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Research Article

Synthesis and biodistribution in mice of a new ^{99m}Tc nitrido complex for brain imaging

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Summary

The bis(*N*-cyclopentyl dithiocarbamato) nitrido technetium-99 m complex ^{99m}TcN(CPEDTC)$_2$ was synthesized by the reduction of $^{99m}TcO_4^-$ into $[^{99m}Tc{\equiv}N]^{2+}$ with stannous chloride in the presence of succinic dihydrazide and propylenediamine tetraacetic acid, followed by the addition of sodium *N*-cyclopentyl dithiocarbamate monohydrate. The radiochemical purity (RCP) of the product was over 90% as measured by thin layer chromatography(TLC) and high performance liquid chromatography(HPLC). In vitro studies showed that the complex possessed good stability under physiological conditions. Its partition coefficient studies indicated it was a good lipophilic complex. The electrophoresis results showed the complex was neutral. The biodistribution results in mice indicated that ^{99m}TcN(CPEDTC)$_2$ was significantly retained into the brain. The brain uptake(ID%/g) was 3.58, 5.26, 3.73 and 2.72 and the brain/blood ratios were 0.79, 1.69, 1.59 and 1.58 at 5, 30, 60 and 90 min post-injection, respectively. These results suggested potential usefulness of the complex as a new brain perfusion imaging agent. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: technetium-99 m; $[^{99m}TcN]^{2+}$ core; brain imaging agent

Introduction

In recent years, the preparation of ^{99m}Tc radiopharmaceuticals with the $[^{99m}Tc{\equiv}N]^{2+}$ core at tracer level and in sterile and pyrogen-free conditions has been extensively investigated.[1] The application of this synthetic procedure to the preparation of Tc-99m nitrido radiopharmaceuticals with dithiocarbamates ligands, $[R(R')NCS_2]^-$, has led further to discover a new class of imaging agents. For example, bis(*N*-ethoxy, *N*-ethyl dithiocarbamato) nitrido

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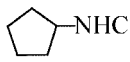
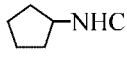
technetium-99m complex $^{99m}TcN(NOET)_2$ exhibits high myocardial uptake and significant redistribution behavior in various animals and in humans.[2–4] It was found that the biodistribution of these complexes were strongly affected by the nature of the lateral group R and R′ bound to the $>NCS_2$ moiety.[1] In order to extend the investigation of the biological properties of the class of technetium-99m nitrido complexes, we report here the synthesis and biodistribution of bis(*N*-cyclopentyl dithiocarbamato) nitrido technetium-99m complex $^{99m}TcN(CPEDTC)_2$ as a potential cerebral imaging agent. The new complex exhibited significant brain localization and good retention in mice, suggesting its potentiality as a brain perfusion imaging agent.

Results and discussion

Chemistry of bis(N-cyclopentyl dithiocarbamato) nitrido technetium-99 m complex $^{99m}TcN(CPEDTC)_2$

The preparation of bis(*N*-cyclopentyl dithiocarbamato) nitrido technetium-99 m complex $^{99m}TcN(CPEDTC)_2$ can be carried out by the following method:

$$[^{99m}TcO_4]^- + H_2NNHCOCH_2CH_2CONHNH_2 + SnCl_2.2H_2O + (HO_2CCH_2)_2NCH(CH_3)CH_2N(CH_2CO_2H)_2 \longrightarrow [^{99m}TcN]_{int}^{2+}$$

$$[^{99m}TcN]_{int}^{2+} + \text{  } \longrightarrow {}^{99m}TcN(\text{  })_2$$

In the reaction, $H_2NNHCOCH_2CH_2CONHNH_2$ plays the role of an efficient donor of nitride nitrogen atoms(N^{3-}). $SnCl_2 \cdot 2H_2O$ behaves as a reducing agent. The presence of $((HO_2CCH_2)_2NCH(CH_3)CH_2N(CH_2CO_2H)_2)$ is required in order to prevent precipitation of Sn^{2+} in the form of insoluble tin salts.

The method is based on the reaction of $[^{99m}TcO_4]^-$ with succinic dihydrazide in the presence of stannous chloride as reducing agent to form a technetium-99 m nitrido intermediate. The $[^{99m}Tc{\equiv}N]_{int}^{2+}$ is a suitable substrate for substitution reaction with sodium *N*-cyclopentyl dithiocarbamate monohydrate at room temperature to give the final product.

