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Radiocomplexation and biological characterization of the ^{99m}TcN-trovafloxacin dithiocarbamate: a novel methicillin-resistant *Staphylococcus aureus* infection imaging agent

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Abstract The radiolabeling of trovafloxacin dithiocarbamate (TVND) with technetium-99m using $[^{99m}Tc\text{-}N]^{2+}$ core was investigated and biologically assessed as prospective infection imaging agent. The achievability of the ^{99m}TcN-TVND complex as a future MRSA infection radiotracer was investigated in artificially methicillin-resistant *Staphylococcus aureus* (MRSA) infected male Sprague–Dawley rats (MSDR). Radiochemically the ^{99m}TcN-TVND complex was characterized in terms of radiochemical purity (RCP) in saline, in vitro permanence in serum, in vitro binding with MRSA and biodistribution in living and heat killed MRSA infected MSDR. Radiochemically the complex showed stability in saline with a 97.90 ± 0.22% yield and serum at 37 °C up to 4 h. The ^{99m}TcN-TVND complex showed saturated in vitro binding with MRSA. Normal in vivo uptake in the MRSA infected MDRS was observed with a five fold uptake in the infected muscle as compared to inflamed and normal muscles. The high RCP values, in vitro permanence in serum, better in vitro binding with MRSA, biodistribution behavior and the target to non-target (infected to inflamed muscle) ratios posed the ^{99m}TcN-TVND complex as a promising MRSA infection radiotracer.

Keywords Trovafloxacin dithiocarbamate · $[^{99m}Tc\text{-}N]^{2+}$ core · MRSA infection

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Introduction

The recognition of acute, subacute and chronic infection and its discrimination from inflammation for appropriate management of patients is highly significant for clinicians. The functional imaging modality of nuclear medicine outweigh the latest structural imaging techniques like high resolution computerized tomography (CT) and magnetic resonance imaging (MRI) by its capability of detecting early disease with accurate discrimination from non-infective pathology. However, selection of the right technique is always a serious matter of concern for the clinicians with special reference to the fever of unknown origin [1, 2].

The available infection imaging agents, developed in the last two decades, have facilitated the clinician in the early diagnoses of infection and its appropriate treatment [3–13]. We have also reported a number of novel technetium-99m (^{99m}Tc) labeled infection radiotracers [14–19]. The radiochemical purity (RCP), in vitro stability in saline and serum, in vitro binding with pathogens and biodistribution results forced us to keep on searching for newer and better infection imaging agents.

Trovafloxacin (TVN), {1,5,6-α-7-(6-amino-3-azabicyclo[3.1.0]hex-3-yl)-1-(2,4-difluorophenyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid} (Fig. 1a) is a new synthetic fluoroquinolone broad spectrum antibiotic which is found to be more effective against both Gram-positive (G+ive) and Gram-negative (G−ive) bacteria. The TVN has proven very persuasive against *Cocci* (G+ive) and especially against methicillin-resistant *Staphylococcus aureus* (MRSA). The TVN through inhibition of the bacterial DNA gyrase terminate the growth of MRSA [20, 21].

The ^{99m}Tc-nitridio complexes played a vital role in the radiosynthesis of newer and promising agents intended for various nuclear medicine diagnostic tests [3]. In

