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Synthesis and biological evaluation of a novel ^{99m}Tc nitrido radiopharmaceutical with deoxyglucose dithiocarbamate, showing tumor uptake

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ABSTRACT

The deoxyglucose dithiocarbamate (DGDTC) was synthesized and radiolabelled with $[^{99m}TcN]^{2+}$ intermediate to form the ^{99m}TcN–DGDTC complex. The radiochemical purity of the ^{99m}TcN–DGDTC complex was over 90%, as measured by TLC and by HPLC, without any notable decomposition at room temperature over a period of 6 h. The partition coefficient and electrophoresis results indicated that this complex was hydrophilic and neutral. The biodistribution of ^{99m}TcN–DGDTC in mice bearing S 180 tumor showed that the complex accumulated in the tumor with high uptake and good retention. The tumor/blood and tumor/muscle ratios increased with time and reached 2.32 and 1.68 at 4 h post-injection, suggesting it would be a promising candidate for tumor imaging.

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As well known, glucose is an important molecule for metabolism. [^{18}F]fluorodeoxyglucose [^{18}F]FDG, an analogue of natural glucose, is more actively taken up in tumor cells than in normal cells, thus making it possible to detect the tumor.[1–3] Currently, [^{18}F]FDG has become the 'working horse' of positron emission tomography (PET) imaging. However, the short half life, limited availability and high cost of the [^{18}F] isotope restrict its wide application in clinical practice, especially in developing countries. By comparison, technetium-99m has been the isotope of choice for development of novel radiopharmaceuticals owing to its short half-life, optimal γ-energy, inexpensive cost and diverse coordination chemistry. Therefore, achieving a ^{99m}Tc labelled glucose derivative as a single photon emission computed tomography (SPECT) analogue of PET tracer [^{18}F]FDG is considered to be of great interest. Recently, several ^{99m}Tc labelled glucose derivatives have been reported.[4–8] Among them, ^{99m}Tc labelled ethylenedicysteine-deoxyglucose (^{99m}Tc-ECDG) is the most promising candidate as a tumor imaging agent. However, it has some defects such as relative lower tumor uptake and slow blood clearance. The preparation of novel ^{99m}Tc labelled glucose surrogates is still considered to be necessary and highly challenging.

In recent years, the $[^{99m}Tc{\equiv}N]^{2+}$ core constitutes a group isoelectronic with $[^{99m}Tc{=}O]^{3+}$ and $[O{=}^{99m}Tc{=}O]^{+}$. The nitrido N^{3-} ligand is considered to be the strongest π donor known, and may act as a terminal or bridging atom which stabilizes the metal at high oxidation states. Because the $[^{99m}TcN]^{2+}$ core exhibits a very high chemical stability towards oxidation–reduction reactions and pH variations and the presence of the $[^{99m}TcN]^{2+}$ core in the molecular structure of a radiopharmaceutical may dramatically alter its biological behaviour, the preparation of ^{99m}Tc nitrido complexes at the tracer level and in sterile and pyrogen-free conditions has been extensively investigated,[9] thus opening the door for the exploration of the biological behavior of a new class of potential diagnostic agents. The $[^{99m}TcN]^{2+}$ core has been found to complex well with ligands containing sulfur atoms, as in dithiocarbamates. So far, a number of Tc-99m nitrido radiopharmaceuticals with dithiocarbamate ligands for heart, brain, tumor and infection imaging have been reported.[9–12] Therefore, it may be of great interest to probe the ^{99m}Tc nitrido chemistry with other dithiocarbamate ligands to develop new better diagnostic agents.

2-Amino-2-deoxy-D-glucose is a highly attractive substance. Its molecular structure has an active amine group, thus making it suitable to react with carbon disulfide in NaOH solutions to form the corresponding dithiocarbamate product. It can be used to form stable $[^{99m}TcN]^{2+}$ complex on the basis of efficient binding of the group to four sulfur atoms. Herein, we report the synthesis of deoxyglucose dithiocarbamate and its ^{99m}Tc labelling using the ^{99m}TcN core. To the best of our knowledge, this report constitutes the first of its kind in using the deoxyglucose dithiocarbamate in the preparation of ^{99m}TcN complex as targeted agent for tumor imaging.

The sodium salt of deoxyglucose dithiocarbamate (DGDTC) was prepared by reacting 2-amino-2-deoxy-D-glucose hydrochloride with an equivalent amount of carbon disulfide in NaOH solutions.[9] The reaction is schematically shown in Scheme 1.[13]

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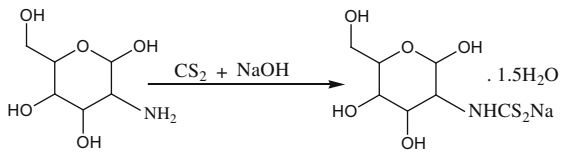


Scheme 1. Synthesis of DGDTC.

