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Radiosynthesis and biodistribution of ^{99m}TcN–Garenoxacin dithiocarbamate complex a potential infection imaging agent

Syed Qaiser Shah · Aakif Ullah Khan · Muhammad Rafiullah Khan

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Abstract Garenoxacin (GXN) was modified to its dithiocarbamate followed by radiolabeling with technetium-99m (^{99m}Tc) through $[^{99m}Tc\text{-}N]^{2+}$ core. The suitability of the ^{99m}TcN–Garenoxacin dithiocarbamate (GXND) complex as a potential multiresistant *Staphylococcus aureus* (MDRSA) and penicillin-resistant *Streptococci* (PRSC) infection radiotracer was assessed in artificially infected rats (AFRT). The radiolabeled complex was investigated for its radiochemical purity (RCP), permanence in serum using HPLC and TLC methods. In vitro binding with MDRSA and PRSC was performed at 37 °C. The ^{99m}TcN–GXND showed maximum RCP of 98.00 ± 0.22% and remained more than 90% stable up to 4 h. The ^{99m}TcN–GXND showed saturated in vitro binding with living MDRSA and PRSC, respectively. The complex showed normal biodistribution in healthy rats (HRT), however in AFRT, seven fold uptakes was observed in infected muscle as compared to inflamed and normal muscles. Based on the high RCP, stability in serum, better in vitro binding with bacteria, biodistribution behavior and the target to non-target (infected to inflamed muscle) ratio, we recommend the ^{99m}TcN–GXND complex for in vivo investigation of MDRSA and PRSC infection in human.

S. Q. Shah (✉)
Nuclear Medicine Research Laboratory (NMRL), University of Peshawar, Peshawar, KPK, Pakistan
e-mail: ssqaiser2002@yahoo.com

A. U. Khan
Nuclear Medicine, Oncology and Radiotherapy Institute (NORI), Islamabad, Pakistan

M. R. Khan
Phytopharmaceutical & Neutraceuticals Research Laboratory (PNRL), University of Peshawar, Peshawar, KPK, Pakistan

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Introduction

Infection identification and its discrimination from inflammation is a challenging dilemma in remedial practices. The implications of in time diagnosis on the appropriate management of infectious foci's are very significant [1, 2].

In the last two decades, great advances in infection imaging have been achieved [3–14]. To facilitate the clinicians and to treat the patients appropriately in case of indecisive clinical history, we have recently reported a number of technetium-99m (^{99m}Tc) labeled antibiotics as potential in vivo infection radiotracers [15–20]. Based on our results of high radiochemical purity (RCP) yield, stability in serum, saturated in vitro binding with bacteria, better biodistribution in artificially infected animals, high target to non target ratio and scintigraphy that give clinically useful images of infectious foci. These findings encourage us to investigate new antibiotics for infection imaging with greater promise.

The major reasons for relentless infections are gram positive (G+) bacteria which over the decade gradually extended resistance to the existing drugs thus stimulated the investigation for a better antibiotic. Garenoxacin (GXN) is a des-fluoro(6)-quinolones (1-cyclopropyl-8-(difluoromethoxy)-7-[(1*R*)-1-methyl-2,3-dihydro-1*H*-isoindol-5-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid) (Fig. 1a) that emerged as an effective antibiotic against multiresistant *Staphylococcus aureus* (MDRSA) and penicillin-resistant *Streptococci* (PRSC), posing its suitability as promising agent against both the pathogen [21, 22].

