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Synthesis and Biological Evaluation of the ^{99m}tcn-Gemifloxacin Dithiocarbamate Complex: A Novel *Streptococcus Pneumoniae* Infection Imaging Agent

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Abstract

Synthesis and biological evaluation of the ^{99m}TcN-Gemifloxacin dithiocarbamate (^{99m}TcN-GIND) complex was investigated in terms of radiochemical stability (RCP) in saline, serum, *in-vitro* binding with *Streptococcus pneumoniae* (*S. pneumoniae*) and biodistribution in male Wistar rats artificially infected with living and heat killed *S. pneumoniae*. The maximum RCP was 98.25 ± 0.30% at 30 min and decreased to 91.25 ± 0.34% within 240 min. The complex showed stable behavior (*in-vitro*) in serum at 37°C with a 14.35% undesirable side products within 16 h. The complex showed 71.25% *in-vitro* binding *S. pneumoniae*. The uptake of the complex in the infected muscle was six times higher than the inflamed and normal muscles of the MWR infected with living *S. pneumoniae*. The promising (*in-vitro* and *in-vivo*) radiochemical and biological behavior posed the ^{99m}TcN-GIND complex as a potential radiotracer for *S. pneumoniae* infection.

Keywords: Gemifloxacin dithiocarbamate (GIND); ^{99m}TcN-GIND complex; *Streptococcus pneumonia*; Infection

Introduction

In the early stages, the identification of infection and its discrimination from inflammation is a critical apprehension of the medical community worldwide. The Nuclear Medicine Imaging (NMI) technology has prevailed over the situation after the failure of the sophisticated techniques such as Computerized Tomography (CT), Magnetic Resonance Imaging (MRI) etc [1,2].

The existing and our recently reported infection imaging agents have shown promising results. The *in-vitro* and *in-vivo* results of our recently developed kits encouraged us to seek for more stable and specific infection imaging agents [3-15].

Recently, it has been reported that gemifloxacin (GIN) [7-[(4Z)-3-(aminomethyl)-4-methoxyimino-pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid] (Figure 1a) is a new broad spectrum antibiotic effective against *Streptococcus pneumoniae* (*S. pneumoniae*), *Haemophilus influenzae, Haemophilus parainfluenzae,* or *Moraxella catarrhalis* including multi-drug resistant strains, *Haemophilus influenzae, Moraxella catarrhalis, Mycoplasma pneumoniae, Chlamydia pneumoniae,* or *Klebsiella pneumoniae* [16,17].

In continuation to our ongoing study, in the present investigation, the conversion of GIN (Figure 1a) to GIND (Figure 1b) as tetradentate chelator and its radio labeling with technetium-99m through $[^{99m}TcN]^{2+}$core has been investigated. The ^{99m}TcN-GIND complex was further evaluated in terms of radiochemical stability in saline, serum, *in-vitro* binding with *S. pneumoniae*, percent absorption in the artificially infected MWR with *S. pneumoniae*.

Experimental Methods

Materials

Gemifloxacin (GIN) (Shanghai Sciencya Biotechnology Co., Ltd. Shanghai, China), 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

Radiosynthesis of the ^{99m}TcN-Gemifloxacin dithiocarbamate: Gemifloxacin dithiocarbamate (GIND) (Figure 1b) was prepared by using the method described earlier [15]. Thereafter, the ^{99m}TcN-GIND complex (Figure 1a) was synthesized by mixing 0.5 mL (1-2 mCi) of sodium pertechnetate ($Na^{99m}TcO_4$-) with 0.05 mg of stannous chloride dihydrate, 5.0 mg each propylenediamine tetra-acetic acid (PDTA) and succinic dihydrazide (SDH). The reaction mixture is then incubated at room temperature for 10 min. Then 2 mg of GIND (dissolve in normal saline (NS)) was added to the reaction mixture followed by incubation for 10 min at room temperature.

