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Synthesis and biodistribution of a novel ^{99m}TcN complex of ciprofloxacin dithiocarbamate as a potential agent for infection imaging

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ABSTRACT

The ciprofloxacin dithiocarbamate (CPFXDTC) was synthesized and radiolabeled with $[^{99m}TcN]^{2+}$ intermediate to form the ^{99m}TcN-CPFXDTC complex in high yield (>95%). No decomposition of the complex at room temperature was observed over a period of 6 h. Its partition coefficient indicated that it was a good lipophilic complex. The bacterial binding assay studies showed ^{99m}TcN-CPFXDTC had a better binding affinity as compared with ^{99m}Tc-ciprofloxacin. Biodistribution results in induced infection mice showed ^{99m}TcN-CPFXDTC had higher uptake at the sites of infection and better abscess/blood ratio than that of ^{99m}Tc-ciprofloxacin, suggesting ^{99m}TcN-CPFXDTC would be a novel potential infection imaging agent.

Scintigraphic imaging of infection and inflammation is a powerful diagnostic tool in the management of patients with infectious or inflammatory diseases. Radiolabeled leukocytes are still considered the gold standard to detect infectious and inflammatory lesions in patients. However, its preparation is time-consuming, laborious and has risk associated with handling of potentially contaminated blood. There is great interest in the development of new radiopharmaceuticals for infection imaging.

^{99m}Tc 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. Recently, ^{99m}Tc-ciprofloxacin, which has a 4-fluoroquinolone backbone, has been proposed as a sensitive and specific tool for distinction between bacterial infection and sterile inflammation. Ciprofloxacin is an antibiotic of which the microbiological activity is mediated by inactivation of bacterial DNA gyrase. It has shown many advantages over ^{99m}Tc-labeled leukocytes for diagnostic scans, in that it is more specific for bacterial infection, is more convenient to prepare, and obtains better image quality.[1–4] However, the problems of ^{99m}Tc-ciprofloxacin preparation discussed in the literature [5–7] are concerned with its low radiochemical yield. A significant amount of colloid ($^{99m}TcO_2$) is formed to cause the RCP of the product low. Moreover, the chemical structure of ^{99m}Tc-ciprofloxacin is uncertain and the preparation of ^{99m}Tc-ciprofloxacin needs heating and the final product requires additional purification.[6] These procedures are very cumbersome for routine clinical use.

In recent years, the preparation of ^{99m}Tc nitrido complexes at the tracer level and in sterile and pyrogen-free conditions has been extensively investigated,[8] 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.[9,10] Herein, we report the synthesis of ciprofloxacin dithiocarbamate and its ^{99m}Tc labeling using the ^{99m}TcN core. To the best of our knowledge, this report constitutes the first of its kind in using the ciprofloxacin dithiocarbamate in the preparation of ^{99m}TcN complex as targeted agent for infection imaging.

The sodium salt of ciprofloxacin dithiocarbamate (compound **1**) was prepared by reacting ciprofloxacin with an equivalent amount of carbon disulfide in NaOH solutions.[9] The reaction is schematically shown in Scheme 1.[11] The molecular structure of ciprofloxacin has a piperazinyl group, thus making it suitable to react with carbon disulfide in NaOH solutions at low temperature to form the corresponding dithiocarbamate product in moderate yield.

The preparation of ^{99m}TcN-CPFXDTC was carried out using the following procedure in Scheme 2.[12]

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 the compound **1** at room temperature to give the final complex ^{99m}TcN-CPFXDTC.

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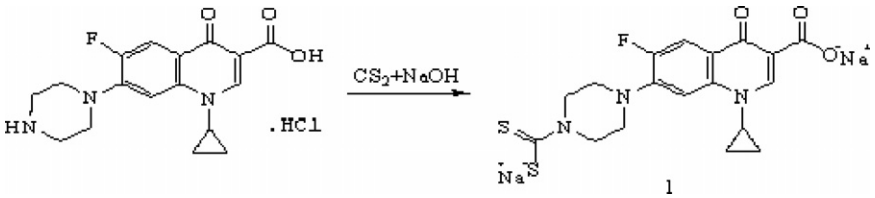
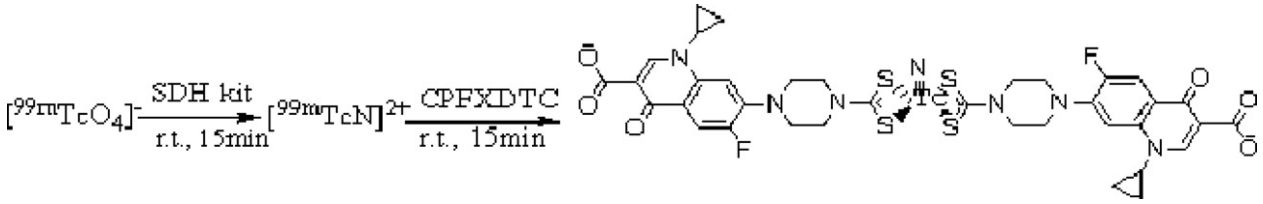


Scheme 1. Synthesis of compound **1**.



