(CO)_3$-OH-PP-CS_2. Biodistribution results showed that radio tracer had moderate brain uptake (0.32 ± 0.03 %ID/g at 30 min post injection), **Conclusion:** This complex may lead to a further development of a radiotracer with specific binding to 5-HT_{1A} receptor.

Keywords: Serotonin, ^{99m}Tc-carbonyl, DWAY, Brain imaging.

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INTRODUCTION

The neurotransmitter serotonin (5-HT) is involved in important physiological processes in the human brain, and changes in its receptors density cause several neurological disease, such as depression, schizophrenia and Alzheimer (1). New radioligands as a diagnostic tool for evaluation of neuroreceptors in the central nervous system (CNS), either by positron emission tomography (PET) or single-photon emission computed tomography (SPECT) were developed in the past years (2).

Recently many compounds as PET radiotracers for imaging of 5-HT_{1A} receptor have been studied (3-12). Despite the great variety of $5HT_{1A}$ radioligands with PET, there is still a scope for the development of technetium labeled 5-HT_{1A} ligands for SPECT imaging due to the availability of low cost $^{99}Mo/^{99m}Tc$ generator, favorable physical characteristics of ^{99m}Tc ($t_{1/2}$ of 6 h, γ 140 keV 89% abundance) and high specific activity of radionuclide. (1, 2).

The (2-methoxyphenyl)piperazine (MPP), residue of WAY 100635, which is known to have high affinity to the 5-HT_{1A} receptor, was selected as the ideal structure for the design of potential radiotracers (11, 8). Many ^{99m}Tc labeled complexes carrying MPP moiety with high affinity for the $5HT_{1A}$ receptor have been reported (13-17). However, most of those agents challenge with some problems such as low initial brain accumulation and high non-specific uptake.

The preparation of a novel ^{99m}Tc labeled MPP analogue is still considered to be necessary and highly interesting.

Over the last years the radio chemistry of ^{99m}Tc-tricarbonyl complexes has been extended to find useful application in radiopharmacy and nuclear medicine. ^{99m}Tc-tricarbonyl precursor $[^{99m}Tc(CO)_3(H_2O)_3]^+$ is prepared easily and three water molecules can be replaced by ligands that contain suitable donor atoms to forms stable complexes (18). The $[^{99m}Tc(CO)_3(H2O)_3]^+$ core represents a stable, but substitution-reactive precursor with a d^6 electronic configuration. Unlike the Tc(V) oxo core, it does not necessarily require coligands to stabilize the +I oxidation state and is independent of temperature, PH and time (19). In previous studies it was reported that dithiocarbamates form strong complexes with $M(CO)_3^+$ fragment, which are quite stable in wide pH range. Dithiocarbamates are soft Lewis bases and have high affinity for soft acids [like Tc(I) and Re(I)] (17, 20).

Among 5-HT_{1A} receptor ligands, [^{11}C] DWAY the desmethylated analogue of [^{11}C] WAY-100635 (21) showed a significantly higher radioactivity signal (22). Based on past studies, Defraiteur et al. developed a desmethyl analogue of [^{18}F]*p*-MPPF: [^{18}F]*p*-DMPPF, which showed a better brain penetration than that of [^{18}F]*p*-MPPF (23).

Based on the above mentioned article, in this work the 2-(piperazin-1-yl)phenol (DWAY fragment) was chosen and conjugated to the technetium chelate dithiocarbamate unit. The synthesis of 2-(piperazin-1-yl)phenol Dithiocarbamate performed and the labeling of ligand by $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core was carried out. It's in vitro and in vivo stability, partition coefficient and biodistributions were investigated.

METHODS

The 2-(piperazin-1-yl) phenol (2PP), carbon disulfide, potassium hydroxide and stannous chloride dehydrate were purchased from Aldrich chemical company. Technetium-99m as sodium pertechnetate ($Na^{99m}TcO_4$) was obtained from an in-house $^{99}Mo/^{99m}Tc$ generator using 0.9% saline. High resolution fast bombardment Mass spectroscopy was performed using an Agilent 1100/ Bruker Daltonic (Ion trap) VL instrument. Monitoring of all reactions was performed with analytical reverse-phase high performance liquid chromatography (RP-HPLC) on a JASCO 880-PU intelligent pump HPLC system (Tokyo, Japan) equipped with a multiwavelength detector and a flow-through Raytest-Gabi g-detector. CC 250/4.6 Nucleosil 120-5 C-18 column from Teknokroma was used for HPLC. The gradient systems consisted of 0.1% trifluoroacetic acid/water (Solvent A) and acetonitrile (Solvent B). For analytical HPLC, Gradient I was used: 0 min 95% A (5% B), 5 min 95% A (5% B), 25 min 0% A (100% B), 30 min 0% A (100% B), flow = 1 mL/min, γ = 280 nm.

