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Synthesis and biological evaluation of a novel $^{99m}Tc(CO)_3$ complex of ciprofloxacin dithiocarbamate as a potential agent to target infection

Junbo Zhang *, Shijian Zhang, Haixun Guo, Xuebin Wang

Key Laboratory of Radiopharmaceuticals (Beijing Normal University), Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, PR China

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ABSTRACT

The ciprofloxacin dithiocarbamate (CPFXDTC) was radiolabeled with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ intermediate to form the $^{99m}Tc(CO)_3$–CPFXDTC complex in high yield. The $^{99m}Tc(CO)_3$–CPFXDTC complex was characterized by HPLC and its stability in serum was studied. Its partition coefficient indicated that it was a lipophilic complex. The bacterial binding efficiency of $^{99m}Tc(CO)_3$–CPFXDTC was almost the same as that of ^{99m}TcN–CPFXDTC, and was higher than that of ^{99m}Tc–ciprofloxacin. Biodistribution results in induced infection mice showed $^{99m}Tc(CO)_3$–CPFXDTC had higher uptake at the sites of infection and better abscess/blood and abscess/muscle ratios than those of ^{99m}Tc–ciprofloxacin and ^{99m}TcN–CPFXDTC. Single photon emission computed tomography (SPECT) static imaging study in infected rabbits demonstrated the uptake in the left thigh infection lesion was observable, while no accumulation in the right thigh muscle was found. These results suggested $^{99m}Tc(CO)_3$–CPFXDTC would be a promising candidate for further evaluation as infection imaging agent.

Infection is an important problem that needs accurate and prompt diagnosis for early management to avoid serious complications. Computed tomography (CT) and magnetic resonance imaging (MRI) have proved to be useful in detecting infections. However, these methods depend solely on morphologic changes, so infectious and inflammatory foci cannot be detected in an early stage because of the lack of substantial anatomical changes at this time. In contrast to CT and MRI, nuclear medicine techniques do not rely on morphologic changes but are based on physiochemical processes in tissues thus making them suitable to visualize infectious foci in early phases.[1] Currently, nuclear medicine imaging of infection and inflammation has been a powerful diagnostic tool in the management of patients with infectious or inflammatory diseases. Radiolabeled leukocytes are still the radiopharmaceutical agents of choice used in the diagnosis of focal bacterial infection and inflammation. However, this technique is time-consuming, needs a sterile environment, and has risk associated with handling of potentially contaminated blood. There is still of great interest in the development of new radiopharmaceuticals for infection imaging.

Since the discovery of ^{99m}Tc in the late 1930s and its introduction into nuclear medicine via the $^{99}Mo/^{99m}Tc$ generator in the 1950s, ^{99m}Tc has been in the forefront of radiopharmaceutical development. 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. It has shown many advantages over ^{99m}Tc-labeled leukocytes for diagnostic scans, in that it is more specific for bacterial infection, is easier to prepare, and acquires better image quality.[2–5] However, the problems of ^{99m}Tc–ciprofloxacin preparation discussed in the literature[6–8] are concerned with its low radiochemical yield and additional purification.[7]

In order to seek novel ^{99m}Tc infection imaging agents, we have recently reported the synthesis of ciprofloxacin dithiocarbamate (CPFXDTC) and its ^{99m}Tc labeling using the ^{99m}TcN core as targeted agent for infection imaging.[9] The ^{99m}TcN–CPFXDTC complex 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. However, the infected muscle-to-normal muscle ratio of the ^{99m}TcN–CPFXDTC (1.78) is much lower than that of the ^{99m}Tc–ciprofloxacin (4.28). Therefore, ongoing research is in progress to solve this problem.

In 1998, Alberto and coworkers first reported the one-step synthesis of the fac-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ complex by direct reduction of $[^{99m}TcO_4]^-$ with sodium borohydride in aqueous solution in the presence of carbon monoxide.[10] Moreover, Mallinckrodt Inc. has developed a kit (Isolink™) preparation of fac-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ core by boranocarbonate reduction from $[^{99m}TcO_4]^-$. This has prompted an explosion of research interest about technetium tricarbonyl complexes. The advantages of the ^{99m}Tc tricarbonyl synthon were related to its ease of preparation, readily substituted water molecules of the precursor fac-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ by a variety of functional groups, small size and inertness. Since technetium is present in the +1 oxidation state in a low spin d^6 electronic

* Corresponding author. Tel.: +86 10 62208126; fax: +86 10 62205562.
E-mail address: zhjunbo@bnu.edu.cn (J. Zhang).