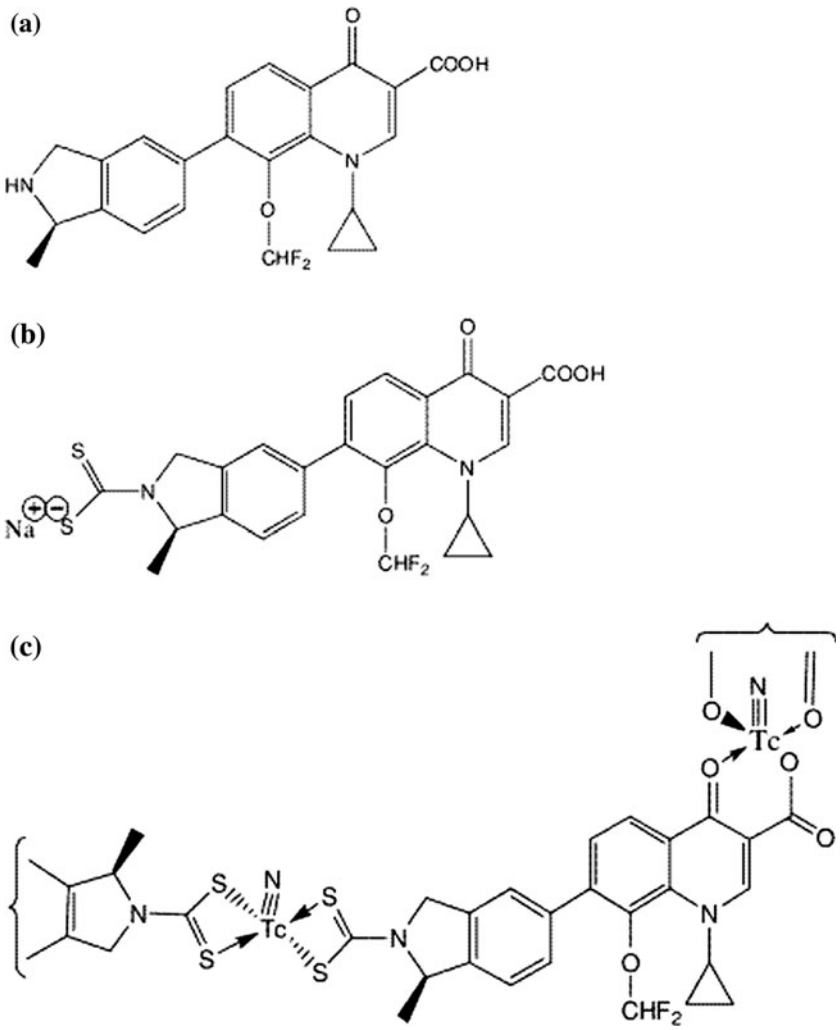

Fig. 1 **a** Chemical structure of GXN. **b** GXND. **c** Proposed structure of ^{99m} TcN–GXND complex

In continuation to our ongoing investigation, now we report the garenoxacin dithiocarbamate (GXND) as the tetradentate chelator for the radiosynthesis of the ^{99m}TcN–GXND complex (Fig. 1c). In the present study, viability of the ^{99m}TcN–GXND complex to trace soft tissue infections in animal models was assessed. The suitability of the complex was further examined in terms of in vitro permanence in saline and serum, in vitro binding with MDRSA and PRSC, biodistribution in AFRT.

Experimental

Materials

Garenoxacin from (Bristol-Myers Squibb, Syracuse, UK), TLC (Merck), 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 of GXND

Garenoxacin dithiocarbamate was synthesized by mixing 3.625 g of GXN with 1.2 g NaOH followed by the addition of 11 mL of 40% tetrahydrofuran in a sterilized flask. After stirring for 10 min in ice-bath 1 mL carbon disulfide was added to the reaction mixture followed by 8 h continuous shaking in an ice-bath. The solvent was removed under low pressure and the product was recovered.

Synthesis of ^{99m}TcN–GXND complex

Sodium pertechnetate ($Na^{99m}TcO_4^-$) freshly eluted, 0.5 mL (1–2 mCi) was added to 0.05 mg $SnCl_2{\cdot}2H_2O$, 5.0 mg PDTA, 5.0 mg SDH. The mixture was incubated at

room temperature for 10 min and thereafter 2 mg of GXND was added.