Determination of partition coefficient (P): The ^{99m}TcN-GIND complex, octanol and phosphate buffer (PB) in equivalent quantity was vortexed 5 min at room temperature. The blend was then centrifuged at 5000 g for 10 min. Next, 0.1 mL of the mixture was drawn at different periods and measured for activity in well counter interface with scalar count rate meter (WCSCRM). The following equation was used for the measurement of the partition coefficient (*P*).

$$P = \frac{(CPM\ in\ octanol\ -\ CPM\ in\ background)}{(CPM\ in\ buffer\ -\ CPM\ in\ background)}$$

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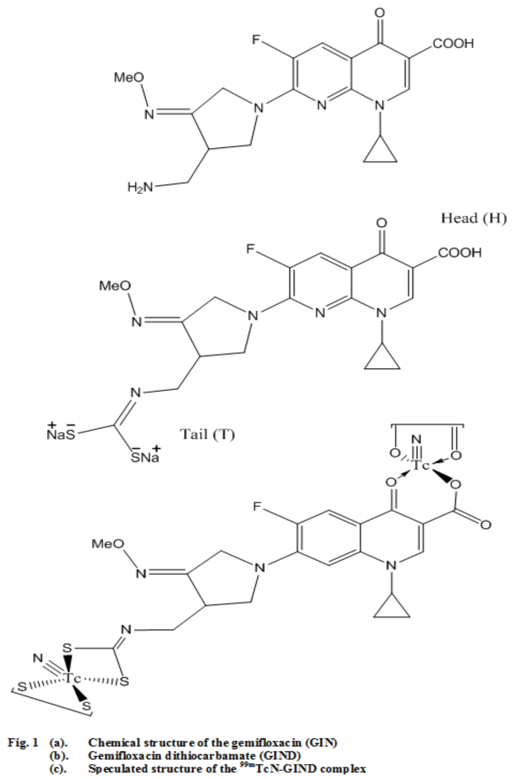

Figure 1: (a) Chemical structure of the gemifloxacin (GIN)
(b) Gemifloxacin dithiocarbamate (GIND)
(c) Speculated structure of the ^{99m}TcN-GIND complex.

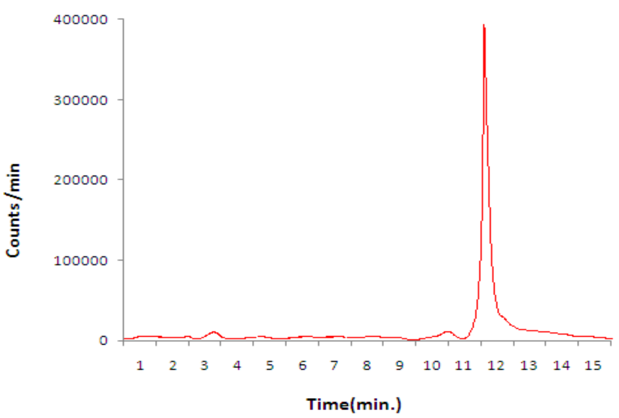

Figure 2: HPLC Chromatogram of ^{99m}TcN-GIND complex.

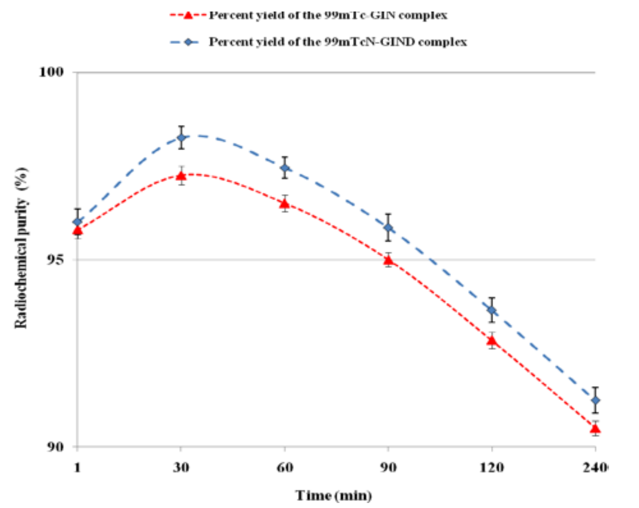

Figure 3: Radiochemical stability of the ^{99m}TcN-GIND and ^{99m}Tc-GIN in normal saline at different intervals.

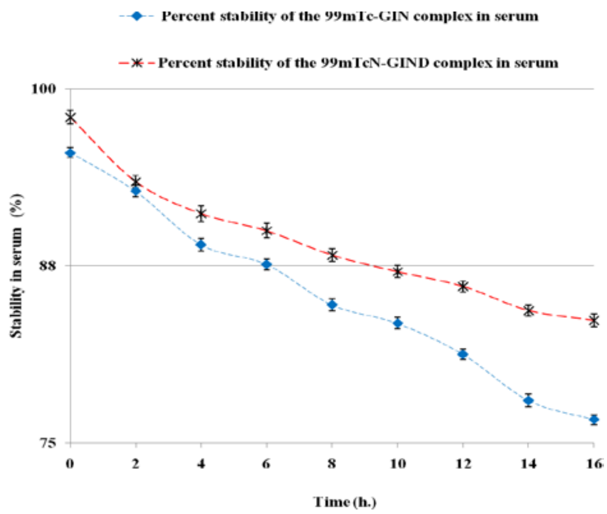

Figure 4: *In-vitro* stability of the ^{99m}Tc-GIN and ^{99m}TcN-GIND in serum at 37°C.