The preparation of ^{99m}TcN–DGDTC was carried out using the following procedure in Scheme 2.[14]

In SDH kit, SDH plays the role of an efficient donor of nitride nitrogen atoms (N^{3-}) and $SnCl_2{\cdot}2H_2O$ behaves as a reducing agent. The presence of PDTA 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 SDH in the presence of stannous chloride as reducing agent to form a technetium-99m nitrido intermediate. The $[^{99m}Tc{\equiv}N]^{2+}$ is a suitable substrate for the substitution reaction with DGDTC at room temperature to give the final complex ^{99m}TcN–DGDTC.

The radiochemical purity of the complex was routinely checked by TLC and HPLC.[14] By TLC, in saline, $^{99m}TcO_4^-$, and ^{99m}TcN–DGDTC remained at the origin while $[^{99m}Tc{\equiv}N]^{2+}_{int}$ migrated with the front. In acetonitrile, $^{99m}TcO_4^-$ migrated at $R_f = 0.3–0.5$, while ^{99m}TcN–DGDTC and $[^{99m}Tc{\equiv}N]^{2+}_{int}$ remained at the origin. The HPLC pattern of ^{99m}TcN–DGDTC is shown in Figure 1. It was observed that the retention time of $^{99m}TcO_4^-$ was 1.8 min and $[^{99m}Tc{\equiv}N]^{2+}_{int}$ was 12.6 min, while that of ^{99m}TcN–DGDTC was found to be 13.3 min. The mean radiochemical purity of the product was over 90% immediately after the preparation.

Based on a previous characterization of the molecular structure of bis(*N*-ethoxy, *N*-ethyl dithiocarbamato)nitrido technetium-99m complex $^{99m}TcN(NOEt)_2$,[15] it seems reasonable to presume that the structure of ^{99m}TcN–DGDTC is similar to that of $^{99m}TcN(NOEt)_2$, having 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. Clearly, its detailed structure needs to be determined by further research work.

The stability of the complex was assayed by measuring the radiochemical purity at different times after preparation. No decomposition of the complex occured over 6 h at room temperature, suggesting that the complex was stable in the reaction mixture at room temperature. The partition coefficient was determined by mixing the complex with an equal volume of 1-octanol and phosphate buffer (0.025 mol/L, pH 7.4) in a centrifuge tube. The partition coefficient ($\log P$) value of ^{99m}TcN–DGDTC was −1.30, suggesting it was hydrophilic. This value is greater than that of ^{99m}Tc-S-DG ($\log P$: −2.92), ^{99m}Tc-MAG$_3$-DG ($\log P$: −2.19) and ^{99m}Tc-MAMA-BA-DG ($\log P$: −1.71),[7] indicating ^{99m}TcN–DGDTC is less hydrophilic than the other three complexes. The paper electrophoresis was done by the literature method.[10] The electrophoresis results showed that the ^{99m}TcN–DGDTC complex remained at the point of spotting (percentage of radioactivity >90%), suggesting it was a neutral complex.

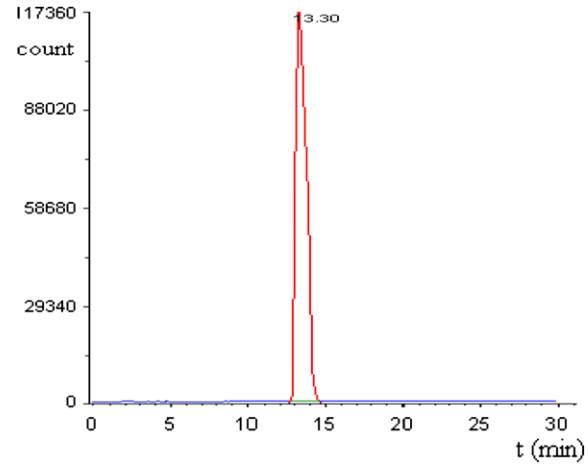


Figure 1. HPLC pattern of ^{99m}TcN–DGDTC.

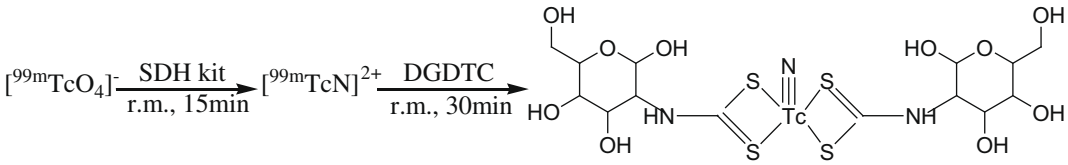
Biodistribution studies were performed in Kunming male mice (weighing 18–20 g) bearing S 180 tumor, which grew to a leg diameter of 10–15 mm. ^{99m}TcN–DGDTC (0.1 ml, 7.4×10^5 Bq) was injected via a tail vein and the injected radioactivity was measured with a well-type NaI(Tl) detector. The mice were sacrificed at 0.5 h, 2 h and 4 h post-injection. The tumor, other organs of interest and blood were collected, weighed and measured for radioactivity. The results were expressed as the percent uptake of injected dose per gram of tissue (%ID/g). All biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation.

