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Radiosynthesis and biological evaluation of ^{99m}TcN-sitafloxacin dithiocarbamate as a potential radiotracer for *Staphylococcus aureus* infection

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Abstract Sitafloxacin dithocarbamate (SFDE) was synthesized, radiolabeled with technetium-99m (^{99m}Tc) using $[^{99m}Tc\text{-}N]^{2+}$ core and evaluated its biological efficacy as a potential radiotracer for *Staphylococcus aureus* (*S. aureus*) infection in artificially infected rats (AIRT) and rabbits (AIRB). The radiochemical stability of the ^{99m}Tc labeled SFDE (^{99m}TcN-SFDE) in saline and serum was determined by radio-HPLC and TLC methods, respectively. After, 1 min of reconstitution the value of radiochemical purity (RCP) was 99.00 ± 0.20% and was remained more than 90% unwavering even after 240 min of the radiolabeling. The ^{99m}TcN-SFDE complex showed similar radiochemical permanence behavior in serum at 37 °C. The complex showed almost six fold higher specific in vitro binding with living than heat killed *S. aureus*. Biodistribution behavior was evaluated in *S. aureus* AIRT and whole body imaging (WBI) in AIRB, respectively. Seven fold up take was observed in infected muscle of the AIRT as compared to inflamed and normal muscles. The disappearance of activity from blood and appearance in urinary system indicated normal route of excretion of the complex. Scintigraphically, it was confirmed that the labeled SFDE was higher accumulated in the infected muscle higher than in inflamed and normal muscle. The high radiochemical stability in saline and serum, specific in vitro binding with *S. aureus,* precise in vivo distribution in *S. aureus* AIRT and targeted WBI in AIRB confirmed the possibility of the ^{99m}TcN-SFDE complex as a potential and promising *S. aureus* infection radiotracer.

Keywords Sitafloxacin dithiocarbamate · $[^{99m}Tc\text{-}N]^{2+}$ core · *Staphylococcus aureus* · Infection

Introduction

The localization and discrimination of in vivo infection from non-infective inflammatory processes and tumors at early stages with scintigraphic procedures is a fast growing area of research [1]. The scintigraphic procedures are proven clinically vital in the early understanding of the disease processes at molecular levels without the inconvenient invasive techniques, if a specific ligand is provided [2].

A number of infection imaging agents were reported including the most promising ^{111}In or ^{99m}Tc labeled leukocytes after the development of ^{67}Ga-citrate. However, due to lack of wide availability, high radiation burden, cumbersome and time consuming preparation procedure etc. have opened new ways for the development of newer and better radiopharmaceuticals for in vivo localization of infection [3, 4].

The extensive evaluation of the ^{99m}Tc-nitridio complexes has been recently explored as a novel radiolabeling technique for the development of newer and better radiopharmaceuticals. The complexation of $[^{99m}TcN]^{2+}$ core with ligand containing sulfur atom was found more stable [5, 6].

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In continuation to our ongoing investigations [7–11] and other reported studies on technetium-99m (^{99m}Tc) labeled antibacterial agents [12–20], the present investigation is focused on the modification of sitafloxacin (STF) to sitafloxacin dithiocarbamate (SFDE) (Fig. 1a) and its radiolabeling with ^{99m}Tc via $[^{99m}TcN]^{2+}$ core. The percent radiochemical purity (% RCP) yield, in vitro stability in saline and serum, in vitro binding with bacteria, and biodistribution in artificially infected rats and scintigraphic were evaluated.