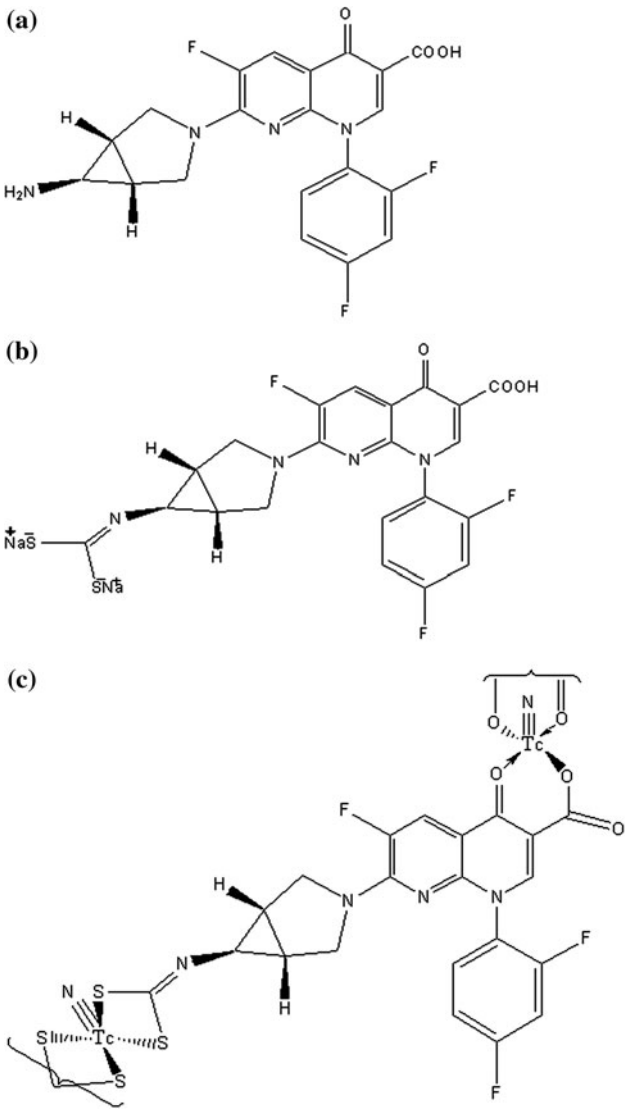

Fig. 1 **a** Chemical structure of Trovafloxacin(TVN). **b** Trovafloxacin dithiocarbonate(TVND). **c** Proposed structure of the ^{99m}TcN-TVND complex

continuation to our ongoing research, now we are reporting the radiolabeling of TVN with ^{99m}Tc using $[^{99m}TcN]^{2+}$ core for more stable complexation. In the current investigation, achievability of the ^{99m}TcN-TVND radiocomplex to map out the deep tissue infections in artificially MRSA infected MSDR was evaluate. The aptness of the ^{99m}TcN-TVND radiocomplex was also observed in terms of in vitro eternalness in normal saline at room temperature and in serum at 37 °C, in vitro binding with MRSA, biodistribution in artificially MRSA infected MSDR.

Experimental

Materials

Trovafloxacin (TVN) (Pfizer Inc. New York, USA), TLC (Merk), succinic dihydrazide (SDH), propylenediamine tetra-acetic acid (PDTA) 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 Gamma camera GKS-1000 (GEADE Nuclearmedizine system, Germany).

Method

Preparation and tricarbonyl complexation of trovafloxacin dithiocarbamate

Trovafloxacin (TVN) was converted to trovafloxacin dithiocarbamate (TVND) using the reported method [19]. Thereafter, 0.5 mL sodium pertechnetate ($Na^{99m}TcO_4^-$) freshly eluted was mixed with 0.10 mg $SnCl_2{\cdot}2H_2O$, 5.0 mg propylenediamine tetra-acetic acid (PDTA), 5.0 mg succinic dihydrazide (SDH) in a sterilized vial. The preparation was incubated at room temperature for 10 min and 2 mg of TVND was added after gentle swirling.

Partition coefficient of the ^{99m}TcN-TVND complex

The partition coefficient of the ^{99m}TcN-TVND complex was determined using the reported method [22]. Briefly, equivalent volume of the ^{99m}TcN-TVND complex, octanol and phosphate buffer (PB) was vortexed for 5 min at room temperature. The preparation was then centrifuged at 5000 rpm/min for 15 min. Subsequently, at different intervals aliquots were haggard and counted for activity using well counter interface with scalar count rate meter. Using the following formula partition coefficient was calculated.

$$P = \frac{(\text{Counts per min in octanol} - \text{counts per min in background})}{(\text{Counts per min in buffer} - \text{counts per min in background})}$$

HPLC analysis of the ^{99m}TcN-TVND complex

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 was used to characterized the ^{99m}TcN-TVND complex using C-18 column (4.6 × 150 mm) as a stationary phase and water (W)/methanol (M) as mobile phase. Freshly prepared ^{99m}TcN-TVND complex 10 µL was injected into the C-18 column and 1 mL/min mobile phase was applied for 15 min [for 0–2 min (100% W), 2–5 min (100–75% W), 5–7 min (75–66% W), 7–10 min (34–100 M), 10–12 min (100% M) and 12–15 min (100% M to 100% W)]. The radio-fractions collected at different intervals were counted for activity using single well counter interface with scalar count rate meter.

In vitro stability of the ^{99m}TcN-TVND complex in serum

The ^{99m}TcN-TVND radiocomplex was investigated for its in vitro stability in serum using TLC method. 1.8 mL serum was incubated with 0.2 mL of the ^{99m}TcN-TVND radiocomplex at 37 °C for 16 h. At different intervals during incubation aliquots were drawn and applied on a TLC strip. Thereafter, the strip was developed in saline and CH_2Cl_2:CH_3OH (9:1) (v/v). Thereafter, The TLC strips were divided into two at R_f 5 and measured for percent in vitro stability using single well counter interface with scalar count rate meter.