The results of biodistribution of ^{99m}TcN–DGDTC are shown in Table 1. As described in Table 1, ^{99m}TcN–DGDTC has a high uptake at the site of tumor and good tumor retention. The blood and muscle clearance is faster than that of the tumor so that the tumor/blood and tumor/muscle ratios increase with time. The kidney uptake is much higher than the hepatic. Early renal activity reflects urinary elimination of this complex. As seen from Table 2, this is

Table 1
Biodistribution of ^{99m}TcN–DGDTC in mice bearing S 180 tumor (%ID/g)[a]

Tissue	0.5 h	2 h	4 h
Heart	1.76 ± 0.16	1.58 ± 0.92	0.68 ± 0.13
Liver	3.84 ± 0.90	4.08 ± 1.70	2.00 ± 0.34
Lung	4.06 ± 0.87	2.17 ± 0.42	1.49 ± 0.14
Kidney	10.33 ± 0.92	11.33 ± 3.56	7.71 ± 1.40
Brain	0.17 ± 0.03	0.23 ± 0.11	0.12 ± 0.07
Muscle	1.54 ± 0.52	1.66 ± 0.88	0.69 ± 0.26
Blood	2.36 ± 0.13	1.43 ± 0.94	0.50 ± 0.04
Tumor	1.48 ± 0.23	1.66 ± 0.63	1.16 ± 0.57
T/N ratio	0.96	1.00	1.68
T/B ratio	0.63	1.16	2.32

T/N = tumor-to-muscle, T/B = tumor-to-blood.
[a] All data are the mean percentage ($n = 3$) of the injected dose of ^{99m}TcN–DGDTC per gram of tissue, ± the standard deviation of the mean.



Scheme 2. Preparation procedure and proposed structure of ^{99m}TcN–DGDTC.

Table 2
Comparison of biodistribution of ^{99m}TcN–DGDTC with some ^{99m}Tc glucose derivatives and [^{18}F]FDG

Complex	^{99m}TcN–DGDTC	^{99m}Tc-ECDG	^{99m}Tc-S-DG	^{99m}Tc-MAG$_3$-DG	^{99m}Tc-MAMA-BA-DG	^{99m}Tc-DTPA-DG	[^{18}F]FDG
Animal	Kunming mice	Nude mice	TA-2 mice	TA-2 mice	TA-2 mice	Nude mice	Nude mice
Tumor type	S 180 tumor	A 549 tumor	MA 891 tumor	MA 891 tumor	MA 891 tumor	MCF-7 tumor	MCF-7 tumor
Time p.i. (h)	4	4	4	4	4	4	4
Tumor uptake (%ID/g)	1.16 ± 0.57	0.41 ± 0.16	1.06 ± 0.18	0.82 ± 0.06	0.21 ± 0.03	1.59 ± 0.04	1.42 ± 0.12
T/N ratio	1.68	2.73	3.29	4.35	2.25	4.30	0.36
T/B ratio	2.32	0.52	0.82	0.94	0.94	3.24	7.12
Reference	Present study	4	7	7	7	8	8

comparable to the results of some ^{99m}Tc radiolabelled glucose derivatives and [^{18}F]FDG in mice bearing tumor. The tumor uptake and the T/B ratio of ^{99m}TcN–DGDTC is higher than that of ^{99m}Tc-ECDG, ^{99m}Tc-S-DG, ^{99m}Tc-MAG$_3$-DG and ^{99m}Tc-MAMA-BA-DG, but the T/N ratio of the former is less than that of the latter. As compared with ^{99m}Tc-DTPA-DG, a relative lower T/B and T/N ratios of ^{99m}TcN–DGDTC are observed. However, the synthesis of the DGDTC ligand is easier than that of the DTPA-DG ligand and the chemical structure of ^{99m}TcN–DGDTC can be well defined because it belongs to ^{99m}TcN dithiocarbamate complexes, whereas the chemical structure of ^{99m}Tc-DTPA-DG is poorly defined due to its complicated coordination structure. By comparison, ^{99m}TcN–DGDTC is superior to [^{18}F]FDG with regard to T/N ratio, whereas [^{18}F]FDG has a higher T/B ratio than ^{99m}TcN–DGDTC.