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configuration, the fac-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ core can be used to prepare complexes that are chemically inert, providing a convenient platform for probe development.[11] As a successful example, ^{99m}Tc(CO)$_3$-MIBI as a potential good myocardial imaging agent has been intensively investigated.[12,13] Bearing in mind the presence of the [^{99m}Tc(CO)$_3$]$^+$ core in the molecular structure of a radiopharmaceutical may possibly alter its biological behavior. This background encouraged us to synthesize the ^{99m}Tc(CO)$_3$–CPFXDTC complex for finding a good infection imaging agent. In this study, the synthesis and biological evaluation of the ^{99m}Tc(CO)$_3$–CPFXDTC complex as a potential agent to target infection are reported.

The sodium salt of ciprofloxacin dithiocarbamate was prepared by reacting ciprofloxacin with an equivalent amount of carbon disulfide in NaOH solutions.[9] The reaction is schematically shown in Scheme 1.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}Tc(CO)$_3$–CPFXDTC was carried out using the following procedure in Scheme 2.[14]

The CPFXDTC ligand is a bidentate chelators, having two sulfur atoms that are well known to be an efficient moiety for ^{99m}Tc labeling. The H$_2$O molecule in the fac-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ precursor is readily displaced by functional groups (sulfur atoms) in the dithiocarbamate ligand. The ^{99m}Tc(CO)$_3$–CPFXDTC complex could be prepared by substituting the CPFXDTC ligand for the two H$_2$O molecules of the fac-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ precursor. Clearly, further studies should be performed, using macroscopic levels of the long-lived ^{99}Tc, to determine and characterize the structure of the ^{99m}Tc(CO)$_3$–CPFXDTC complex.

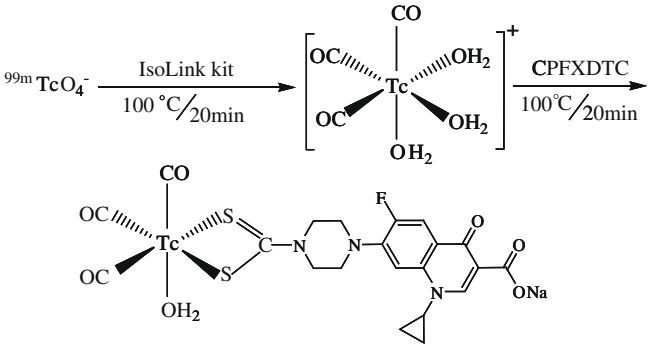
The radiochemical purity of the complex was routinely checked by HPLC.[14] The retention time of [^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ was 3.1 min, while that of ^{99m}Tc(CO)$_3$–CPFXDTC was found to be 19.6 min. Single peak suggested only one product (^{99m}Tc(CO)$_3$–CPFXDTC) was formed. The mean radiochemical purity of the product was >95% immediately after the preparation. The specific radioactivity of the product was calculated to be (16.82–336.4) MBq/nmol.

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 the complex was stable in the reaction mixture at room temperature. The stability in mouse plasma was determined by incubating 0.1 mL of ^{99m}Tc(CO)$_3$–CPFXDTC (3.7 MBq) in the solution of 0.5 mL murine plasma at 37 °C for 1 h and 3 h. Plasma proteins were precipitated by adding acetonitrile and removed by centrifugation. The supernatant was injected in HPLC to determine the stability of the complex. Figure 1A indicated that the complex was stable during the 1h incubation period in murine plasma. However, Figure 1B showed some (about 15%) decomposition occurred of ^{99m}Tc(CO)$_3$–CPFXDTC after incubation in murine plasma at 37 °C for 3 h. This decomposition is likely due to the substitution of the coordinated water molecule by the functional groups of murine plasma.

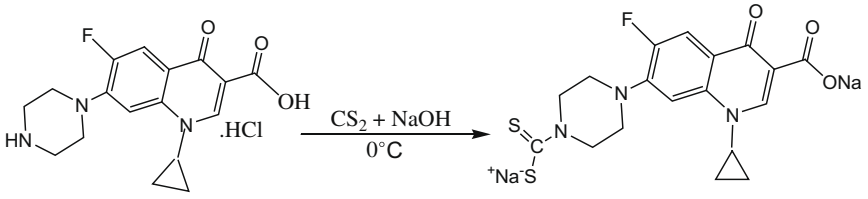
The partition coefficient was determined by mixing the complex with an equal volume of 1-octanol and phosphate buffer (0.025 mol/L, pH 7.4) in a centrifuge tube. The measurements were repeated five times and reported as an average of five measurements plus the standard deviation. The partition coefficient (logP) value of ^{99m}Tc(CO)$_3$–CPFXDTC was calculated as 0.40 ± 0.02 (n = 5). As compared to ^{99m}TcN–CPFXDTC and ^{99m}Tc–ciprofloxacin, the logP of the ^{99m}Tc(CO)$_3$–CPFXDTC complex was less than that of the ^{99m}TcN–CPFXDTC complex (logP = 1.02), but was more than that of ^{99m}Tc–ciprofloxacin (logP = −1.08).This facts suggested ^{99m}Tc(CO)$_3$–CPFXDTC and ^{99m}TcN–CPFXDTC were lipophilic, whereas ^{99m}Tc–ciprofloxacin was hydrophilic. The results also demonstrated the incorporation of the [^{99m}Tc(CO)$_3$]$^+$ core into the dithiocarbamate ligand may decrease the lipophilicity of the complex as compared to its [^{99m}TcN]$^{2+}$ analogue.