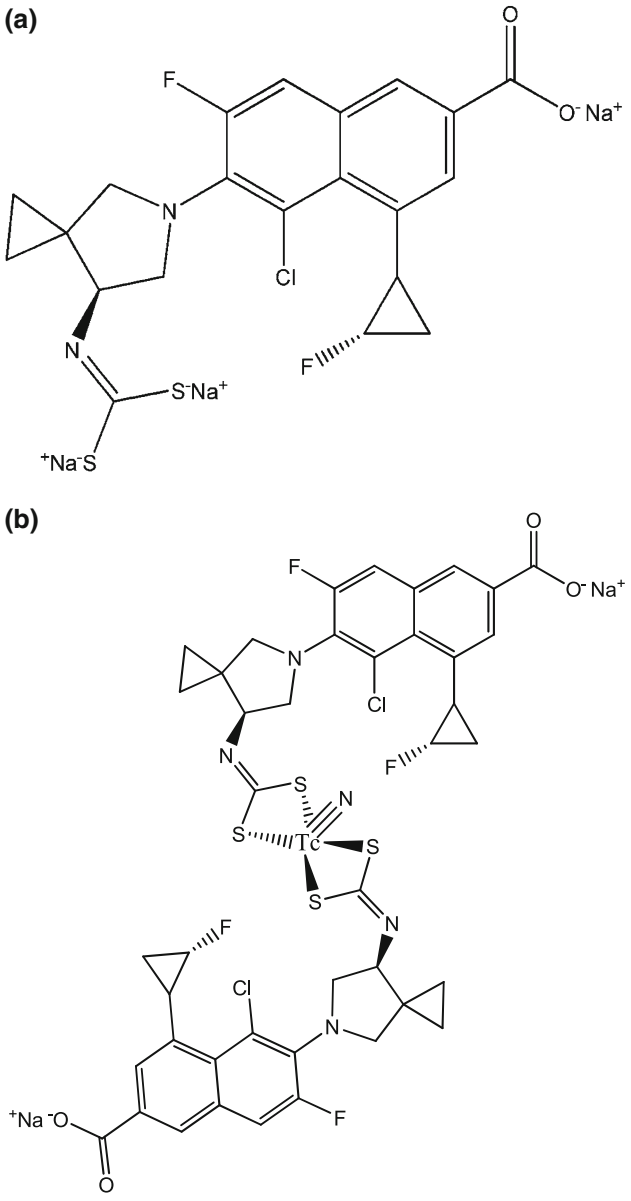

Fig. 1 **a** Chemical structure of sitafloxacin dithiocarbamate (SFDE). **b** Proposed structure of the ^{99m}TcN-SFDE complex

Experimental

Materials

Sitafloxacin (STF) (Daiichi Sanko, Japan), succinic dihydrazide (SDH), propylenediamine tetra acetic acid (PDTA) (Aldrich, USA) and all the other chemicals and solvents of analytical grade (Sigma)., RP-HPLC (Shimadzu, Japan), well counter, scalar count rate meter (Ludlum, USA), Dose calibrator (Capintech USA) and the Gamma camera (GEADE Nuclearmedizine system, Germany).

Method

Synthesis of sitafloxacin dithiocarbamate (SFDE)

Sitafloxacin (STF) was modified to sitafloxacin dithiocarbamate (SFDE) using the reported method [14] with slight modification, briefly, sodium hydroxide (NaOH) 1.2 gm was mixed with 3.625 gm of STF.HCl in a clean sterilized flask having 11 mL of 40% tetrahydrofuran. The mixture was stirred for 10 min in ice-bath followed by addition of 1 mL carbon disulfide (CS_2). The mixture was stirred continuously for 8 h in an ice-bath. The solvent was removed under reduced pressure and the product was recovered.

Radiosynthesis of ^{99m}TcN-SFDE complex

^{99m}TcN-SFDE complex was prepared by adding 1 mCi (0.5 mL) of freshly eluted sodium pertechnetate ($Na_2^{99m}TcO_4$) to a preparation containing 0.05 mg stannous chloride dihydrate, 5.0 mg PDTA and 5.0 mg SDH. After 10 min, 2 mg (1 mL saline) of SFDE was added to the preparation followed by incubation at room temperature for 10 min.

