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SYNTHESIS, CHARACTERIZATION AND BIODISTRIBUTION OF A ^{99m}Tc NITRIDO COMPLEX AS A POTENTIAL BRAIN PERFUSION IMAGING AGENT

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SUMMARY

The bis(N-cyclohexyl-dithiocarbamato) nitrido technetium-99m complex [^{99m}TcN-(CHDTC)$_2$] (CHDTC:N-cyclohexyl dithiocarbamato) has been synthesized through a ligand-exchange reaction. The two-step procedure involved the initial reaction of $^{99m}TcO_4^-$ with succinic dihydrazide(SDH) in the presence of stannous chloride as reducing agent and propylenediamine tetraacetic acid(PDTA) as complexant, followed by the addition of sodium N-cyclohexyl dithiocarbamate dihydrate. The radiochemical purity of the complex was over 90% by thin layer chromatography. It was stable over 6h at room temperature. Its partition coefficient indicated that it was a good lipophilic complex. Biodistribution in mice demonstrated that the complex accumulated in brain with high uptake and good retention. The brain uptake(ID%/g) was 2.91,5.88 and 5.91 at 5,30 and 60min post-injection respectively. The ratio of brain/blood in mice was high, 2.10 at 1hr post-injection. The results for the complex suggested that it could be a potential brain perfusion imaging agent.

Key words: Technetium-99m , [^{99m}TcN]$^{2+}$ core, sodium N-cyclohexyl dithiocarbamate dihydrate, brain uptake, lipophilic, brain imaging agent

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INTRODUCTION

In recent years, the preparation of ^{99m}Tc radiopharmaceuticals with the $[^{99m}TcN]^{2+}$ core at tracer level and in sterile and pyrogen-free conditions has been extensively investigated.[1-3] For example, bis(N-ethoxy,N-ethyl dithiocarbamato) nitrido technetium-99m complex $[^{99m}TcN(NOEt)_2]$ exhibits high myocardial uptake in humans and shows a behavior similar to that of thallium-201 as it undergoes redistribution in myocardial ischemic patients under stress/rest conditions.[4] It is currently under preliminary clinical evaluation as a tracer for myocardial perfusion. Until now, however, there has not been a good brain perfusion imaging agent containing the $[^{99m}TcN]^{2+}$ core for clinical use. Bellande[5] successfully synthesized an unsymmetrical complex $[^{99m}Tc][TcN(MePS_2)(Et_2PS_2)]$ which was retained in the brain of rats for a prolonged time, but the ratio of brain/blood in rats was low so that it could not be a good brain perfusion imaging agent. Now, ^{99m}Tc-d,l-HMPAO(hexamethylpropylene amine oxime)[6] and ^{99m}Tc-l,l-ECD(ethyl cysteinate dimer)[7] are accepted as valuable tracer agents for the determination of regional cerebral blood flow, but they are complexes containing the $[^{99m}Tc{=}O]^{3+}$ core. In order to develop a new class of brain perfusion imaging agents which contain $[^{99m}TcN]^{2+}$ core, we report here the synthesis, characterization and biodistribution of bis(N-cyclohexyl dithiocarbamato) nitrido technetium-99m complex $[^{99m}TcN(CHDTC)_2]$ as a potential brain perfusion imaging agent. It was found that the complex exhibited significant brain localization and good retention in mice , suggesting that it could be potentially useful as a brain perfusion imaging agent.

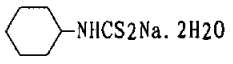
EXPERIMENTAL

Materials

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

Synthesis of sodium N-cyclohexyl dithiocarbamate dihydrate

Sodium N-cyclohexyl dithiocarbamate dihydrate was prepared by reacting cyclohexylamine with an equivalent amount of carbon disulfide in NaOH solutions[3]. Thus, sodium hydroxide(0.1mol) was dissolved in water, the solution cooled in an ice-salt bath and then added to cyclohexylamine(0.1mol) under stirring, followed by carbon disulfide(0.1mol).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-cyclohexyl dithiocarbamate dihydrate(yield,51.5%). IR/cm^{-1}:3350(-OH),3200(-NH),2940(-CH_2),1480(C-N),990(C=S). Elemental analysis: for



Calculated:C:36.05%,H:6.87%,N:6.01%; Found:C:36.04%,H:6.74%,N:5.93% .