Radioactivity measurements were carried out using Na (Tl) scintillation counter (ORTEC Model 4001 M Minibin & Power Supply).

Synthesis

The potassium salt of 2-(piperazine-1-yl) phenol Dithiocarbamate was synthesized by the reaction of 2-(piperazine-1-yl) phenol with an equivalent amount of carbon disulfide in KOH solution. A solution of potassium hydroxide (100 mg, 1.78 mmol) was added to 2-(piperazin-1-yl) phenol (205 mg, 1.15 mmol) in about 7 ml Ethanol in the ice bath and stirred then carbon disulfide (200 µl) was added dropwise to this solution. The mixture stirred for an hour in ice bath below 5 ºC. A dark precipitate slowly appeared. The solvent was removed by evaporation under reduced pressure and the precipitate then washed by ether. The product was recrystalized from ethanol/diethyl ether to give a semi white crystals of 2-(piperazine-1-yl) phenol Dithiocarbamate. Mass analysis was used to confirm the production of the desired complex.

^{99m}Tc-tricarbonyl

The precursor $[^{99m}Tc\ (CO)_3(H_2O)_3]^+$ was prepared according to the reported procedure (18).

In a closed vial 4.5mg Na_2CO_3, 5.5mg $NaBH_4$ and 20 mg sodium potassium tartarate were added and the vial was flushed with CO and after that the 1100 MBq activity that was eluted from a commercial $^{99}Mo/^{99m}Tc$ generator was added and heated to 95 °C for 30 min. After cooling the vial to room temperature, with 1 N HCl was neutralized to PH = 7. Radiochemical purity of the precursor was checked by reversed-phase HPLC.

Radiolabeling

One mg of the potassium salt of 2-(piperazin-1-yl)phenol Dithiocarbamate dissolved in 0.5 ml of phosphate buffer (pH = 7) in a vial. Then, $[^{99m}Tc\ (CO)_3(H2O)_3]^+$ precursor (100 µl, 370 MBq) was added and the reaction solution was heated at 80 °C for 30 min. The complex prepared was characterized by HPLC.

Partition coefficient

The octanol/water partition coefficient of complex was measured following 1 min vigorous vortex mixing of 1 ml of octanol and 0.9 ml saline (pH = 7), with approximately 100 µl of radiotracer in a micro centrifuge tube. The tubes were centrifuged at 500 rpm for 5 min and the counts in 100 µl aliquots of both organic and inorganic layers were determined by use of a NaI well-type γ-counter.

The partition coefficient (P) was calculated using the following equation: P = (cpm in octanol – cpm in background)/(cpm in water – cpm background).

The reported octanol/water partition coefficient represents the mean (± standard deviation) of the three measurements.

Stability

The stability of the complex was evaluated by monitoring the radiochemical purity (RCP) at different time points (15 min, 1, 3 and 6 h) using the following procedures. In a propylene test tube an aliquot of complex was incubated at room temperature (25 °C) for different time periods.

The RCP was determined for each time by HPLC. To determine the in vitro serum stability, 100 µl of radiolabeled complex was incubated with 1 ml human serum and its further incubation at 37 °C for 1 hour, 4 hrs and 6 hrs. Ethanol was added to the above solution.

The precipitate was separated by centrifugation. The supernatant was injected in HPLC to determine the stability of the complex.

Biodistribution

The in vivo biodistribution study of ^{99m}Tc-tricarbonyl-complex was carried out according to the relevant national regulations using male Wistar rats (5-6 week old). Complex (about 7.4 MBq in 100 µl solution) after HPLC purification was injected through the tail vein. The rats were sacrificed at different post injection times (2-60min) 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. The blocking studies were also done for two complexes by using of 8-OH-DPAT, the putative $5HT_{1A}$ blocker. Rats received a tail vein injection of a solution (50 µl) of 8-OH-DPAT (2 mg/kg) 1 min before administration of the radiotracer and dissected 30 min after injection. At 5 min after injection, accumulation of the tracer in brain area was also assessed by planar scintigraphy under ether anesthesia.