The radiochemical purity of the complex was routinely checked by TLC and HPLC. R_f values for some selected complexes are shown in Table 1(Polyamide strip). HPLC retention times(Rt) for some selected complexes were reported as follows: $[^{99m}TcO_4]^-$: 3 min; $[^{99m}Tc{\equiv}N]_{int}^{2+}$: 2.6 min; $^{99m}TcN(CPEDTC)_2$:

Table 1. (R_f values for some selected complexes)

	$[^{99m}TcO_4]^-$	$^{99m}TcO_2 \cdot nH_2O$	$[^{99m}Tc{\equiv}N]_{int}^{2+}$	$^{99m}TcN(CPEDTC)_2$
Saline	0.1	0.1	0.7 ~ 1.0	0.1
CH_2Cl_2:CH_3OH = 9:1(V/V)	0.1	0.1	0.1	0.9 ~ 1.0

17.2 min. The HPLC pattern of ^{99m}TcN(CPEDTC)$_2$ is shown in Figure 1. The single peak suggested only one product was formed. The mean radiochemical purity of the product was 97 $\pm$ 2% immediately after the preparation.

Bis(diethyldithiocarbamato) nitrido technetium-99 m complex ^{99m}TcN(DDC)$_2$ is neutral and has a square pyramidal geometry with an apical Tc≡N bond and two monoanionic dithiocarbamate ligands spanning the four positions in the basal plane through the four sulfur atoms.[5] Because the sodium salt of *N*-clyclopentyl dithiocarbamate and the sodium salt of diethyldithiocarbamate all belong to the dithiocarbamate ligands, it seems reasonable to presume that the structure of the bis(*N*-cyclopentyl dithiocarbamato) nitrido technetium-99 m complex ^{99m}TcN(CPEDTC)$_2$ is similar to that of the bis(diethyldithiocarbamato) nitrido technetium-99 m complex ^{99m}TcN(DDC)$_2$. The proposed structure of ^{99m}TcN(CPEDTC)$_2$ is shown in Figure 2. Its structure needs to be further investigated.

Partition coefficient (log P) of the complex

The partition coefficient (log *P*) of the complex was 1.47 and 1.45 at pH7.0 and pH7.4, respectively, showing no great difference between pH7.0 and pH7.4. The log *P* of the complex is within the range of values quoted for lipophilicity

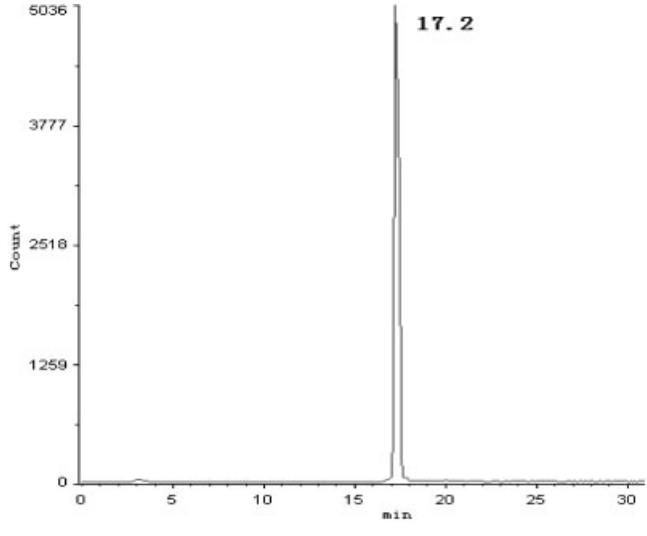


Figure 1. The HPLC pattern of ^{99m}TcN(CPEDTC)$_2$

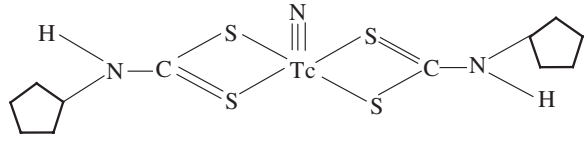


Figure 2. The proposed structure of ^{99m}TcN(CPEDTC)$_2$

(0.9–2.5) that are suitable for crossing the blood brain barrier (BBB). The complex is easily adsorbed on the walls of the vials and syringes. In order to decrease this adsorption, γ-cyciodextrin was added to the final solution. As compared with ^{99m}Tc-d,l-HMPAO,[6] the $\log P$ of the ^{99m}TcN(CPEDTC)$_2$ complex is greater than that of the ^{99m}Tc-d,l-HMPAO ($\log P = 1.20$).