In order to compare its bacterial binding to that of ^{99m}TcN–CPFXDTC and ^{99m}Tc–ciprofloxacin at the same conditions, binding of ^{99m}Tc(CO)$_3$–CPFXDTC to bacteria was assessed by a procedure previously developed by our group.[15]The efficiency of bacteria binding of ^{99m}Tc(CO)$_3$–CPFXDTC was 65.2 ± 3.47% (n = 3), almost identical to that of^{99m}TcN–CPFXDTC (60%)[9] and higher than that of ^{99m}Tc–ciprofloxacin (40%).[7] However, the limitations of the bacterial binding experiment without a lipophilic control studies should be noted here.

Male Kunming mice weighing 18–20 g were used in all of the animal studies. About 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}Tc(CO)$_3$–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 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}Tc(CO)$_3$–CPFXDTC are shown in Table 1. As noted from Table 1, ^{99m}Tc(CO)$_3$–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. The liver, lung, spleen



Scheme 2. Preparation procedure and proposed structure of ^{99m}Tc(CO)$_3$–CPFXDTC.



Scheme 1. Synthesis of ciprofloxacin dithiocarbamate.

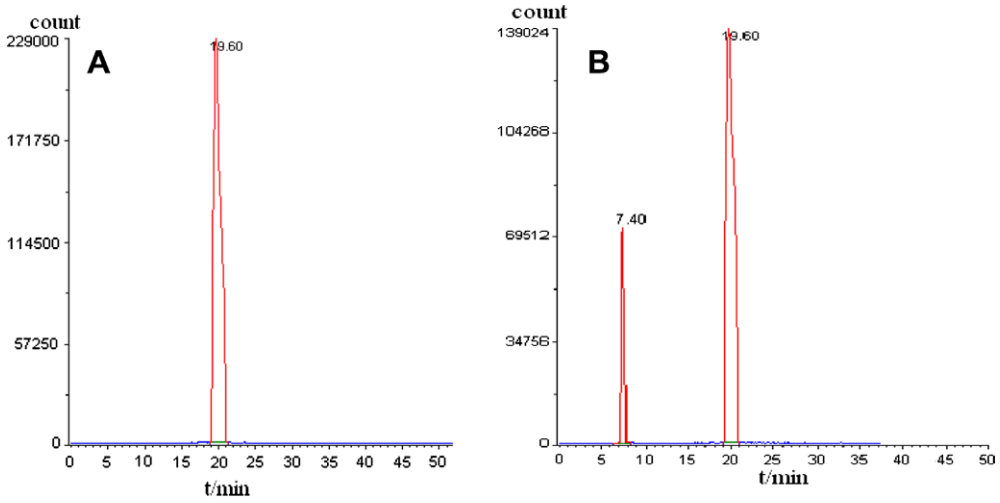


Figure 1. HPLC pattern of $^{99m}Tc(CO)_3$–CPFXDTC after incubation in murine plasma for 1 h (A) and 3 h (B).

Table 1
Biodistribution in mice of $^{99m}Tc(CO)_3$–CPFXDTC[a], ^{99m}TcN–CPFXDTC[b] and ^{99m}Tc–ciprofloxacin[c].