Radiochemical purity (RCP) and characterization: Shimadzu (SCL-10 AVP) HPLC system fitted with (SDP-10 AVP) UV detector operating at 254 nm, (Packard 500 TR series) flow scintillation analyzer, binary pump an online degasser and C-18 (4.6×150 mm) column was used for the radiochemical purity determination and radiocharacterization of the ^{99m}TcN-GIND complex using the reported method [15]. Briefly, 5 µL of the ^{99m}TcN-GIND complex was injected in to the C-18 column of the HPLC system followed by elution of 1 mL/min for 15 min using Water:methanol (W:M) as the mobile phase for 0-3 min (100:00), 3-5 min (60:40), 5-8 min (55:45), 8-10 (25:75), 10-13 (00:100) and 13-15 (50:50). The radiofractions collected during 15 min of elution were measured for activity using WCSCRM.

Radiochemical stability in serum: In serum the stability of the ^{99m}TcN-GIND complex was evaluated using RTLC technique. The ^{99m}TcN-GIND complex (0.2 mL) with 1.8 mL of the serum was incubated at 37°C for 16 h. During the incubation, aliquots at 0, 2, 4, 6, 8, 10, 12, 14 and 16 h were taken and applied to the TLC strips. Next, the strips were developed in saline and CH_2Cl_2:CH_3OH (9:1) (v/v). Thereafter, the developed strips were divided into two equal parts and measured for activity using WCSCRM.

***In vitro* binding with *Streptococcus pneumoniae*:** *In vitro* binding of the *Streptococcus pneumoniae* with ^{99m}TcN-GIND complex was investigated by the reported method [18]. Briefly, to a test tube containing 0.1 mL of the sodium phosphate buffer (Na-PB), 10 MBq of the freshly prepared complex was poured followed by the addition of 0.8 mL (50%, v/v) 0.01 M acetic acid containing approximately 1×10^8

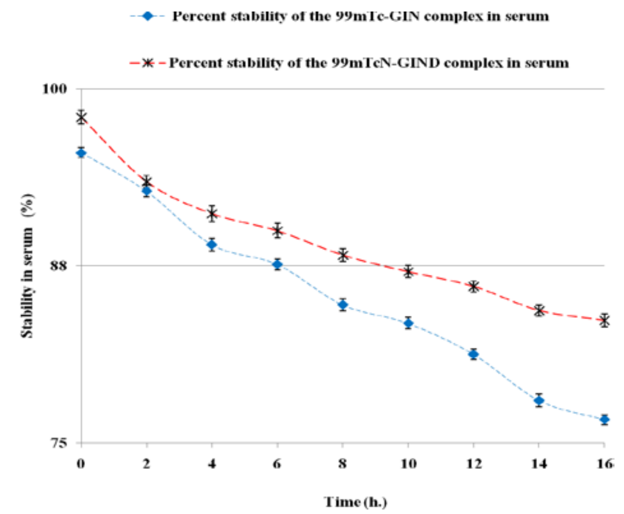

Figure 5: *In-vitro* binding of the ^{99m}TcN-GIND and ^{99m}Tc-GIN complexes.

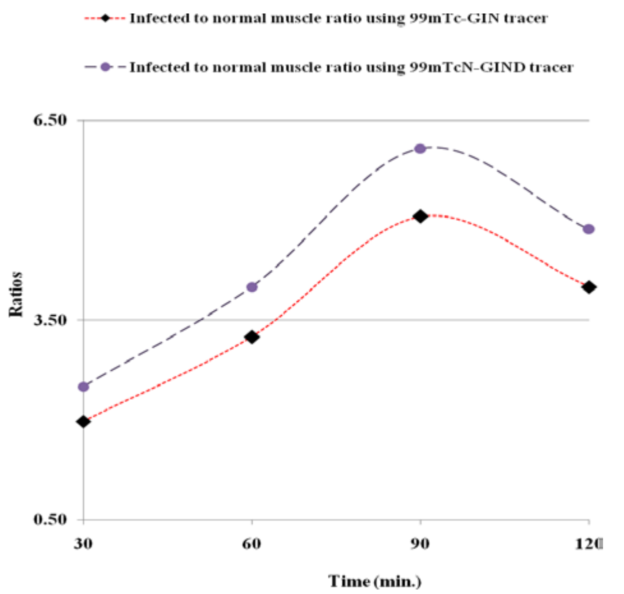

Figure 6: Analysis of infected to normal muscle ratios of the ^{99m}Tc-GIN and ^{99m}TcN-GIND at different intervals.