Preparation of bis(N-cyclohexyl dithiocarbamato) nitrido technetium-99m complex [^{99m}TcN(CHDTC)$_2$]

The preparation of the complex was carried out using the following procedure[8-9]: A kit was prepared as follows: 250mg of succinic dihydrazide(SDH) and 250mg of propylenediamine tetraacetic acid(PDTA) was dissolved in 10ml 0.5mol/L sodium hydroxide solution in a 100ml glass beaker, and then 35ml of distilled water was added to the beaker. The pH of the solution was adjusted to 7~8 by using 2mol/L hydrochloride acid. Then 0.1ml of stannous chloride solution(formed by dissolving 25mg of stannous chloride dihydrate in 1ml of 2mol/L hydrochloride acid) was injected into the beaker. The volume of the solution was adjusted to 50ml. The solution was sterilized by membrane filtration and each 1ml was dispensed in 50 sterile, pyrogen-free capped vials. The vials were in a freeze-dried form and under nitrogen.

1ml of saline containing [$^{99m}TcO_4$]$^-$ (15MBq) was added to a kit containing 0.05mg of stannous chloride dihydrate, 5.0mg of succinic dihydrazide(SDH), 5.0mg of propylenediamine tetraacetic acid(PDTA). The mixture was kept at room temperature for 15 minutes. Successively, 4.0mg of sodium N-cyclohexyl dithiocarbamate

dihydrate dissolved in 1.0ml water was then added and the reaction allowed to stand for 10min at room temperature.

The radiochemical purity of the product was evaluated by thin layer chromatography and ranged from 90%-99%. The thin layer chromatography was performed on a polyamide strip and eluted with saline and CH_2Cl_2:CH_3OH=9:1(V/V) respectively.

The stability of the complex was determined at room temperature by measuring radiochemical purity values at different times (1,3 and 6hr) after preparation.

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 (0.025M,pH7.4) in a centrifuge tube. The mixture was vortexed at room temperature for 1min and then centrifuged at 5000r/min for 5min. 0.1Ml samples from the octanol and aqueous layers were pipetted into other test tubes 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 logP.

Biodistribution study

A solution of bis(N-cyclohexyl dithiocarbamato) nitrido technetium-99m complex [^{99m}TcN(CHDTC)$_2$] (0.1ml,740KBq) was administered via a tail vein to Kunming mice(18~20g) and the injected radioactivity measured with a well type NaI(Tl) detector. Mice were sacrificed at 5,30 and 60min 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).

RESULTS AND DISCUSSION

Chemistry of bis(N-cyclohexyl dithiocarbamato) nitrido technetium-99m complex [^{99m}TcN(CHDTC)$_2$]

The preparation route for bis(N-cyclohexyl dithiocarbamato) nitrido technetium-99m complex [^{99m}TcN(CHDTC)$_2$] is as follows:

$$(1)[^{99m}TcO_4]^- + H_2NNHCOCH_2CH_2CONHNH_2 + SnCl_2.2H_2O + (HO_2CCH_2)_2NCH(CH_3)CH_2N(CH_2CO_2H)_2 \rightarrow [^{99m}Tc{\equiv}N]_{int}^{2+}$$

$$(2)[^{99m}Tc{\equiv}N]_{int}^{2+} + \bigcirc\!\!-NHCS_2Na.\,2H_2O \rightarrow {}^{99m}TcN(\bigcirc\!\!-NHCS_2)_2$$

In the reaction, succinic dihydrazide($H_2NNHCOCH_2CH_2CONHNH_2$) plays the role of an efficient donor of nitride nitrogen atoms (N^{3-}) and $SnCl_2.2H_2O$ behaves as reducing agent. The presence of propylenediamine tetraacetic acid ($(HO_2CCH_2)_2N$-$CH(CH_3)CH_2N(CH_2CO_2H)_2$) 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-99m nitrido intermediate.The $[^{99m}Tc{\equiv}N]_{int}^{2+}$ is a suitable substrate for substitution reaction with sodium N-cyclohexyl dithiocarbamate dihydrate at room temperature to give the final complex bis(N-cyclohexyl dithiocarbamato) nitrido technetium-99m [^{99m}TcN(CHDTC)$_2$].