RESULTS AND DISCUSSION

The Dithiocarbamate derivative of DWAY was required for subsequent complexation with the ^{99m}Tc-core. The amino group of DWAY was simply derivatized to dithiocarbamate using carbon disulfide in the presence of KOH. The reaction was monitored by HPLC and the reaction product was recristalized and the yield was 58%. The mass spectra data were used to characterize the dithiocarbamate. The ESI mass spectrum (m/z, percent abundance) was as follows: 291 $[M+K]^+$, 100%.

To prepare the technetium-99m complex of dithiocarbamate, ^{99m}Tc-carbonyl core was first prepared. The ^{99m}Tc-tricarbonyl was prepared according to the above mentioned procedure and then used for complexation. The $[^{99m}Tc\ (CO)_3(H_2O)_3]^+$ core is a suitable substrate for the substitution reaction with the sodium potassium salt of dithiocarbamate-DWAY at room temperature to give the final complex (Fig. 1). Standardization and optimization studies for obtaining maximum complexation yield showed that the radiolabeling yield depends on the reaction pH. The reaction was favorable at neutral condition (pH=7) leading to >90% complexation. Radiochemical purity of the ^{99m}Tc-tricarbonyl core and ^{99m}Tc-tricarbonyl-ligand were determined by reversed phase HPLC using the gradient systems consisted of 0.1% trifluoroacetic acid/water (Solvent A) and acetonitrile (Solvent B). The labeling yield of $[^{99m}Tc\ (CO)_3(H_2O)_3]^+$ was >95% (Fig. 2). The ^{99m}Tc-tricarbonyl-ligand was characterized by HPLC which prepared in >90% yield at specific activity of 0.12 GBq/µmol (Fig. 3).

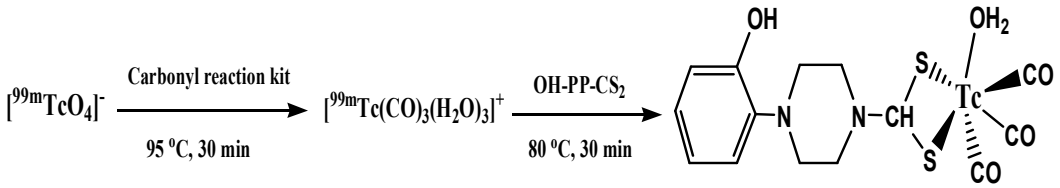

Fig 1. Preparation procedure and proposed structure of $^{99m}Tc(CO)_3$-OH-PP-CS_2.

The HPLC retention times for some selected complexes were as follows: $^{99m}TcO_4^-$: 4.75 min, $[^{99m}Tc\ (CO)_3(H_2O)_3]^+$: 15.87 min, $^{99m}Tc(CO)_3$-OH-PP-CS_2: 19.58 min.

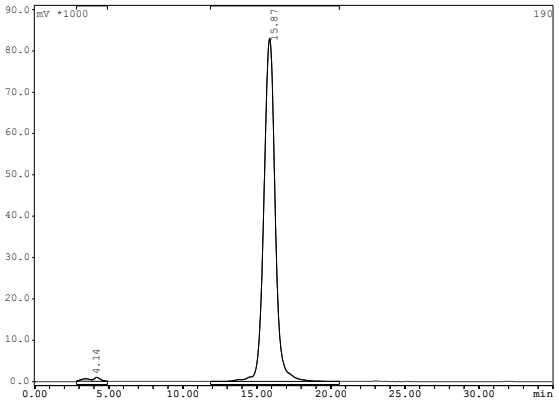

Fig 2. RP-HPLC profile of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor.