In summary, novel DGDTC ligand was successfully synthesized and ^{99m}TcN–DGDTC was prepared in high yields through a ligand-exchange reaction, which can be easily used for the preparation of a radiopharmaceutical through a freeze-dried kit formulation. The significant tumor localization, good retention and high tumor/blood and tumor/muscle ratios of the ^{99m}TcN–DGDTC complex in mice bearing tumor exhibited favorable properties, suggesting that further investigations of the biological behavior of this complex may lead to identify useful candidates for tumor imaging.

Acknowledgments

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References and notes

1. Conti, P. S.; Lilien, D. L.; Hawley, K.; Keppler, J.; Grafton, S. T.; Bading, J. R. *Nucl. Med. Biol.* **1996**, *23*, 717.
2. Rigo, P.; Paulus, P.; Kaschten, B. J.; Hustinx, R.; Bury, T.; Jerusalem, G.; Benoit, T.; Foidart-Willems, J. *Eur. J. Nucl. Med.* **1996**, *23*, 1641.
3. Pauwels, E. K. J.; Ribeiro, M. J.; Stoot, J. H. B. M.; McCready, V. R.; Bourguignon, M.; Maziere, B. *Nucl. Med. Biol.* **1998**, *25*, 317.
4. Yang, D. J.; Kim, C. G.; Azhdarinia, A.; Yu, D. F.; Oh, C. S.; Bryant, J. L.; Won, J. J.; Kim, E. E.; Podoloff, D. A.; Schechter, N. R. *Radiology* **2003**, *226*, 465.
5. Schibli, R.; Dumas, C.; Petrig, J.; Garcia-Garayoa, E.; Schubiger, P. A.; Spadola, L.; Scapozza, L. *Bioconjugate Chem.* **2005**, *16*, 105.
6. Oh, S. J.; Ryu, J. S.; Yoon, E. J.; Bae, M. S.; Choi, S. J.; Park, K. B.; Moon, D. H. *Appl. Radiat. Isot.* **2006**, *64*, 207.
7. Chen, X. J.; Li, L.; Liu, F.; Liu, B. L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5503.
8. Chen, Y.; Huang, Z. W.; He, L.; Zheng, S. L.; Li, J. L.; Qin, D. L. *Appl. Radiat. Isot.* **2006**, *64*, 342.
9. Pasqualini, R.; Duatti, A.; Bellande, E.; Comazzi, V.; Brucato, V.; Hoffschir, D.; Fagret, D.; Comet, M. *J. Nucl. Med.* **1994**, *35*, 334.
10. Zhang, J. B.; Wang, X. B.; Liu, J. *Appl. Radiat. Isot.* **2005**, *62*, 33.
11. Chu, T. W.; Li, R. J.; Hu, S. W.; Wang, Y.; Liu, X. Q.; Wang, X. Y. *J. Radioanal. Nucl. Chem.* **2004**, *261*, 199.
12. Zhang, J. B.; Guo, H. X.; Zhang, S. J.; Lin, Y.; Wang, X. B. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5168.
13. The synthetic procedures and the spectral data for DGDTC are as follows. Sodium hydroxide (0.02 mol) was dissolved in water, the solution cooled in an ice–salt bath and then added to 2-amino-2-deoxy-D-glucose hydrochloride (0.01 mol) whilst stirring, and carbon disulfide (0.01 mol) then added. The mixture was stirred for 2 h in an ice-salt bath. Most of the solvent was removed under reduced pressure, and the precipitate collected by filtration. The yellow crude product was recrystallized from methanol to give the compound DGDTC (yield, 19%).The compound DGDTC was characterized by IR, ESI–MS and Elemental analysis. IR (KBr)/cm^{-1}:3427 (νOH), 2925 (νCH$_2$), 1038 (νC=S). ESI–MS: 254 ([M-Na]). Anal. Calcd for $C_7H_{12}NO_5S_2Na{\cdot}1.5H_2O$: C, 27.63; H, 4.93; N, 4.61. Found: C, 28.00; H, 5.20; N, 4.85.
14. The preparation procedure for ^{99m}TcN–DGDTC and the TLC, HPLC analysis conditions are as follows. One milliliters 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, 5.0 mg of DGDTC dissolved in 1.0 ml water was then added and the reaction allowed to stand for 30 min at room temperature. The TLC was performed on a polyamide strip and eluted with saline and acetonitrile respectively. HPLC analysis was carried out with a reversed-phase column (Kromasil 100-5C, 250 × 4.6 mm), Shimadzu SCL-10AVP series, working at a flow rate of 1.0 ml/min. Water (A) and acetonitrile (B) mixtures were used as the mobile phase and the following gradient elution technique was adopted for the preparation (0 min 100% B, 60 min 5% B).
15. Pasqualini, R.; Duatti, A. *J. Chem. Soc., Chem. Commun.* **1992**, 1354.