Stability of the complex

The radiochemical purity at different times after preparation is shown in Figure 3. From Figure 3, the complex was seen to have high stability in the medium of synthesis, suggesting it possesses a great stability under physiological conditions. The stability of the ^{99m}TcN(CPEDTC)$_2$ complex was superior to that of the ^{99m}Tc-d,l-HMPAO[6] (It was decomposed at room temperature at 1 h after preparation).

Paper electrophoresis

The paper electrophoresis pattern of ^{99m}TcN(CPEDTC)$_2$ showed that the complex remained at the point of spotting(Percentage of radioactivity: 98.16%), suggesting that it is a neutral complex.

Biodistribution of the complex in mice

Biological distribution results in mice for the complex are shown in Table 2. Results of biodistribution of ^{99m}TcN(CPEDTC)$_2$, ^{99m}Tc-d,l-HMPAO[7] were shown in Table 3 for comparison.

From Table 2, the complex was seen to have a significant brain uptake. The brain uptake(ID%/g) was 3.58, 5.26, 3.73 and 2.72 and the brain/blood ratios

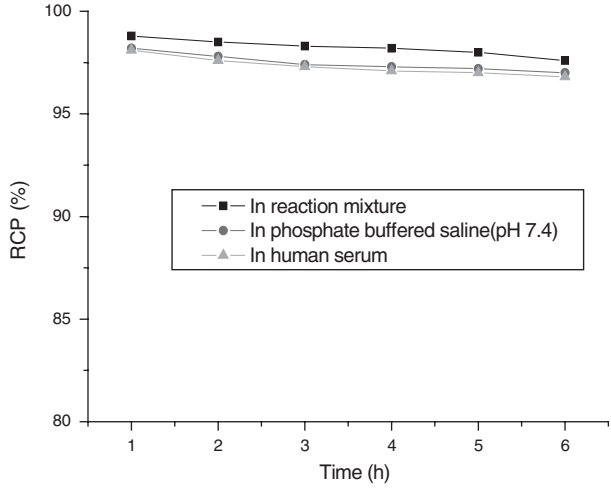


Figure 3. The stability of ^{99m}TcN(CPEDTC)$_2$

Table 2. Biodistribution in mice of ^{99m}TcN(CPEDTC)$_2$

t/min	5	30	60	90
Blood	4.56 ± 0.20	3.11 ± 0.17	2.35 ± 0.55	1.72 ± 0.60
Brain	3.58 ± 0.40	5.26 ± 0.36	3.73 ± 1.23	2.72 ± 0.35
Heart	18.47 ± 2.04	9.80 ± 1.02	6.08 ± 2.04	5.40 ± 0.93
Liver	32.76 ± 3.11	27.42 ± 3.70	26.02 ± 6.12	24.05 ± 8.39
Kidney	21.03 ± 1.08	10.33 ± 0.98	7.40 ± 2.44	6.95 ± 0.50
Lung	10.90 ± 1.44	7.85 ± 1.58	5.02 ± 1.82	5.52 ± 0.78
Brain/blood	0.79	1.69	1.59	1.58

Results were given as the percentage dose/g, for an average of three mice, ± SD(Standard deviation).

Table 3. Comparison of biodistribution of ^{99m}TcN(CPEDTC)$_2$ and ^{99m}Tc-d,l-HMPAO in mice

	^{99m}TcN(CPEDTC)$_2$			^{99m}Tc-d,l-HMPAO		
t/min	5	30	60	5	30	60
Brain	3.58 ± 0.40	5.26 ± 0.36	3.73 ± 1.23	4.00 ± 1.46	4.17 ± 0.24	4.13 ± 1.13
Blood	4.56 ± 0.20	3.11 ± 0.17	2.35 ± 0.55	6.43 ± 1.17	3.90 ± 0.87	3.23 ± 1.12
Brain/blood	0.79	1.69	1.59	0.62	1.07	1.28

were 0.79, 1.69, 1.59 and 1.58 at 5, 30, 60 and 90 min post-injection, respectively. The complex also exhibited high myocardial uptake. The heart uptake(ID%/g) was 18.47, 9.80, 6.08 and 5.40 at 5, 30, 60 and 90 min post-injection, respectively. As compared with ^{99m}TcN(NOET)$_2$,[8] the heart/blood, heart/liver and heart/lung ratios of ^{99m}TcN(CPEDTC)$_2$ were 2.59, 0.23 and 1.21 at 60 min post-injection. The heart/blood, heart/liver and heart/lung ratios of ^{99m}TcN(NOET)$_2$ were 3.36, 0.44 and 0.82 at 60 min post-injection. The heart/blood, heart/liver ratios of ^{99m}TcN(CPEDTC)$_2$ were lower than that of ^{99m}TcN(NOET)$_2$, but the heart/lung ratio of the former was superior to that of the latter. Five minutes post-injection, the hepatic uptake reached its peak activity of 32.76(%ID/g) and remained high. It showed the hepatobiliary system was the major route of excretion of the administered radioactivity.