Radiochromatography

HPLC was used to determine the percent radiochemical purity (% RCP) of the ^{99m}TcN-SFDE complex using the reported method with minor modifications [9]. Briefly, 5 µL of the preparation was injected into C-18 column (4.6 × 150 mm, 5 µM) of the shimadzu SCL-10 AVP system, equipped with SDP-10 AVP UV detector operating at 254 nm, Packard 500 TR series flow scintillation analyzer, binary pump, and online degasser followed by 1 mL/min water:acetonitrile (1:9) elution. The fractions collected separately were checked for activity using well counter interface with scalar count rate meter (WCSR). The preparation was examined at 30, 60, 90 and 120 min after radiolabeling for % RCP.

Stability in serum

TLC with two mobile systems was used for in vitro stability determination of the ^{99m}TcN-SFDE complex in human serum. The ^{99m}TcN-SFDE complex, 0.2 mL was incubated with 1.8 mL serum for 120 min at 37 °C. Thereafter, 1 µL of the preparation was applied to the TLC strip and after drying developed in two different mobile systems. The one is saline in which $^{99m}TcO_4^-$, $^{99m}TcO_2$ and the complex stayed at the application point while the intermediate $[^{99m}TcN]^{2+}$ moiety move with the solvent front. However, in acetone as mobile phase the complex and $[^{99m}TcN]^{2+}$ moiety stayed at the origin**.** The TLC strips developed in different mobile phases were analyzed for activity using well counter.

In vitro binding with *Staphylococcus aureus*

In vitro binding behviour of the ^{99m}TcN-SFDE complex was investigated using the reported method [21]. Briefly, 10 MBq of the ^{99m}TcN-SFDE in 0.1 mL of sodium phosphate buffer (Na-PB) was transferred to a clean and sterilized test tube. Thereafter, 0.8 mL of 50% (v/v) 0.01 M acetic acid in a Na-PB containing approximately 1×10^8 colony forming units (CFU) of *S. aureus,* were added. The mixture was then incubated at 4 °C for 1 h (pH 5). The mixture after 1 h was centrifuged for 5 min at 2000 rpm. The bacterial pellets were resuspended after the removal of supernatant in 1 mL Na-PB and recentrifuged. The *S. aureus* pellets after removal of the supernatant analyzed for % activity using well counter.

Animal model and biodistribution

Healthy, Sprague–Dawley male rats (weight rage 150–200 g) 18 in number were selected and placed in two groups. The rats of group 1 and 2 were infected intramuscularly (I.M.) with 0.5 mL living *S. aureus* and heat killed *S. aureus* (right thighs), respectively. After 12 h, 0.5 mL of sterile turpentine oil was injected I.M. into the left thigh of all the model rats followed by intravenous (I.V.) injection of 0.1 mL (37 MBq) of ^{99m}TcN-SFDE. The model rats were sacrificed in accordance with the guide lines of the Nuclear Medicine Research Laboratory, University of Peshawar. Percent injected absorbed dose (% I.D/g) in one gram of blood, *S. auras* infected (SAI), inflamed (NT), normal (NR) muscle, liver, spleen, stomach, intestine, and kidney of the model rats were calculated using gamma well counter.

Whole body imaging

The imaging silhouette of the ^{99m}TcN-SFDE was evaluated in animal model New Zealand white rabbits (weight range 3.0–4.0 kg). In saline, 1 mL containing 1×10^8 CFU of the *S. aureus* was intramuscularly administered to the right thigh of the healthy rabbit followed by 1 mL sterile turpentine oil (with a 12 h gap) to the left thigh. Thereafter, 0.4 mL (111 MBq) of ^{99m}TcN-SFDE was I.V. injected through ear vein of the model rabbit. Whole body imaging of the model rabbit was processed using gamma camera (GC) equipped with low energy general purpose collimator (LEGPC).

Results and discussion

The SDH in a kit acts as an efficient source nitride nitrogen (N^{3-}) and stannous chloride as reducing agent and the PDTA avert the formation of insoluble salt of tin. The SDH in the presence of stannous chloride react with sodium pertechnetate to give an intermediate $[^{99m}TcN]^{2+}$ core that under substitution reaction with SFDE, yield ^{99m}TcN-SFDE complex.