colony forming units (CFU) of *Streptococcus pneumoniae* and incubated for 1 h at 4°C with a pH 5. The blend was centrifuged at 2000 rpm for 10 min followed by decanting of the supernatant. Next, the remaining was mixed with 2 mL of Na-BP. The reaction mixture was once again recentrifuged at 2000 rpm for 10 min. The pellets so obtained were measured for *in-vitro* uptake (%).

Biodistribution in infected MWR: The absorption (%) of the ^{99m}TcN-GIND complex in (per gram) blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of the MWR infected with living and heat killed *Streptococcus pneumoniae* (*S. pneumoniae*) was investigated at 30, 60, 90 and 120 min. Twelve MWR (weight, 150–170 g) were preferred and separated into two groups (**A** and **B**) having six MWR in each group. Intramuscularly (I.M.) to the left thigh, 0.2 mL of sterile turpentine oil was injected to each MWR. Next, group **A** (MWR) were injected (I.M.) with 0.2 mL of living *S. pneumoniae* (containing around 1×10^{8} CFU) to the right thigh. Likewise, group **B** (MWR) was injected with 0.2 mL of heat killed *S. pneumoniae*. After 18 h, intravenously 0.5 mL (18.5 MBq) of the labeled GIND was administered to the MWR of group **A** and **B**. Subsequently, the group A and B (MWR) were sacrificed in accordance with the regulations of the Nuclear Medicine Research Laboratory (NMRL), University of Peshawar (Part-I and II). Absorption (percent per gm) in blood, liver, spleen, stomach, intestine, kidney, and infected muscle, inflamed and normal muscle was calculated using WCSCRM.

Results and Discussion

Radiochemistry and geometry

Gemifloxacin dithiocarbamate (GIND) (Figure 1b) was synthesized from gemifloxacin (GIN) (Figure 1a) using the procedure described earlier [15]. The coordinating groups (sulfur atoms, carboxyl and hydroxyl) of the tetradentate GIND under substitution reaction gave a stable complex of GIND and technetium-99m using the $[^{99m}TcN]^{2+}$core as shown in Figure 1c. The structure of the ^{99m}TcN-GIND complex was proposed on the likeness with bis (diethyldithiocarbamato) nitride ^{99m}Tc complex [19]. The intermolecular complexation could be any permutation of HH-TT, HT-TH etc.

The speculated geometry of the ^{99m}TcN-GIND complex is pyramidal having a TcN:Ligand ratio of 1:1. The ^{99m}TcN-GIND complex showed two radiopeaks at 2.9 and 11.7 min of retention as depicted in HPLC radiochromatogram (Figure 2). The radiopeak at 2.9 min of retention characterized to the $[^{99m}TcN]^{2+}$ intermediate and the 11.7 correspond to the radiochemical yield of the ^{99m}TcN-GIND complex.

Radiochemically the ^{99m}TcN-GIND complex showed stability in normal saline upto 240 min as shown in Figure 3. The maximum value of the radiochemical stability observed was 98.25 ± 0.30% at 30 min. The value of the radiochemical stability decreased to 91.25 ± 0.34% within 240 min.

Partition coefficient

The P value observed for the ^{99m}TcN-GIND complex was 1.02 ± 0.01 suggesting lipophilicity.

Radiochemical stability in serum

The ^{99m}TcN-GIND complex showed *in-vitro* stability in serum upto 4 h more than 90% as shown in Figure 4. Thereafter, the growth of undesirable species (de-tagging) lowered the stability value by 16.50% within 16 h.