Scheme 2. Preparation procedure and proposed structure of ^{99m}TcN-CPFXDTC.

The radiochemical purity of the complex was routinely checked by TLC and HPLC.[12] By TLC, in saline, $^{99m}TcO_4^-$, $^{99m}TcO_2{\cdot}nH_2O$ and ^{99m}TcN-CPFXDTC remained at the origin while $[^{99m}Tc{\equiv}N]^{2+}_{int}$ migrated with the front. In CH_2Cl_2:CH_3OH = 9:1 (V/V), ^{99m}TcN-CPFXDTC migrated with the front while $^{99m}TcO_4^-$, $^{99m}TcO_2{\cdot}nH_2O$ and $[^{99m}Tc{\equiv}N]^{2+}_{int}$ remained at the origin. The HPLC pattern of ^{99m}TcN-CPFXDTC is shown in Figure 1. It was observed that the retention time of $[^{99m}Tc{\equiv}N]^{2+}_{int}$ was 2.9 min, while that of ^{99m}TcN-CPFXDTC was found to be 23.9 min. Single peak suggested only one product (^{99m}TcN-CPFXDTC) was formed. The mean radiochemical purity of the product was 95 ± 4% immediately after the preparation.

As compared with the preparation of ^{99m}Tc-ciprofloxacin, ^{99m}TcN-CPFXDTC can be prepared at room temperature and its preparation does not need heating and additional purification, thus making it more suitable for routine clinical use.

Based on a previous characterization of the molecular structure of bis(diethyldithiocarbamato) nitrido technetium-99 m complex ^{99m}TcN(DDC)$_2$,[13] it seems reasonable to presume that the structure of ^{99m}TcN-CPFXDTC is similar to that of ^{99m}TcN(DDC)$_2$, 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, further studies should be performed, using macroscopic levels of the long-lived ^{99}Tc, to determine and characterize the structure of ^{99m}TcN-CPFXDTC.

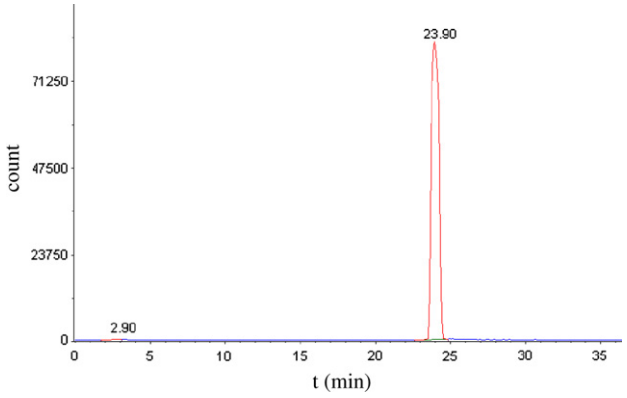


Figure 1. HPLC pattern of ^{99m}TcN-CPFXDTC.

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 possesses a great stability 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*) values of ^{99m}TcN-CPFXDTC and ^{99m}Tc-ciprofloxacin were calculated as 1.02 and −1.08, respectively, suggesting the former was lipophilic, whereas the latter was hydrophilic.

Binding of ^{99m}TcN-CPFXDTC to bacteria was assessed by the method.[14] The efficiency of bacteria binding of ^{99m}TcN-CPFXDTC was nearly 60%, much higher than that of ^{99m}Tc-ciprofloxacin (40%),[6] suggesting ^{99m}TcN-CPFXDTC had a better bacteria binding affinity.

Male Kunming mice weighing 18–20 g were used in all of the animal studies. 0.05 ml of 0.1 mol/L Na–PBS (pH 7.4) containing approximately 1×10^{10}/ml viable Staphylococcus aureus was injected into the left thigh muscle of the mice. Twenty-four hours later, 0.1 ml of ^{99m}TcN-CPFXDTC (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 3 and 4 h post-injection. The infected muscle, 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-CPFXDTC are shown in Table 1. As described in Table 1, ^{99m}TcN-CPFXDTC has a high uptake at the site of infection and good target/non-target ratio. The high concentration in the liver suggests that the hepatobiliary system is the major route of excretion of the administered radioactivity. As compared with ^{99m}Tc-ciprofloxacin,[15] ^{99m}TcN-CPFXDTC shows much higher infected muscle uptake (3.21%ID/g) than that of ^{99m}Tc-ciprofloxacin (1.24%ID/g) at 4 h post-injection. The T/B ratio of ^{99m}TcN-CPFXDTC (1.76) is better than that of ^{99m}Tc-ciprofloxacin (0.82), but the T/N ratio of the former (1.78) is much lower than that of the latter (4.28).