MRSA in vitro uptake

In vitro binding of the ^{99m}TcN-TVND radiocomplex with MRSA was investigated using the reported method [23]. Briefly, the ^{99m}TcN-TVND complex, 0.2 mL (10 MBq) was moved through a sterilized syringe to a tube containing 0.1 mL sodium phosphate buffer (Na-PB). Thereafter, 0.8 mL (50%, v/v) 0.01 M acetic acid (AA) containing approximately 1×10^8 colony forming units (CFU) of MRSA were added to the tube followed by incubation at 4 °C for 1 h. The pH of the preparation was adjusted to pH 5 and the preparation was centrifuged at 2000 rpm for 15 min. The supernatant was discarded and the pellets were resuspended in 2 mL of Na-PB and repeated the centrifugation at 2000 rpm for 15 min. Finally, the pellets were counted for activity with single well counter interface with scalar count rate meter.

Biodistribution

Male Sprague–Dawley rats (MSDR) (weight range, 180–220 g) were used for the determination of the percent absorbed dose of the ^{99m}TcN-TVND radiocomplex. Twelve healthy MSDR were selected and divided into two groups (**A** and **B**) of six rats each. MSDR (group **A** and **B**) were intramuscularly (I.M.) administered 0.2 mL sterile turpentine oil to the left thigh. 0.2 mL of living MRSA in saline containing approximately 1×10^8 colony forming units (cfu) were injected to the right thigh of the group **A** and similarly heat killed MRSA to the group **B** MSDR, respectively. After 24 h 0.5 mL (37 MBq) of the freshly prepared radiotracer was intravenously (I.V.) injected to the MSDR of group **A** and **B.** Subsequently, the MSDR of group **A** and **B** animals were sacrificed as per rules of the Nuclear Medicine Research Laboratory Part-I and II. The absorbed dose per gram in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed muscle and normal muscle was calculated using single well counter interface with scalar count rate meter.

Results and discussion

Radiocomplexation and characterization of the ^{99m}TcN-TVND complex

Trovafloxacin (Fig. 1a) was converted to its dithiocarbamate derivative (Fig. 1b) and tagged by treatment with an intermediate $[^{99m}TcN]^{2+}$ core using the method described earlier [19]. The proposed structure of ^{99m}TcN-TVND radiocomplex (Fig. 1c) with the tetra-dentate ligand will have square planner pyramidal geometry with TcN: Ligand ratio of 1:1.

The HPLC radiochromatogram of the ^{99m}TcN-TVND complex is shown in Fig. 2 giving radio-peaks at retention time (RT) 3.9 and at 12.2 min. The peak at 3.9 min of RT correspond to $[^{99m}TcN]^{2+}$ intermediate and the one at 12.2 min to the ^{99m}TcN-TVND complex.

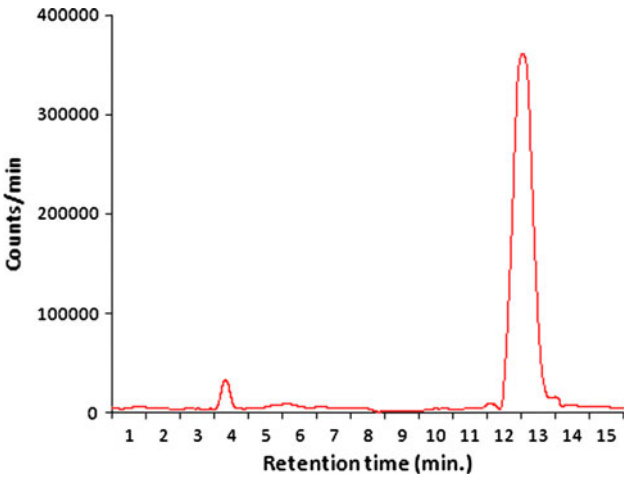

Fig. 2 HPLC radiochromatogram of the ^{99m}TcN-TVND complex

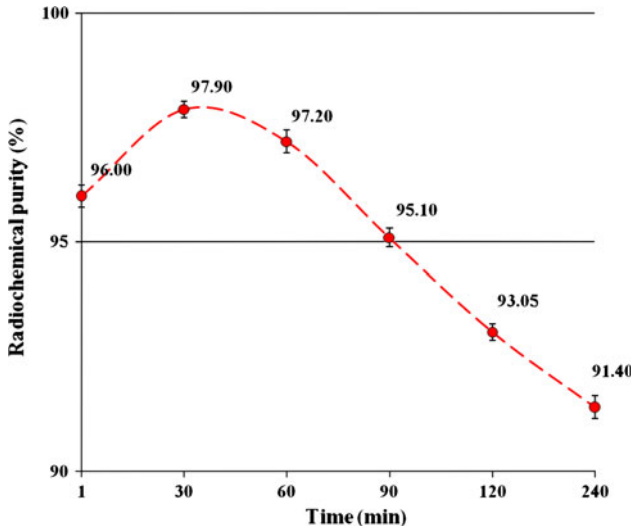

Fig. 3 Stability of the ^{99m}TcN-TVND complex in normal saline at different intervals