In vitro binding with *Streptococcus pneumoniae*

^{99m}TcN-GIND complex showed saturated *in-vitro* binding with *Streptococcus pneumoniae* at different intervals as shown in Figure 5. The maximum value of the *in-vitro* binding was 65.00% and the min was 47.00%

Biodistribution in infected MWR

The absorption (%) of the ^{99m}TcN-GIND complex in (per gram) blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of the MWR infected with living and heat killed *Streptococcus pneumoniae* (*S. pneumoniae*) is given in Table 1. The appearance of the ^{99m}TcN-GIND activity in blood was initially high which was reduced to 4.00 ± 0.18% from 19.50 ± 0.15% within 120 min of the I.V administration. Almost similar behavior was seen in liver, spleen, stomach and intestines of the MWR no matter infected with living or heat killed *S. pneumoniae*. In kidneys an opposite behavior was seen where the activity of the ^{99m}TcN-GIND complex was low in the

Organs/tissues (gm)	Uptake of the ^{99m}TcN-GIND							
	Group A (living *Streptococcus pneumoniae*)				Group B (heat killed *Streptococcus pneumoniae*)			
	30	60	90	120	30	60	90	120
Infected muscle	6.25 ± 0.18	12.00 ± 0.20	15.20 ± 0.17	12.15 ± 0.16	2.50 ± 0.15	3.00 ± 0.18	3.50 ± 0.16	3.00 ± 0.16
Inflamed muscle	4.50 ± 0.16	4.00 ± 0.18	3.50 ± 0.14	3.00 ± 0.17	4.25 ± 0.18	4.00 ± 0.14	3.50 ± 0.17	3.00 ± 0.15
Normal muscle	2.50 ± 0.14	3.00 ± 0.16	2.50 ± 0.19	2.50 ± 0.20	2.50 ± 0.18	3.00 ± 0.16	2.50 ± 0.17	2.50 ± 0.20
Blood	18.55 ± 0.20	10.80 ± 0.16	8.00 ± 0.00	4.75 ± 0.15	19.00 ± 0.17	10.65 ± 0.16	7.90 ± 0.18	4.50 ± 0.20
Liver	19.00 ± 0.16	11.50 ± 0.20	9.30 ± 0.18	6.00 ± 0.15	18.40 ± 0.20	11.45 ± 0.15	9.10 ± 0.20	6.10 ± 0.14
Spleen	8.70 ± 0.18	7.50 ± 0.20	6.40 ± 0.14	4.20 ± 0.18	8.65 ± 0.14	7.30 ± 0.17	6.25 ± 0.20	4.00 ± 0.18
Kidney	8.00 ± 0.14	17.40 ± 0.20	20.10 ± 0.16	23.75 ± 0.14	8.25 ± 0.20	19.00 ± 0.14	21.30 ± 0.17	24.00 ± 0.00
Stomach & intestines	8.50 ± 0.20	7.45 ± 0.16	6.75 ± 0.12	4.10 ± 0.00	8.75 ± 0.14	8.00 ± 0.18	7.10 ± 0.19	4.30 ± 0.18

Table 1: Biodistribution of the ^{99m}TcN-GIND complex in artificially infected MWR with *Streptococcus pneumoniae*.

beginning of the I.V administration. The activity of the ^{99m}TcN-GIND went up from 8.00 ± 014% to 23.75 ± 0.14% within 120 min. Marginal difference was noted in the uptake of the ^{99m}TcN-GIND complex in kidneys of the MWR infected by living or heat killed *S. pneumoniae*. The ^{99m}TcN-GIND complex showed higher uptake in the infected muscle than the inflamed and normal muscle of the MWR infected by living *S. pneumoniae* while no significant difference was observed in the infected, inflamed, and normal muscles of the group B (MWR) infected by heat killed *S. pneumoniae*.

The appearance of the activity of the ^{99m}TcN-GIND complex in urinary system and disappearance from the circulatory system substantiated the regular path of the excretion of the ^{99m}TcN-GIND complex from the MWR. Figure 6 gives comparative analysis of infected to normal muscle ratios using ^{99m}Tc-GIN and ^{99m}TcN-GIND complex at different intervals. Significantly higher uptake ratio was seen in case of ^{99m}TcN-GIND as compared to ^{99m}Tc-GIN complex.

Conclusion

The ^{99m}TcN-GIND complex was radiochemically characterized and biologically evaluated in MWR artificially infected with living and heat killed *S. pneumoniae*. The complex showed radiochemical stability in saline, serum, saturated *in-vitro* binding with *S. pneumoniae* and promising biodistribution in MWR with almost six time higher accumulation in the infected muscle as compared to inflamed and normal muscles. Based on the radiochemical stability, *in-vitro* binding with *S. pneumoniae* and six time higher absorption in the infected muscle of the MWR, validated the feasibility of the ^{99m}TcN-GIND complex as prospective infection imaging agent.

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