Characterization of ^{99m}TcN–GXND complex

^{99m}TcN–GXND complex was characterized by RP-HPLC chromatography using 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. 10 µL of the labeled complex was injected into C-18 column (4.6 × 150 mm^2). The flow rate of 1 mL/min was applied for 15 min using water (W) and methanol (M) as mobile phase. 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–100% W). Single well counter interface with scalar count rate meter (SWCSR) was employed for measuring the radiochemical activity in each fraction collected in a sterile vial.

Serum stability

In vitro stability of the ^{99m}TcN–GXND complex was evaluated in serum by incubating 0.2 mL of the preparation with 1.8 mL serum at 37 °C. Aliquots, at different intervals post incubation were obtained and for thin layer chromatography executed on polyamide strip. The strip was developed in saline and CH_2Cl_2:CH_3OH (9:1) (v/v) for the determination of various components of the complex. The developed strips were measured for activity using SWCSR.

Binding with MDRSA and PRSC

MDRSA and PRSC in vitro up take was assessed by adopting the reported method with slight modification [23]. Briefly, 10 MBq of ^{99m}TcN–GXND was transferred to a sterilized test tube containing 0.1 mL sodium phosphate buffer (Na-PB) followed by addition of 0.8 mL (50%, v/v) 0.01 M acetic acid containing approximately 1 × 10^8 colony forming units (CFU) of MDRSA and PRSC. The mixture was incubated at 4 °C for 1 h and the pH was adjusted to pH 5 followed by centrifugation with 2,000 rpm for 10 min. Thereafter, the supernatant was removed and the pellets were resuspended in 2 mL of Na-PB and repeated the centrifugation with the same spin speed. Finally, the pellets were counted for activity using SWCSR.

Biodistribution

Twenty healthy (Sprague–Dawley male rat: weight range, 180–220 g) were selected and arranged in four groups (G-I, G-II, G-III, and G-IV) of five rats each. Intramuscularly (IM) sterile turpentine oil 0.2 mL was injected to the left thigh of all the selected rats. G-I was IM (right thigh) infected with living MDRSA (1 × 10^8 cfu) and G-II with heat killed. Similarly, G-III and G-IV were IM infected with living and heat killed PRSC (1 × 10^8 cfu), respectively. After, 24 h, 0.5 mL (37 MBq) of the ^{99m}TcN–GXND were injected intravenously to all the animals (G-I to G-IV). Thereafter, the animals were killed (in accordance with the rules and regulation stipulated in the manual of the Nuclear Medicine Research Laboratory Part-I and II). Percent absorbed dose per gram (each) in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed muscle and normal muscle was determined using SWCSR.

Result and discussion

Radiochemistry and HPLC characterization of ^{99m}TcN–GXND

The tetradentate GXND (two sulfur atoms, a carboxyl and hydroxyl group) under substitution reaction with an intermediate [^{99m}TcN]$^{2+}$core yield ^{99m}TcN–GXND complex as given in Fig. 1c. By similarity with the structure of bis(diethyldithiocarbamato) nitride technetium-99m complex [24]. We propose a similar structure for our complex (Fig. 1c), with a square pyramidal geometry having a TcN:Ligand ratio of 1:1.

Figure 2 showed two peaks, one at 4.4 and the second one at 12.6 min. The radio-peaks at 4.4 represents [^{99m}TcN]$^{2+}$ intermediate and of 12.6 min the ^{99m}TcN–GXND complex.