Radiochromatography

The HPLC radio-chromatogram (Fig. 2) of the ^{99m}TcN-SFDE showed two distinctively variable peaks with retention time 3.2 and 10.5 min. The radioactivity peak at 3.2 and 10.5 min represents the intermediate $[^{99m}TcN]^{2+}$ core and ^{99m}TcN-SFDE, respectively. The RCP of the ^{99m}TcN-SFDE complex determined at various intervals after reconstitution is shown in Fig. 3. The maximum RCP observed was 99.00 ± 0.20% at 1 min.

By analogy with the molecular structure of bis(diethyldithiocarbamato) nitride ^{99m}Tc complex [22]. It seems

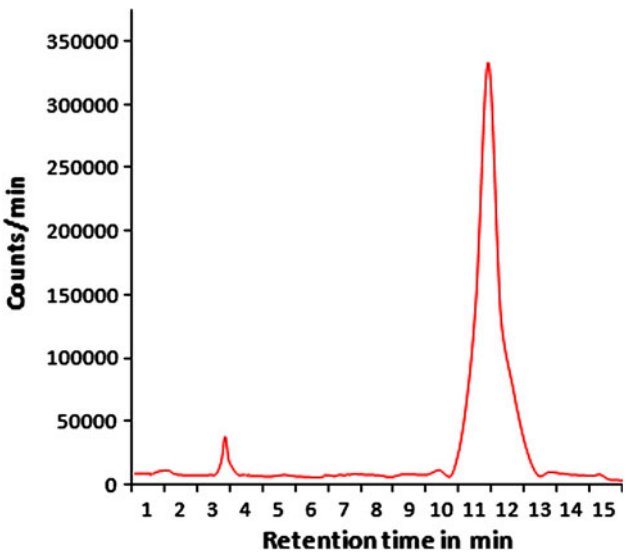

Fig. 2 HPLC profile of ^{99m}TcN-SFDE complex

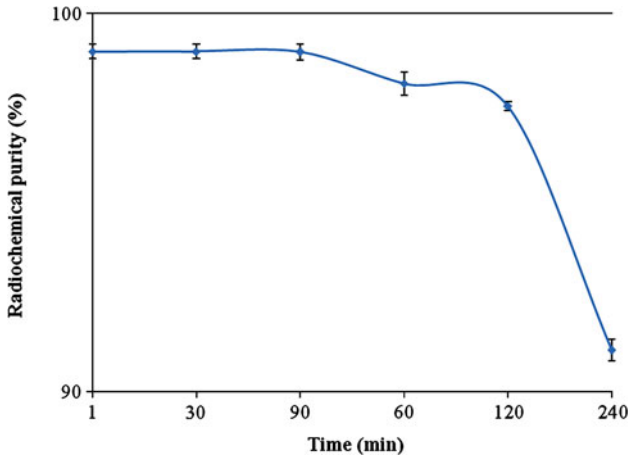

Fig. 3 Stability of ^{99m}TcN-SFDE complex in saline at different intervals

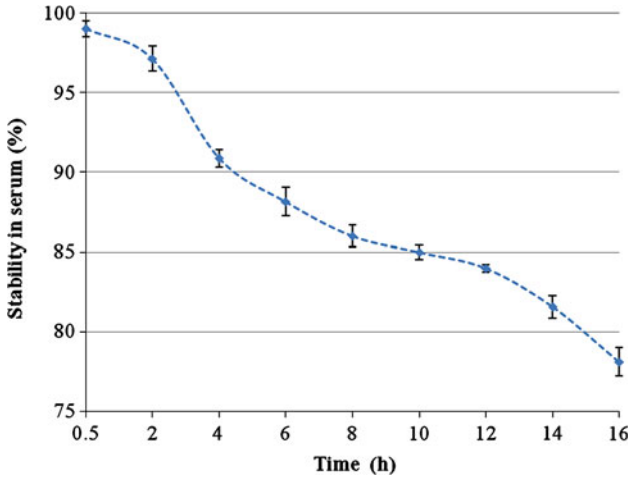

Fig. 4 Stability of ^{99m}TcN-SFDE complex in serum at 37 °C at different intervals