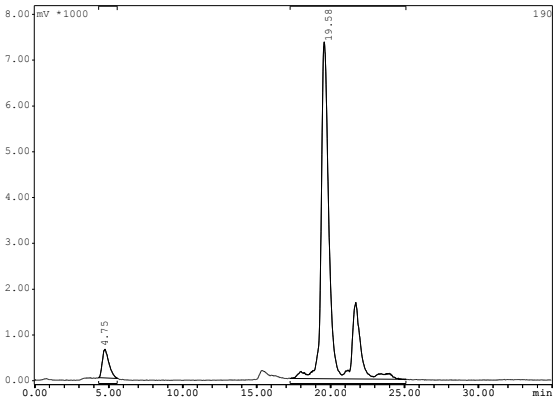

Fig 3. RP-HPLC profile of the $^{99m}Tc(CO)_3$-OH-PP-CS_2.

The partition coefficient of the radiolabeled complex was determined by distribution in octanol and water, and the lipophilicity (log P) of $^{99m}Tc(CO)_3$-OH-PP-CS_2 was found to be 0.74. The log p of the complex was within the range of values quoted for lipophilicity (0.5-2.5) which is suitable for crossing the blood brain barrier. The radiochemical purity of the ^{99m}Tc-tricarbonyl-ligand was nearly constant (>90%) over the observed period of 6 h. No decomposition of the complex was observed in this time period, suggesting its high stability in the reaction mixtures at room temperature. In serum stability studies, nearly 15% of the activity was associated with the precipitate obtained after ethanol addition, indicating the low binding of complex with serum proteins. The ethanol fraction was characterized by HPLC where a single peak was observed at the same time (19.58 min, > 90%) as that of the complex, indicating no decomposition of the complex and therefore its stability under 37 °C incubation.

Biological evaluation of $^{99m}Tc(CO)_3$-OH-PP-CS_2 complex was performed in male Wistar rats. The results are shown in Table 1. ^{99m}Tc-tricarbonyl-complex showed significant liver and kidney accumulation within 2 min (11.01 ± 1.02 %ID/g and 8.99 ± 1.47 %ID/g respectively) with moderate clearance at 60 min post injection (6.51 ± 0.38 %ID/g and 11.73 ± 1.19 %ID/g respectively), suggesting the hepatobiliary and urinary systems are the major routes of excretion of the administered radioactivity. As the log p of the tricarbonyl-complex shows, this manner of acting is probably due to more lipophilicity of this radio tracer. This complex had moderate brain uptake (0.32 ± 0.03 %ID/g at 5 min and 0.21 ± 0.06 %ID/g at 60 min post injection) and good retention compared to other studies done by other researchers as stated in our references. Initial brain uptake and retention may be is related to lipophilicity and in vivo stability of the complex. Although the blood uptake was not so high at the beginning (3.62 ± 0.11 %ID/g) and almost 63% of the radioactivity was cleared out after 60 min post injection (1.35 ± 0.28 %ID/g).

The distribution of the activity in different regions of brain was also performed in Wistar rats (Table 1). For complex $^{99m}Tc(CO)_3$-OH-PP-CS_2 the radioactivity concentration of hippocampus (Hipp) at

Table 1. Biodistribution of $^{99m}Tc(CO)_3$-OH-PP-CS_2 in normal rat (%ID/g ± SD, n=3).

Organs	Time (min)				
	2	15	30	30 Block	60
Blood	3.62±0.11	2.45±0.35	2.15±0.22	1.64±0.25	1.35±0.28
Kidney	8.99±1.47	11.25±1.28	12.29±1.45	14.97±2.08	11.73±1.19
Spleen	1.25±0.14	1.28±0.14	1.38±0.27	1.82±0.45	1.38±0.35
Intestine	3.01±0.20	3.81±0.51	3.87±0.37	6.09±1.41	8.39±1.65
Liver	11.01±1.02	8.58±0.96	8.61±0.89	6.74±1.97	6.51±0.38
Lung	2.98±0.25	2.55±0.19	1.71±0.49	1.38±0.39	1.41±0.29
Heart	1.45±0.20	1.02±0.58	0.98±0.23	0.96±0.78	0.85±0.91
Brain	0.29±0.02	0.30±0.04	0.32±0.03	0.29±0.02	0.21±0.06
Brain Regions:					
Cortex	0.23±0.09	0.29±0.01	0.24±0.03	0.16±0.02*	0.25±0.04
Hippocampus	0.36±0.03	0.37±0.05	0.41±0.04	0.23±0.02*	0.35±0.09
Cerebellum	0.21±0.07	0.19±0.04	0.13±0.03	0.11±0.02	0.11±0.08
Hipp/CB Ratio	1.71	1.94	3.15	2.09	3.18

* P value of less than 0.05 was considered statistically significant.