Tissue	$^{99m}Tc(CO)_3$–CPFXDTC 4 h p.i.	^{99m}TcN–CPFXDTC 4 h p.i.	^{99m}Tc–ciprofloxacin 4 h p.i.
Infected muscle	3.93 ± 0.74	3.21 ± 0.66	1.24 ± 0.06
Normal muscle	0.88 ± 0.16	1.80 ± 0.29	0.29 ± 0.03
Blood	0.92 ± 0.28	1.82 ± 0.27	1.51 ± 0.02
Heart	1.47 ± 0.21	5.06 ± 0.78	0.67 ± 0.07
Liver	30.01 ± 1.79	34.65 ± 5.93	20.08 ± 0.10
Lung	33.52 ± 9.71	21.11 ± 6.80	2.09 ± 0.15
Kidney	4.05 ± 0.77	9.21 ± 0.78	6.16 ± 0.05
Bone	2.39 ± 0.71	6.21 ± 1.26	No data
Spleen	20.22 ± 2.98	No data	5.50 ± 0.01
Intestine	1.17 ± 0.41	No data	0.61 ± 0.05
T/N ratio	4.47	1.78	4.28
T/B ratio	4.27	1.76	0.82

T/N, infected muscle-to-normal muscle, T/B, infected muscle-to-blood.
[a] All data are the mean percentage ($n = 5$) of the injected dose of $^{99m}Tc(CO)_3$–CPFXDTC per gram of tissue, ±the standard deviation of the mean.
[b] The data are quoted from reference 9.
[c] The data are quoted from reference 16.

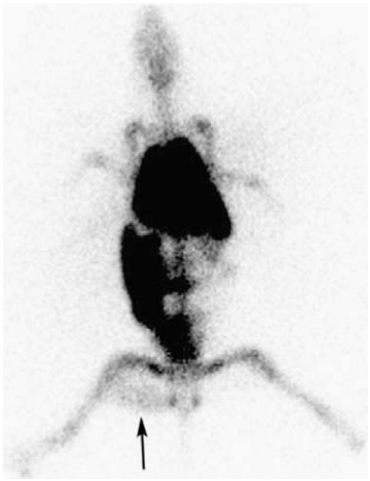


Figure 2. SPECT image was taken at 2-h post-injection of $^{99m}Tc(CO)_3$–CPFXDTC in infected rabbits.

uptakes are appreciable and this would be a major disadvantage with $^{99m}Tc(CO)_3$–CPFXDTC as compared to ^{99m}Tc labeled white blood cell. By comparison, $^{99m}Tc(CO)_3$–CPFXDTC shows higher infection uptake than that of ^{99m}TcN–CPFXDTC and ^{99m}Tc–ciprofloxacin at 4 h post-injection. The infection uptake of $^{99m}Tc(CO)_3$–CPFXDTC is high and the normal muscle and blood uptakes of $^{99m}Tc(CO)_3$–CPFXDTC are low so that the T/B and T/N ratios of $^{99m}Tc(CO)_3$–CPFXDTC are better than those of the other two complexes.

Three male rabbits weighing 2.5 kg was used in SPECT imaging studies. About 1.0 mL of 0.1 mol/L Na–PBS (pH 7.4) containing approximately 1×10^{10}/mL viable *S.* aureus was injected into the left thigh muscle of the rabbit. A week later, 1.0 mL of $^{99m}Tc(CO)_3$–CPFXDTC (185 MBq) was injected via the ear vein. Whole body static image (Fig. 2) was acquired by SPECT at 2 h post-injection. The imaging results showed the uptake in the left thigh infection lesion was obvious, while no accumulation in the right thigh muscle was found.

In summary, labeling of CPFXDTC via the ^{99m}Tc-carbonyl precursor could be achieved in high yields (>95%). The biodistribution study in mice showed that the $^{99m}Tc(CO)_3$–CPFXDTC complex had a higher infection uptake and better abscess/blood and abscess/muscle ratios than those of ^{99m}TcN–CPFXDTC and ^{99m}Tc–ciprofloxacin. In the present case, the $^{99m}Tc(CO)_3$–CPFXDTC complex did reveal good biological features as an infection imaging agent, justifying further investigations in animals and humans.

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14. The preparation procedure for $^{99m}Tc(CO)_3$–CPFXDTC and the HPLC analysis conditions are as follows. Briefly, 1 mL of saline containing $[^{99m}TcO_4]^-$ (activity ranging from 37 MBq to 740 MBq) was added to an Isolink™ kit and the vial was heated at 100 °C for 20 min. After cooling to room temperature, 0.1 mol/L HCl was added to adjust the pH to approximately 10. Then 1 mL of a water solution containing 1.0 mg of the CPFXDTC ligand was added and the reaction mixture was heated at 100 °C for 20 min. The $^{99m}Tc(CO)_3$–CPFXDTC complex was characterized by HPLC. 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).
15. Binding of $^{99m}Tc(CO)_3$–CPFXDTC to bacteria was assessed by the following method. 0.2 mL of $^{99m}Tc(CO)_3$–CPFXDTC (18.5 MBq/mL) 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. The measurements were repeated three times and reported as an average of three measurements plus the standard deviation.
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