The radiochemical purity of the complex was routinely checked by thin layer chromatography. R_f values for some selected complexes were shown in Table 1(Polyamide strip).

Table 1(R_f values for some selected complexes)

	$[^{99m}TcO_4]^-$	$^{99m}TcO_2.nH_2O$	$[^{99m}Tc{\equiv}N]_{int}^{2+}$	$[^{99m}TcN(CHDTC)_2]$
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

The mean radiochemical purity of the product was 97±2% immediately after the preparation. No decomposition of the complex occured over 6hr at room temperature.

Based on the previous characterization of the molecular structure of bis(N-ethoxy,N-ethyl dithiocarbamato) nitrido technetium-99m complex [^{99m}TcN(NOEt)$_2$], [2] we know that the bis(N-ethoxy,N-ethyl dithiocarbamato) nitrido technetium-99m complex [^{99m}TcN(NOEt)$_2$] 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. Because sodium N-cyclohexyl dithiocarbamate dihydrate and sodium N-ethoxy,N-ethyl dithiocarbamate monohydrate all belong to the sodium salts of the dithiocarbamate ligands, it seems reasonable to presume the structure of bis(N-cyclohexyl dithiocarbamato) nitrido technetium-99m [^{99m}TcN(CHDTC)$_2$] is similar to that of the complex bis(N-ethoxy,N-ethyl dithiocarbamato) nitrido technetium-99m [^{99m}TcN(NOEt)$_2$]. The structure and retention mechanism in brain of bis(N-cyclohexyl dithiocarbamato) nitrido technetium-99m [^{99m}TcN(CHDTC)$_2$] needs to be determined by further research work.

Partition coefficient (logP) of the complex

The partition coefficient (logP) was calculated as 1.50. It shows that the complex is a good lipophilic complex able to cross the intact blood brain barrier(BBB).

Biodistribution of the complex in mice

Biological distribution results in mice for the complex are shown in Table 2.

Table 2 Biodistribution in mice of ^{99m}TcN(CHDTC)$_2$ (x±s, n=3)

(%ID/g)

	5min	30min	60min
heart	23.74±1.81	18.72±0.80	12.51±0.19
liver	63.17±2.17	55.60±5.08	45.39±1.03
lung	23.94±2.14	12.33±2.39	11.09±1.03
kindey	22.78±1.40	20.93±0.10	15.09±0.05
brain	2.91±0.14	5.88±0.27	5.91±0.02
blood	7.15±0.20	4.04±0.14	2.82±0.12
brain/biood	0.14	1.46	2.10

From Table 2, the complex is seen to have a significant brain uptake, and surprisingly the radioactivity localized into the brain 1hr post-injection was higher than that measured 5min post-injection. The brain/blood ratios were 0.14, 1.46, 2.10 at 5, 30 and 60min, respectively. The complex also exhibited high myocardial uptake and good retention. The heart uptake(ID%/g) was 23.74,18.72 and 12.51 at 5,30 and 60min post-injection respectively. The heart/blood, heart/liver and heart/lung ratios were 4.44, 0.28 and 1.13 at 60min post-injection. The heart/liver ratio was low thereby restricting the use of the complex as a myocardial perfusion imaging agent. Five minutes post-injection, the hepatic uptake reached its peak activity of 63.17(%ID/g) and remained high. It showed the hepatobiliary system was the major route of excretion of the administered radioactivity.

CONCLUSION

The bis(N-cyclohexyl dithiocarbamato) nitrido technetium-99m complex [^{99m}TcN-$(CHDTC)_2$] was prepared through a simple and efficient method, which can be easily utilized for the preparation of a radiopharmaceutical through a freeze-dried kit formulation. The significant brain localization , good retention and high brain/blood ratio in mice of the complex exhibited favourable properties, justifying further studies in animals and humans.

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