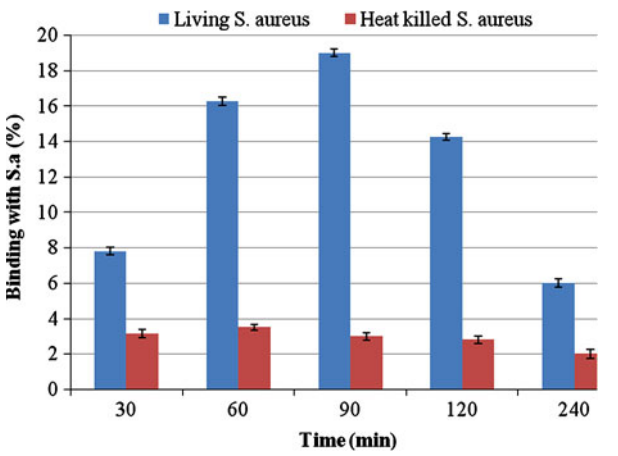

Fig. 5 In vitro binding of the ^{99m}TcN-SFDE complex with living and heat killed *S. aureus*

Table 1 In vivo distribution (in %) of the ^{99m}TcN-SFDE complex in rats at different intervals of I.V. administration (mean ± S.D)

Organs of the *S. aureus* infected rats (gm)	Distribution of ^{99m}TcN-SFDE (in %) at different intervals (in min)							
	Living *S. aureus* infected rats				Heat killed *S. aureus* infected rats			
	30	60	90	120	30	60	90	120
S. auras infected (SAI) muscle	7.80 ± 0.20	16.25 ± 0.23	19.00 ± 0.20	18.50 ± 0.20	3.15 ± 0.22	3.50 ± 0.18	3.00 ± 0.21	2.80 ± 0.23
Inflamed (NT) muscle	3.15 ± 0.21	3.50 ± 0.20	3.00 ± 0.22	2.80 ± 0.19	3.15 ± 0.18	3.50 ± 0.22	3.00 ± 0.22	2.80 ± 0.20
Normal (NR) muscle	2.50 ± 0.21	2.50 ± 0.22	2.50 ± 0.18	2.50 ± 0.20	3.15 ± 0.22	3.50 ± 0.20	3.00 ± 0.20	2.80 ± 0.23
Blood	23.00 ± 0.18	7.25 ± 0.20	6.80 ± 0.18	3.00 ± 0.24	23.50 ± 0.21	8.90 ± 0.22	7.50 ± 0.23	4.00 ± 0.22
Liver	19.50 ± 0.18	12.10 ± 0.21	8.75 ± 0.24	4.50 ± 0.23	21.50 ± 0.24	13.75 ± 0.21	9.00 ± 0.20	6.15 ± 0.19
Spleen	8.15 ± 0.20	5.50 ± 0.24	3.25 ± 0.21	2.80 ± 0.26	8.00 ± 0.20	6.75 ± 0.22	5.00 ± 0.19	3.25 ± 0.18
Kidney	10.00 ± 0.25	20.50 ± 0.23	23.15 ± 0.24	25.50 ± 0.22	10.25 ± 0.19	20.00 ± 0.24	24.10 ± 0.21	26.00 ± 0.24
Stomach & intestines	10.00 ± 0.21	8.50 ± 0.23	6.10 ± 0.24	3.10 ± 0.22	10.50 ± 0.24	8.75 ± 0.19	5.80 ± 0.24	3.25 ± 0.21
SAI/NR muscle ratio	3.12 ± 0.95	6.50 ± 1.05	7.60 ± 1.11	7.40 ± 1.00	1.00 ± 1.00	1.00 ± 0.90	1.00 ± 1.05	1.00 ± 1.00
NT/NR muscle ratio	1.26 ± 1.00	1.40 ± 0.91	1.20 ± 1.22	1.12 ± 0.95	1.00 ± 0.82	1.00 ± 1.10	1.00 ± 1.10	1.00 ± 0.87

reasonable to propose a similar structure for ^{99m}TcN-SFDE complex having a square pyramidal geometry with 1:2 stoichiometry of the ^{99m}TcN:Ligand. The proposed structure of the ^{99m}TcN-SFDE complex is given in Fig. 1b.