From Table 3, the blood uptake of ^{99m}TcN(CPEDTC)$_2$ was lower than that of ^{99m}Tc-d,l-HMPAO, thus the brain/blood ratio of the former was superior to that of the latter. The biodistribution results of ^{99m}TcN(CPEDTC)$_2$ in mice demonstrated that the brain was one of the principal target organs for this compound. This finding was somewhat surprising when compared to the biological properties of ^{99m}TcN(NOET)$_2$. As reported previously,[8] it was found that the latter complex exhibited high myocardial uptake and less brain uptake. It seems difficult to explain the different biological behavior of these two species on the basis of their minor structural differences. We think that the hydrogen atom bonded to the nitrogen atom of ^{99m}TcN(CPEDTC)$_2$ perhaps plays an important role in brain uptake and retention. The view was partially

supported by our recent research work.[9,10] We have synthesized two complexes—$^{99m}TcN(CHDTC)_2$ (CHDTC: *N*-cyclohexyl dithiocarbamato) and $^{99m}TcN(DCHDTC)_2$ (DCHDTC: dicyclohexyl dithiocarbamato), which are different in lateral group bound to the $>NCS_2$ moiety. Different biodistribution results in mice of these two complexes were found. The former had a high brain uptake and good brain/blood ratio (ID%/g: 5.91; brain/blood ratio: 2.10 at 60 min post-injection), while the latter had much less brain uptake and a poor brain/blood ratio (ID%/g: 0.41, brain/blood ratio: 0.03 at 60 min post-injection). The only difference in these two complexes was that $^{99m}TcN(CHDTC)_2$ had a hydrogen atom bound to the $>NCS_2$ moiety, suggesting the hydrogen atom bound to the nitrogen atom of the dithiocarbamate ligand possibly affects the brain uptake and retention.

Experimental

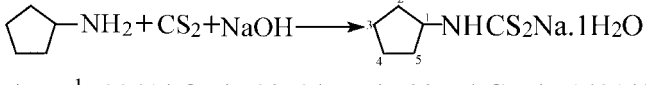
Materials

Succinic dihydrazide(SDH), propylenediamine tetraacetic acid(PDTA) and stannous chloride dihydrate were purchased from Aldrich Chemical Co., USA. $^{99}Mo/^{99m}Tc$ generator was obtained from the China Institute of Atomic Energy, Beijing. All other chemicals were of reagent grade and used without further purification.

IR spectrum was obtained with a AVATAR 360 FT-IR spectrometer using KBr pellets. NMR spectrum was recorded on a Bruker Avance 500(400 MHz) spectrometer with D_2O as a solvent. Elemental analyses was performed on a Vario EL elemental analyzer model. Electrospray ionization(ESI) mass spectrum was obtained with a LCMS-2010 mass spectrometer.

Synthesis of sodium N-cyclopentyl dithiocarbamate monohydrate

Sodium *N*-cyclopentyl dithiocarbamate monohydrate was prepared by reacting cyclopentylamine with an equivalent amount of carbon disulfide in NaOH solutions.[1] Thus, sodium hydroxide(0.1 mol) was dissolved in water, the solution cooled in an ice-salt bath and then added to cyclopentylamine(0.1 mol) under stirring, followed by carbon disulfide(0.1 mol). The mixture was stirred for an hour in an ice-salt bath. The solvent was removed under reduced pressure and the residue was filtered off. The crude product was recrystallized from ethanol/diethyl ether to give white crystals of sodium *N*-cyclopentyl dithiocarbamate monohydrate.



IR/cm^{-1}: 3341(-OH), 3273(-NH), 2957(-CH_2), 1481(C–N), 965(C=S).

^{1}H NMR δ(D_2O): 4.39(q, J = 6.4 Hz, 1H, CH); 2.12(s, 1H, NH); 1.86(m, 2H, 2H + 5H); 1.57(m, 2H, 2H′ + 5H′); 1.47(m, 4H, 3H + 4H).

Elemental analysis: Calculated: C: 35.80%, H: 6.01%, N: 6.96%;

Found: C: 35.73%, H: 6.33%, N: 6.79%.

The ESI mass spectrum(m/z, percent abundance) was as follows: 160 [M^-], 100%.