Table 1
Biodistribution in mice of ^{99m}TcN-CPFXDTC[a] and ^{99m}Tc-ciprofloxacin[b]

Tissue	^{99m}TcN-CPFXDTC		^{99m}Tc-ciprofloxacin
	3 h	4 h	4 h
Infected muscle	2.94 ± 0.47	3.21 ± 0.66	1.24 ± 0.06
Normal muscle	1.57 ± 0.36	1.80 ± 0.29	0.29 ± 0.03
Blood	1.84 ± 0.25	1.82 ± 0.27	1.51 ± 0.02
Heart	3.90 ± 0.92	5.06 ± 0.78	0.67 ± 0.07
Liver	34.54 ± 6.30	34.65 ± 5.93	20.08 ± 0.10
Lung	22.49 ± 7.01	21.11 ± 6.80	2.09 ± 0.15
Kidney	8.11 ± 1.37	9.21 ± 0.78	6.16 ± 0.05
Bone	5.06 ± 0.88	6.21 ± 1.26	No data
T/N ratio	1.87	1.78	4.28
T/B ratio	1.60	1.76	0.82

T/N = infected muscle-to-normal muscle, T/B = infected muscle-to-blood.
[a] All data are the mean percentage ($n = 4$) of the injected dose of ^{99m}TcN-CPFXDTC per gram of tissue, ±the standard deviation of the mean.
[b] The data are quoted from Ref. 15.

In summary, new ciprofloxacin dithiocarbamate was successfully synthesized and ^{99m}TcN-CPFXDTC 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 biodistribution studies in mice showed that the complex accumulated in the infected site with high uptake and good retention, justifying further investigations in animals and humans.

Acknowledgment

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11. The synthetic procedures and the spectral data for compound **1** are as follows. About 1.2 g of crushed sodium hydroxide (0.03 mol) and 3.625 g of ciprofloxacin hydrochloride (0.01 mol) were stirred vigorously in 11 ml of 40% tetrahydrofuran solution for 5 min in an ice-salt bath. To the above solution, 1 ml of carbon disulfide was then added. The mixture was stirred for 2 h in an ice-salt bath. Stirring was continued overnight at room temperature. The solvent was removed under reduced pressure and the residue was filtered off. The yellow crude product was recrystallized from methanol/diethyl ether to give the compound **1** (3.23 g, 66%). The compound **1** was characterized by IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectroscopy. IR (KBr)/cm^{-1}: 3416 (νOH), 1625 (νC=O), 1009 (νC=S), ^{1}H NMR (DMSO-d_6) δ: 8.58 (s, 1H), 7.76 (d, J = 10.78, 1H), 7.37 (s, 1H), 4.57 (br s, 4H), 3.58 (s, 1H), 3.16 (br s, 4H), 1.26 (br s, 2H), 0.99 (br s, 2H). ^{13}C NMR (DMSO-d_6) δ: 215.1, 175.3, 168.8, 153.7, 151.7, 147.9, 144.0, 138.7, 122.1, 111.7, 111.5, 106.0, 50.2, 48.9, 34.6, 8.0. The ESI mass spectrum (m/z, percent abundance) was as follows: 406 $[M-2Na+H]^-$, 100%.
12. The preparation procedure for ^{99m}TcN-CPFXDTC and the TLC, HPLC analysis conditions are as follows. One milliliter 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, 1.0 mg of compound **1** dissolved in 1.0 ml water was then added and the reaction allowed to stand for 15 min at room temperature. 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 (Kromasil 100-5C, 250 × 4.6 mm), Shimadzu SCL-10AVP series, working at a flow rate of 1.0 ml/min. Water (A) and methanol (B) mixtures were used as the mobile phase and the following gradient elution technique was adopted for the preparation (0 min 70% B, 10 min 70% B, 15 min 90% B, 30 min 90% B).
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14. Binding of ^{99m}TcN-CPFXDTC to bacteria was assessed by the following method. 0.2 ml of ^{99m}TcN-CPFXDTC (3.7 MBq) was transferred to a test tube. Then 0.4 ml of 0.01 mol/L acetic acid and 0.4 ml of 0.1 mol/L Na–PBS (pH 7.4) containing approximately 1×10^{10}/ml viable *Staphylococcus aureus* were added. The mixture was incubated for 1 h at 4 °C and then centrifuged for 5 min at 2000 rpm at 4 °C. The supernatant was removed and the bacterial pellet was gently resuspended in 1 ml of 0.1 mol/L Na–PBS (pH 7.4) and recentrifuged as above. The supernatant was removed, and the radioactivity in the bacterial pellet was determined by a gamma counter. The radioactivity related to bacteria was expressed in percent of the added ^{99m}Tc activity bound to viable bacterial in regard to total ^{99m}Tc.
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