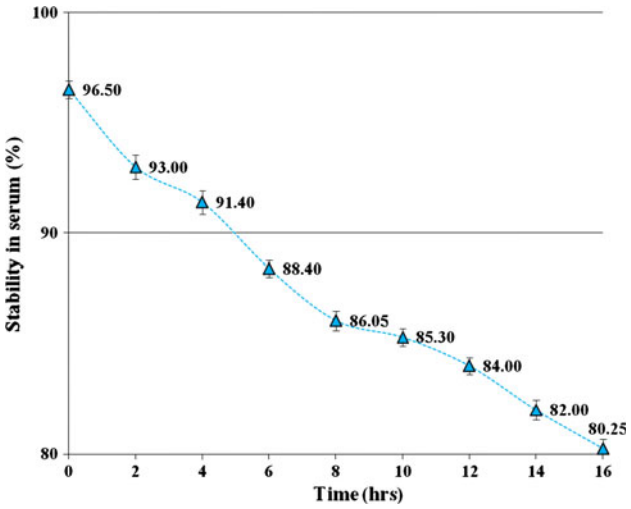

Fig. 4 In vitro stability of the ^{99m}TcN-TVND complex in serum at 37 °C

Radiochemically the ^{99m}TcN-TVND complex showed stable behaviour in normal saline as shown in Fig. 3. The radiochemical purity values of the ^{99m}TcN-TVND complex went down with the time but not less than 90% up to 240 min after reconstitution. The ^{99m}TcN-TVND complex showed maximum RCP value (97.90 ± 0.22%) at 30 min after reconstitution.

Lipophilicity of the ^{99m}TcN-TVND complex

The participation coefficient values calculated for ^{99m}TcN-TVND complex is 1.02 ± 0.04. The *P* value for ^{99m}TcN-TVND complex suggested that the complex is lipophilic.

In vitro stability of ^{99m}TcN-TVND complex in serum

In vitro stability of the ^{99m}TcN-TVND complex in serum at 37 °C up to 16 h is given in Fig. 4. In serum the ^{99m}TcN-TVND complex showed stable profile up to 4 h. The stability went down from 96.50 ± 0.24 to 80.25 ± 0.20% within 16 h.

In vitro MRSA uptake

The in vitro binding of the ^{99m}TcN-TVND complex with MRSA is shown in Fig. 5. Maximum and saturated in vitro binding was observed at 90 min of the incubation.

Biodistribution

The absorbed dose per gram in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed muscle and normal muscle of MSDR artificially infected with living (group **A**) and heat killed MRSA (group **B**) after I.V.

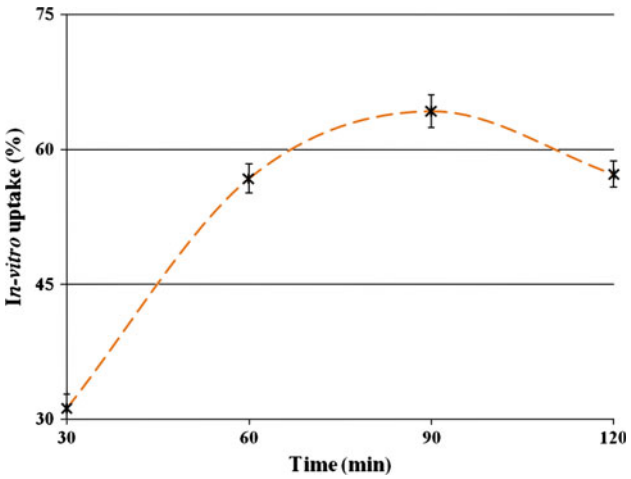

Fig. 5 In vitro binding of MRSA with ^{99m}TcN-TVND radiocomplex

administration of the ^{99m}TcN-TVND complex is given in Table 1. High percentage of the radioactivity was noted in blood after 30 min of the I.V. administration of the ^{99m}TcN-TVND in group **A** and **B**, MSDR. However, this high uptake was found temporary and it went down from 20.50 ± 0.18 to 3.50 ± 0.15% within 120 min. Similar behaviour was observed in case of liver, spleen, stomach and intestines. However, in case of kidneys a reverse behaviour was noted where the activity of the complex was initially low which went up with time from 10.50 ± 0.16 to 23.25 ± 0.16%. Approximately, five fold higher activity of the tagged complex was noted in infected muscle of group **A**, MDSR as compared to inflamed and normal muscles as shown in Fig. 6. However, in group **B**, MDRS no such distinct difference in the infected muscle was seen as compared to the inflamed and normal muscles. The disappearance of