The radiochemical purity observed at different intervals are shown in Fig. 3. It was observed that the complex showed high and longer stability in saline. The maximum

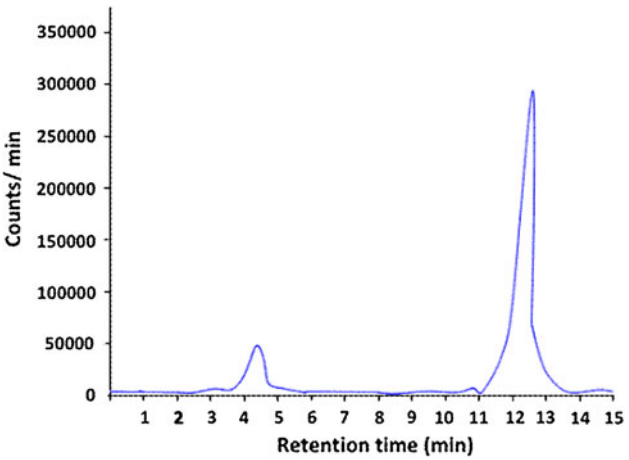

Fig. 2 HPLC radiochromatogram of ^{99m} TcN–GXND complex

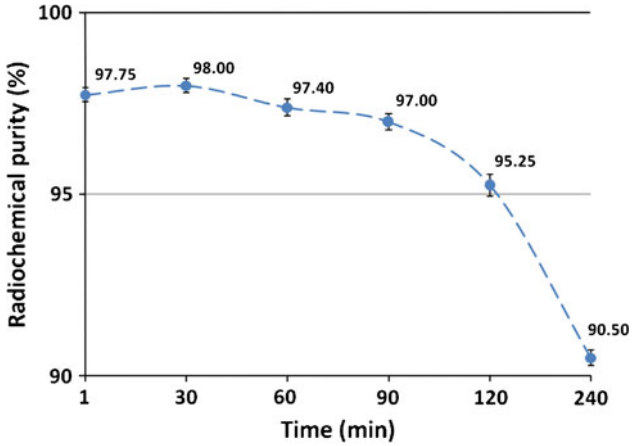

Fig. 3 Radiochemical purity of ^{99m}TcN–GXND complex in saline

RCP value observed was 98.00 ± 0.22% at 1 min and remained more than 90% stable up to 240 min after reconstitution for ^{99m}TcN–GXND.

Stability of ^{99m}TcN–GXND in serum

At 37 °C the complex showed good stability in serum as shown in Fig. 4. The complex was found stable in serum up to 4 h however it went down to 78% after 16 h.

In vitro binding with MDRSA and PRSC

The in vitro binding affinity of the complex with MDRSA and PRSC is shown in Fig. 5. The complex showed saturated in vitro binding with living MDRSA and PRSC respectively.

