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 pneumoniae; 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-flouro-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 $[^{99m}\text{TcN}]$ 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 ($\text{Na}^{99m}\text{TcO}_4^{-}$) 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 ($P$).

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

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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 (00:10) 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$_2$Cl$_2$:CH$_3$OH (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$^4$
Biodistribution in infected MWR: The absorption (%) of the $^{99m}$Tc-GIND complex in (per gram) blood, liver, spleen, intestine, kidney, infected muscle, inflamed and normal muscle of the MWR infected with living and heat killed *Streptococcus 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* ($\approx 10^8$ CFU) to the right thigh. 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 $[^{99m}TcN]^+$ core as shown in Figure 1c. The structure of the $[^{99m}TcN-GIND]$ complex was proposed on the likeness with bis (diethyl dithiocarbamato) nitride $^{99m}$Tc complex [19]. The intermolecular complexation could be any permutation of HH-TT, HT-TH etc.

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.

The speculated geometry of the $^{99m}$TcN-GIND complex is pyramidal having a TcN:Ligand ratio of 1:1. The $^{99m}$Tc-N-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 $[^{99m}TcN]^+$ intermediate and the 11.7 correspond to the radiochemical yield of the $^{99m}$TcN-GIND complex.

Radiochemically the $^{99m}$Tc-N-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 $^{99m}$Tc-N-GIND complex was 1.02 ± 0.01 suggesting lipophilicity.

Radiochemical stability in serum

The $^{99m}$Tc-N-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**

$^{99m}$Tc-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 binding was 47.00%

Biodistribution in infected MWR

The absorption (%) of the $^{99m}$Tc-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* 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 1×10^9 CFU) to the right thigh.
beginning of the LV administration. The activity of the $^{99m}$Tc-GIND went up from 8.00 ± 0.14% to 23.75 ± 0.14% within 120 min. Marginal difference was noted in the uptake of the $^{99m}$Tc-GIND complex in kidneys of the MWR infected by living or heat killed $S$. pneumoniae. The $^{99m}$Tc-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 $^{99m}$Tc-GIND complex in urinary system and disappearance from the circulatory system substantiated the regular path of the excretion of the $^{99m}$Tc-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 $^{99m}$Tc-GIND as compared to $^{99m}$Tc-GIN complex.

### Conclusion

The $^{99m}$Tc-GIND complex was radiochemically characterized and biologically evaluated in MWR artificially infected with living and heat killed $S$. pneumoniae. The complex showed radioactive 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 $^{99m}$Tc-GIND complex as prospective infection imaging agent.

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

| Organs/tissues (gm) | Uptake of the $^{99m}$Tc-GIND | Group B (heat killed $Streptococcus$ pneumoniae) |
|---------------------|-------------------------------|-----------------------------------------------|
|                     | 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 |
| Infamed 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               | 6.00 ± 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 | 6.50 ± 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 |

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