2 min p.i was 0.36 ± 0.03 %ID/g which was increased to 0.41 ± 0.04 %ID/g at 30 min post injection and more than 85 % of its activity was retained in Hipp at 60 min p.i. This is may be due to the clearance of non specific uptake from brain after 60 min post injection, and on the other hand this high retention shows $^{99m}Tc(CO)_3$-OH-PP-CS_2 have specific affinity to 5-HT_{1A} receptor in the brain. For cerebebellum (CB) the uptake was lower than Hipp. The ratio of Hipp/CB was 1.71 at 2 min and 3.18 at 60 min post injection; it shows the accumulation of radioactivity in Hipp. (the area of 5-HT_{1A} receptors).

Biodistribution of the complex with blocking agent in different part of brain also was investigated at 30 min post injection (Table 1). The uptake of Hipp was decreased obviously from 0.41 ± 0.04 %ID/g to 0.23 ± 0.02 %ID/g and the uptake of cortex was also decreased from 0.24 ± 0.03 %ID/g to 0.16 ± 0.02 %ID/g at 30 min post injection but activity in the cerebellum showed no significant decrease. The uptake in hippocampus and cortex was specific and receptor mediated, as shown by the co-injection of blocking agent, indicating that these brain regions are also 5-HT_{1A} receptor positive. Scintigraphic study showed early brain uptake 5 min post injection (Fig. 4).

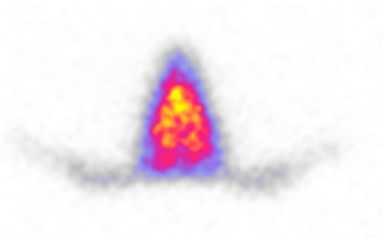

Fig 4. Scintigraphy image of rat head 5 min post injection of $^{99m}Tc(CO)_3$-OH-PP-CS_2 ligand.

The uptake in the whole brain was not similar and in areas with more receptors it was higher which shows that distribution of activity is specific and receptor mediated.

In the past few years, ^{11}C labeled WAY-100635 has been developed and successfully used as PET radiotracer (11). However Studies of the metabolic pathway of [carbonyl-^{11}C]WAY-100635 in humans revealed rapid metabolism to WAY-100634 due to the hydrolysis of the amide bond, which interfere the PET measurements (7-9). In addition, a disadvantage of using carbon-11 ($t_{1/2}$ = 20 min) radiotracers is that these tracers can only be used where both a cyclotron and a PET-camera are in close proximity due to it short half-life. Zhuang and co-workers have shown in a series of benzamido analogs of WAY-100635 a limited tolerance for variations at the amino pyridine position in the native receptor (24). Therefore, research has been directed toward WAY-100635 analogs that were labeled with the longer lived fluorine-18 ($t_{1/2}$ = 110 min) isotope with the label outside the WAY-100634 moiety (25) but in addition to the hydrolysis of the amide bond, deflurination was observed (5). Flowed by successful development of ^{99m}Tc-TRODAT (26, 27), fragments of WAY100635 were selected for developing ^{99m}Tc-labeled 5HT_{1A} receptor imaging agents (14-16). As until now there has no ideal ^{99m}Tc agent for imaging 5-HT_{1A} receptors. Further design of these kind ^{99m}Tc complexes could improve the brain uptake with a high receptor affinity. Based on pharmacokinetic behavior and brain distribution results for our radioconjugate, further ligand modification such as using carbon chain spacer could be considered for improvement of this novel ^{99m}Tc-complex as a brain receptor imaging agent.

CONCLUSION

We have shown design and synthesis of new 5-HT_{1A} receptor imaging agent $^{99m}Tc(CO)_3$-OH-PP-CS_2 with high labeling yield. According to the results of in vivo biodistribution studies, we found that this complex had moderate brain uptake and a good retention time with more favorable properties for further study. Regional brain distribution study showed a clear correlation between distribution of radioactivity and distribution of 5-HT_{1A} receptors in the brain. In this area further studies of the structure modification should be considered to increase regional brain uptake.

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