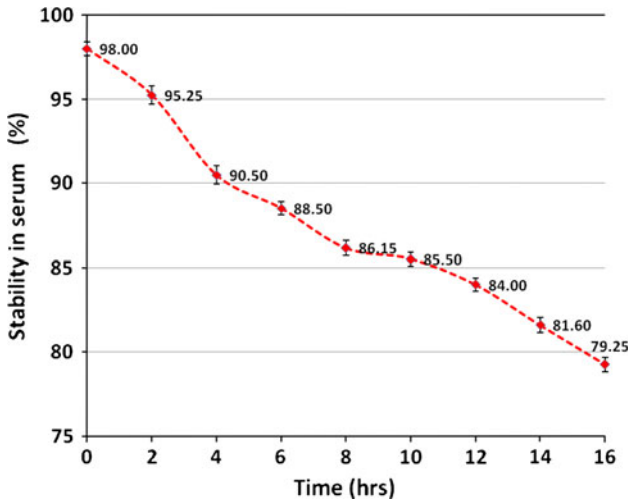

Fig. 4 Stability of the ^{99m}TcN–GXND complex in serum

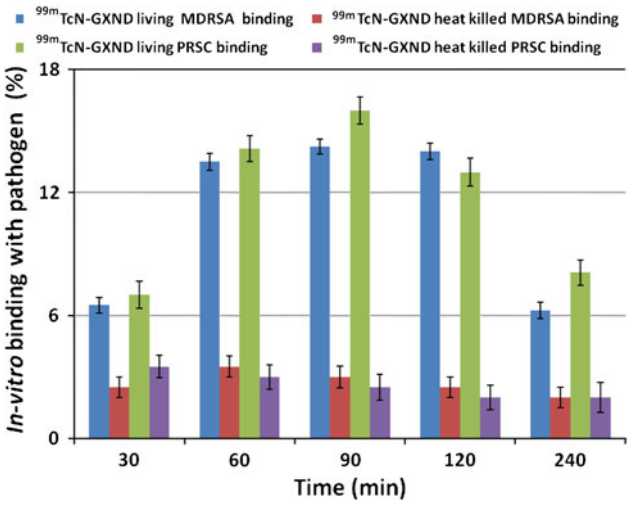

Fig. 5 In vitro binding of ^{99m}TcN–GXND complex with living and heat killed MDRSA and PRSC at different intervals

Biodistribution MDRSA and PRSC infected rats

The percent up take of the ^{99m}TcN–GXND in various organs of the artificially MDRSA and PRSC infected rats (AFRT) are given in Tables 1 and 2. It was observed that activity in blood of the rat infected with MDRSA and PRSC was high but it went down with the passage of time. No significant difference in the activity up take with regards to blood was noted in all animals either infected by MDRSA or PRSC (alive or heat killed). The uptake of ^{99m}TcN–GXND to G-I to G-IV, infected with MDRSA and PRSC gave almost similar profile with a seven fold up take in the target organ as compared to the inflamed and normal muscle. Initially, the higher uptake in liver, spleen, and blood was observed which significantly declined with time. A reciprocal behavior was noted in kidney, where the activity went up with time. The appearance of activity in urinary system and disappearance from the circulatory system confirmed the normal route of excretion. The appearance of activity almost seven fold in the infected muscle further confirmed the feasibility of the radiotracer as specific and potential radiopharmaceutical for the diagnosis of infection caused by MDRSA and PRSC.

Conclusion

Garenoxacin dithiocarbamate radiolabeling with ^{99m}Tc through $[^{99m}\text{Tc-N}]^{2+}$ core was investigated and its feasibility as a potential MDRSA and PRSC infection radiotracer was evaluated in AFRT. Based on the high RCP, stability in serum, better in vitro binding, biodistribution behavior and target to non-target (infected to inflamed

Table 1 Distribution of ^{99m}TcN–GXND complex in various organs of rats artificially infected with MDRSA

Organs/tissues (g)	Distribution of ^{99m}TcN–GXND (in %) at different intervals (in min)							
	Living				Heat killed			
	30	60	90	120	30	60	90	120
Infected muscle	6.00 ± 0.24	12.75 ± 0.22	13.90 ± 0.22	13.50 ± 0.21	2.50 ± 0.25	3.00 ± 0.22	2.50 ± 0.21	2.50 ± 0.21
Inflamed muscle	3.50 ± 0.22	3.50 ± 0.24	3.00 ± 0.20	3.00 ± 0.22	3.50 ± 0.23	3.50 ± 0.20	3.00 ± 0.22	3.00 ± 0.24
Normal muscle	3.00 ± 0.24	2.50 ± 0.22	2.50 ± 0.20	2.50 ± 0.21	2.50 ± 0.21	2.50 ± 0.24	2.50 ± 0.22	2.50 ± 0.24
Blood	22.50 ± 0.22	8.50 ± 0.21	7.00 ± 0.20	3.50 ± 0.24	20.50 ± 0.22	9.50 ± 0.24	8.15 ± 0.22	3.00 ± 0.21
Liver	18.75 ± 0.22	13.00 ± 0.24	9.10 ± 0.21	5.10 ± 0.24	19.50 ± 0.20	13.75 ± 0.20	9.00 ± 0.24	4.55 ± 0.22
Spleen	8.55 ± 0.24	7.00 ± 0.21	5.00 ± 0.22	3.00 ± 0.20	8.25 ± 0.22	6.50 ± 0.21	4.50 ± 0.24	3.5 ± 0.22
Kidney	9.50 ± 0.22	18.50 ± 0.24	21.55 ± 0.22	24.00 ± 0.23	10.00 ± 0.20	17.85 ± 0.22	20.25 ± 0.24	23.50 ± 0.21
Stomach & intestines	8.55 ± 0.20	7.00 ± 0.22	5.75 ± 0.24	3.00 ± 0.21	9.15 ± 0.22	7.75 ± 0.20	6.10 ± 0.20	3.25 ± 0.24
Ratio 1	2.00 ± 1.09	5.10 ± 0.92	5.56 ± 1.10	5.40 ± 0.95	1.00 ± 1.09	1.20 ± 1.10	1.00 ± 0.95	1.00 ± 0.88
Ratio 2	1.17 ± 0.92	1.40 ± 1.09	1.20 ± 1.00	1.20 ± 1.05	1.40 ± 1.10	1.40 ± 0.83	1.20 ± 1.00	1.20 ± 1.00