Serum stability

The in vitro permanence of ^{99m}TcN-SFDE complex in serum at 37 °C after reconstitution is shown in Fig. 4. The complex was found stable more than 90% up to 4 h and the stability went down up to 78.10 ± 0.90% within 16 h.

In vitro binding with *Staphylococcus aureus*

The complex showed saturated in vitro binding to the living but not to the heat killed *S. aureus*. No effect was observed on adding additional SFDE in incubation. The in vitro binding affinity of the ^{99m}TcN-SFDE complex is shown in Fig. 5.

Biodistribution

The uptake of the ^{99m}TcN-SFDE in different organs of the animals infected with living and heat killed *S. aureus* is given in Table 1. The uptake was significantly low in heat killed *S. aureus* infected group of animals as compared to the living. Initially, the activity in blood, liver, spleen, was much higher than in kidney and urine but after 60 and 90 min a reciprocal contour was observed in the group of animals infected with living *S. aureus*. Seven fold uptake in the target organ (SAI) was observed in a groups of 9 animals infected with living as compared to the heat killed *S. aureus*. The distribution of the labeled SFDE in different organs and absconding from blood, liver, spleen, and appearance in the kidney confirmed the normal route of excretion and seven fold accumulations in the infected muscle confirmed the normal route of excretion and promising *S. aureus* in vivo infection radiotracer.

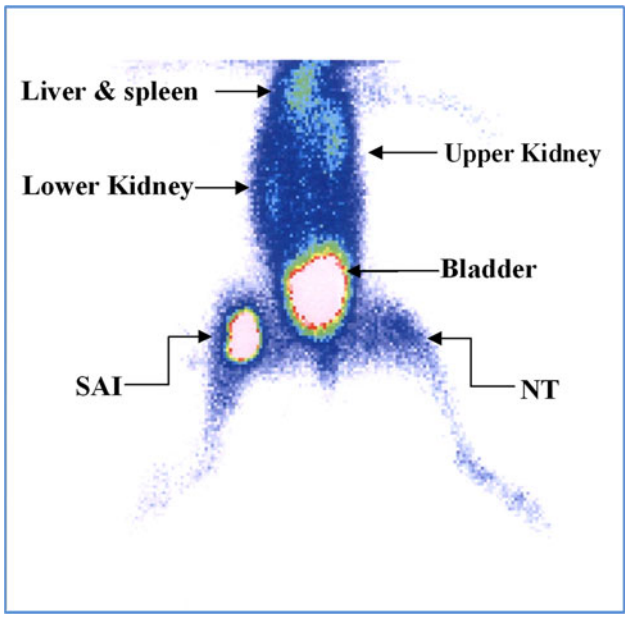

Fig. 6 Whole body image of the artificially *S. aureus* infected rabbits after intravenous (I.V.) injection of ^{99m}TcN-SFDE complex

Scintigraphy

Scintigraphically, our findings confirmed that initially the radiolabeled from blood stream accumulated into the liver and spleen. Thereafter, specifically the ^{99m}TcN-SDEF appeared in the infected sites and renal system. Almost negligible uptake was seen in NT and NR muscle of the infected rabbit as shown in Fig. 6.

Conclusion

Sitafloxacin was modified to sitafloxacin dithiocarbamate and labeled with ^{99m}Tc-N core through ligand exchange reaction and evaluated as a potential *Staphylococcus aureus* (*S. aureus*) infection radiotracer. The complex showed high in vitro permanence in saline and serum, better in vitro binding affinity with *S. aureus,* promising biodistribution behaviour in *S. aureus* infected rats and fine scintigraphic results in *S. aureus* infected rabbits. Based on the above findings we recommended the ^{99m}TcN-SFDE complex for in vivo infection localization in human.

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