Table 1 Percent absorption of the ^{99m}TcN-TVND complex in various organs of MRSA infected MDSR

Organs/tissues (gm)	Percent in vivo absorption at different intervals (min)							
	Alive MRSA				Heat killed MRSA			
	30	60	90	120	30	60	90	120
Infected muscle	6.25 ± 0.18	11.35 ± 0.16	12.85 ± 0.16	11.45 ± 0.20	2.80 ± 0.18	3.00 ± 0.19	3.00 ± 0.18	2.50 ± 0.16
Inflamed muscle	3.00 ± 0.16	3.50 ± 0.17	3.00 ± 0.0.18	3.00 ± 0.16	3.50 ± 0.19	3.50 ± 0.15	3.00 ± 0.20	3.00 ± 0.17
Normal muscle	3.00 ± 0.15	2.50 ± 0.017	2.50 ± 0.19	2.50 ± 0.19	2.50 ± 0.17	2.50 ± 0.18	2.50 ± 0.15	2.50 ± 0.19
Blood	20.50 ± 0.18	9.00 ± 0.16	7.35 ± 0.16	3.50 ± 0.15	21.00 ± 0.20	10.50 ± 0.15	8.10 ± 0.15	3.25 ± 0.20
Liver	16.40 ± 0.18	12.10 ± 0.16	9.25 ± 0.17	5.5 ± 0.20	17.10 ± 0.18	12.25 ± 0.15	9.10 ± 0.18	4.85 ± 0.15
Spleen	9.45 ± 0.17	7.50 ± 0.14	6.10 ± 0.20	3.20 ± 0.16	9.05 ± 0.15	6.90 ± 0.14	4.80 ± 0.20	3.50 ± 0.18
Kidney	10.50 ± 0.16	17.80 ± 0.20	20.75 ± 0.18	23.25 ± 0.16	10.00 ± 0.16	17.55 ± 0.15	21.15 ± 0.14	24.00 ± 0.20
Stomach and intestines	8.30 ± 0.17	6.95 ± 0.19	6.00 ± 0.16	3.20 ± 0.20	9.00 ± 0.14	7.50 ± 0.16	6.20 ± 0.18	3.25 ± 0.18

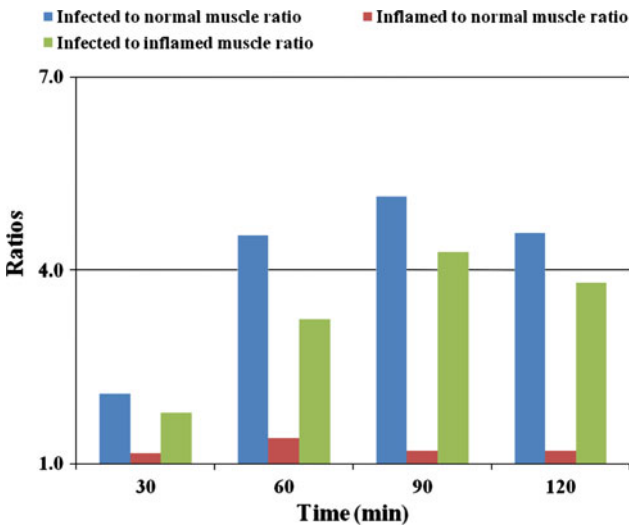

Fig. 6 Infected to normal, infected to inflamed and inflamed to normal muscles ratios

the radiocomplex from circulatory system and appearance in the urinary system suggest the normal route of excretion in group **A** and **B** MDRS. The five fold uptake of the radiocomplex in the infected part of the group **A**, MDRS as compared to inflamed and normal muscles established the achievability of the ^{99m}TcN-TVND complex as prospective MRSA infection radiotracer.

Conclusion

Radiolabeling of trovafloxacin dithiocarbamate (TVND) with technetium-99m using $[^{99m}\text{Tc-N}]^{2+}$ core was studied and its efficacy as a prospective MRSA infection radiotracer was evaluated in MDRS. The high RCP values, in vitro permanence in serum, better in vitro binding with bacteria, better biodistribution behavior with high target to non-target ratios posed the ^{99m}TcN-TVND complex as a promising MRSA infection radiotracer.

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