Ratio 1 Infected/normal muscle, *Ratio 2* Inflamed/normal muscle

Table 2 Distribution of ^{99m}TcN–GXND complex in various organs of rats artificially infected with PRSC

Organs/tissues (g)	Distribution of ^{99m}TcN–GXND (in %) at different intervals (in min)							
	Living				Heat killed			
	30	60	90	120	30	60	90	120
Infected muscle	7.50 ± 0.20	13.25 ± 0.24	14.00 ± 0.20	13.75 ± 0.20	2.50 ± 0.20	3.00 ± 0.24	2.50 ± 0.22	2.50 ± 0.24
Inflamed muscle	4.00 ± 0.24	3.50 ± 0.20	3.00 ± 0.21	3.00 ± 0.19	3.50 ± 0.20	3.00 ± 0.24	3.00 ± 0.21	3.00 ± 0.20
Normal muscle	3.50 ± 0.20	3.00 ± 0.24	2.50 ± 0.22	2.50 ± 0.20	2.50 ± 0.22	2.50 ± 0.20	2.50 ± 0.24	2.50 ± 0.22
Blood	18.75 ± 0.24	10.00 ± 0.21	8.25 ± 0.24	4.25 ± 0.20	19.00 ± 0.20	8.55 ± 0.21	7.25 ± 0.20	3.50 ± 0.24
Liver	16.50 ± 0.20	12.75 ± 0.20	7.55 ± 0.24	4.85 ± 0.20	18.50 ± 0.24	11.00 ± 0.20	8.15 ± 0.20	4.00 ± 0.22
Spleen	7.25 ± 0.24	6.00 ± 0.24	4.55 ± 0.20	3.50 ± 0.22	8.00 ± 0.20	6.75 ± 0.24	5.25 ± 0.20	4.00 ± 0.19
Kidney	10.00 ± 0.21	17.75 ± 0.20	20.15 ± 0.24	22.50 ± 0.20	9.75 ± 0.24	16.50 ± 0.24	19.75 ± 0.20	23.00 ± 0.24
Stomach & intestines	7.50 ± 0.24	6.10 ± 0.22	5.25 ± 0.20	3.50 ± 0.20	8.00 ± 0.24	7.25 ± 0.21	5.75 ± 0.20	3.25 ± 0.20
Ratio 1	2.14 ± 1.00	4.42 ± 1.00	5.60 ± 0.91	5.50 ± 1.00	1.00 ± 0.91	1.20 ± 1.20	1.00 ± 0.92	1.00 ± 1.09
Ratio 2	1.14 ± 1.20	1.17 ± 0.83	1.20 ± 0.95	1.20 ± 0.95	1.40 ± 0.91	1.20 ± 1.20	1.20 ± 0.88	1.20 ± 0.91

Ratio 1 Infected/normal muscle, *Ratio 2* Inflamed/normal muscle

muscle) ratio we recommend the ^{99m}TcN–GXND for in vivo localization of MDRSA and PRSC infection in human.

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