Preparation of bis(N-cyclopentyl dithiocarbamato) nitrido technetium-99 m complex $^{99m}TcN(CPEDTC)_2$

The preparation of the complex was carried out using the following procedure.[11,12] A kit was prepared as follows: 250 mg of succinic dihydrazide(SDH) and 250 mg of propylenediamine tetraacetic acid(PDTA) was dissolved in 10 ml 0.5 mol/l sodium hydroxide solution in a 100 ml glass beaker, and then 35 ml of distilled water was added to the beaker. The pH of the solution was adjusted to 7 by using 2 mol/l hydrochloride acid. Then 0.1 ml of stannous chloride solution(formed by dissolving 25 mg of stannous chloride dihydrate in 1 ml of 2 mol/l hydrochloride acid) was injected into the beaker. The volume of the solution was adjusted to 50 ml. The solution was sterilized by membrane filtration and each 1 ml was dispensed in 50 sterile, pyrogen-free capped vials. The vials were in a freeze-dried form and under nitrogen.

1 ml of saline containing [$^{99m}TcO_4$]$^-$ (15 MBq) was added to a kit containing 0.05 mg of stannous chloride dihydrate, 5.0 mg of succinic dihydrazide(SDH), 5.0 mg of propylenediamine tetraacetic acid(PDTA). The mixture was kept at room temperature for 15 min. Successively, 1 ml of a water solution containing 4.0 mg of the sodium *N*-cyclopentyl dithiocarbamate monohydrate was then added and the reaction was allowed to proceed for 10 min at room temperature.

The radiochemical purity of the product was evaluated by TLC and HPLC. The TLC was performed on a polyamide strip and eluted with saline and CH_2Cl_2:CH_3OH = 9:1(V/V), respectively. HPLC analysis was carried out with a reversed-phase column(ODS-C18, 250 × 4.6 mm, Aldrich), Shimadzu SCL-10AVP series, by using methanol/water(70:30, V/V) as a mobile phase, working at a flow rate of 1.0 ml min^{-1}.

Determination of the partition coefficient for the complex

The partition coefficient was determined by mixing the complex with an equal volume of 1-octanol and phosphate buffer (0.025 mol/l, pH7.0 and pH7.4) in a centrifuge tube. The mixture was vortexed at room temperature for 1 min and then centrifuged at 5000 r/min for 5 min. From each phase 0.1 ml of the aliquot was pipetted and counted in a well γ-counter. The measurement was repeated

three times. Care was taken to avoid cross contamination between the phases. The partition coefficient, *P*, was calculated using the following equation:

P = (cpm in octanol-cpm in background)/(cpm in buffer-cpm in background).

Usually the final partition coefficient value was expressed as log *P*.

Stability study

The stability of the complex in the reaction mixture was assayed by measuring the RCP at different times after preparation at room temperature (25°C). For in vitro stability in phosphate buffered saline (pH 7.4) and human serum, 100 µl of the radiolabelled complex were mixed with 2 ml each of phosphate buffered saline (pH 7.4) and human serum (1 mg/ml), respectively. TLC was carried out to assess the RCP after incubating at 37°C for different time intervals.

Paper electrophoresis

1 µl samples was spotted on a piece of Whatman 1 chromatography paper, saturated with 0.05 mol/l, pH7.4 phosphate buffer, in an electrophoresis bath. Across 12 cm of the strip 150 V was applied for 1.5 h. The strips were dried, and the distribution of radioactivity on the strip was determined.

Biodistribution study

A solution of bis(*N*-cyclopentyl dithiocarbamato) nitrido technetium-99m complex $^{99m}TcN(CPEDTC)_2$(0.1 ml, 740KBq) was administered via a tail vein to Kunming mice(18–20 g) and the injected radioactivity measured with a well type NaI(Tl) detector. Mice were sacrificed at 5, 30, 60 and 90 min post-injection. The organs of interest and blood were collected, weighed and measured for radioactivity. The radioactivity in each organ was expressed as a percentage of the injected dose per gram of organ(%ID/g). All biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation.

Conclusion

Bis(*N*-cyclopentyl dithiocarbamato) nitrido technetium-99 m complex $^{99m}TcN(CPEDTC)_2$ was prepared in sterile and apyrogen conditions by an efficient method, which can be easily used for the preparation of a radiopharmaceutical through a freeze-dried kit formulation. The significant brain localization, good retention and high brain/blood ratio of the complex in mice exhibited favorable properties, suggesting that further investigations of the biological behavior of this complex may lead to identify useful candidates for clinical application.

Acknowledgements

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