Radiocharacterization of the ^{99m}Tc–rufloxacin complex and biological evaluation in *Staphylococcus aureus* infected rat model

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Abstract ^{99m}Tc–rufloxacin (^{99m}Tc–RUN) complex was prepared by reaction of different amounts of reduced sodium pertechnetate with different amount of Rufloxacin (RUN) antibiotic for the in vivo scintigraphic localization of the *Staphylococcus aureus* (*S. aureus*) infectious foci in Male Wister Rats (MWR) model. The ^{99m}Tc–RUN complex was radiochemically and biologically characterized in terms of radiochemical stability in saline, serum, in vitro binding with *S. aureus* and biodistribution in artificially infected with *S. aureus* MWR. The ^{99m}Tc–RUN complex showed stability more than 90% up to 240 min in normal saline with a maximum stability value of 98.10 ± 0.18% at 30 min after reconstitution. At 37 °C the complex showed in vitro permanence in serum up to 16 h with 13.90% side products during incubation. The ^{99m}Tc–RUN complex showed saturated in vitro binding with *S. aureus* at different intervals with a maximum uptake value of 71.50%. Infected to normal muscle, infected to inflamed and inflamed to normal muscles ratios were approximately 6.04, 4.31 and 1.40. Based on the stability of the complex in saline, serum, in vitro binding with *S. aureus* and biodistribution results, the ^{99m}Tc–RUN complex is recommended for in vivo scintigraphic localization of the *S. aureus* in vivo infectious foci in human.

Introduction

Imaging of bacterial infection is still a serious concern of the medical community in spite of the innovation in the diagnostic facilities and expansion in the antibiotics. The role of the advanced diagnostic facilities like Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) has proven inadequate in the diagnosis of infection and its discrimination from inflammation in the early stages. The Nuclear Medicine Scintigraphy (NMS) has been recognized as a promising technique for the diagnosis of infection and its discrimination from inflammation [1, 2].

The available radiopharmaceuticals [3–15] and our recently reported ones [16–24] have supported the NMS and the promising diagnostic results encouraged us to look for more receptive and explicit agents for the localization of infectious foci.

Rufloxacin (RUN) Fig. 1a. 9-fluoro-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-{1,4}thiazino {2,3,4-*ij*} quinoline-6-carboxylic acid is a new quinoline broad spectrum antibiotic intended for the treatment of a wide range of infections caused by various pathogens [25, 26]. In the current investigation the radiolabeling of RUN with a gamma (γ-) emitter technetium-99m (^{99m}Tc) was investigated. The effect of various reacting species on the percent radiochemical purity yield, stability in normal saline at 30, 60, 90, 120 and 240 min, in vitro stability in serum at 37 °C up to 16 h, in vitro binding with *Staphylococcus aureus* (*S. aureus*) and biodistribution in infected (*S. aureus*) rats of the ^{99m}Tc–RUN complex was assessed.

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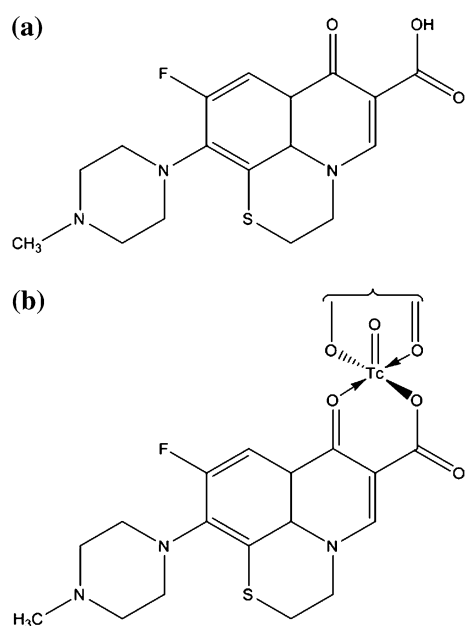
(a)

(b)

Fig. 1 **a** Structure of the rufloxacin (RUN). **b** Proposed structure of the ^{99m}Tc–RUN complex

Experimental

Materials

Rufloxacin (RUN) (Jinan Haohua Industry Co., Ltd Shandong, China), TLC (Merck, Germany) and the other chemicals and solvents of analytical grade (Sigma). RP-HPLC (Shimadzu, Japan) well counter and scalar count rate meter (Ludlum, USA) Dose calibrator (Capintech, USA) and Gamma camera GKS-1000 (GEADE Nuclear-medizine system, Germany).

Methods

Radiosynthesis of the ^{99m}Tc–rufloxacin complex

Rufloxacin (RUN), 0.5–5.0 mg (with 0.5 mg rise) was taken in ten sterilized syringes. After that sodium pertechnetate 0.5–5.0 mCi (with 0.5 mCi increase) were taken in ten different nitrogen gas filled vials. Thereafter, stannous chloride 25–250 µL (with 25 µL: µg/µL 0.01 N HCL) were added to the ten vials followed by injection of RUN through syringes. The pH of the reaction mixtures was set between 5.1 and 6.0 (with 0.1 unit augment). The reaction mixtures were incubated at room temperature followed by filtration through Millipore.

Characterization of the ^{99m}Tc–RUN complex

The ^{99m}Tc–RUN complex was radiocharacterized using the HPLC method reported earlier [22]. Briefly, 10 5 µL of the

^{99m}Tc–RUN complex was loaded on the C-18 column 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. Thereafter, the column was eluted with 1 mL/min of water:acetonitrile (1:9) mixture for 15 min followed by collection of the radio-fractions in separate vials. The radio-fractions were measured for radioactivity in well counter interface with scalar count rate meter (WCSR). The ^{99m}Tc–RUN complex was characterized at 30, 60, 90 and 120 min, subsequent to the reconstitution for determination of various radiospecies.

Radiochemical stability in serum

The in vitro radiochemical stability of the ^{99m}Tc–RUN complex was assessed in serum for 16 h at 37 °C. The ^{99m}Tc–RUN complex, 0.2 mL was incubated with 1.8 mL of serum at 37 °C. Thereafter, 1 µL aliquots of the incubated mixture immediately after incubation and at 2, 4, 6, 8, 10, 12, 14 and 16 h was taken and spotted on TLC strips. The strips were then developed in $CH_2Cl_2:CH_3OH$ (9:1) (v/v) and thereafter divided into two equal parts for measurement of the radiochemical stability using WCSR.

In vitro binding with *S. aureus*

The binding of ^{99m}Tc–RUN complex with *S. aureus* was assessed using the reported method [27]. Briefly, to a sterilized test tube having 0.1 mL of sodium phosphate buffer (Na-PB) was added 0.2 mL of the freshly prepared ^{99m}Tc–RUN complex. Subsequently 0.01 M (0.8 mL) acetic acid (50% v/v) including 1×10^8 colony forming units (CFU) of the *S. aureus* was added to the test tube followed by incubation for 1 h at 4 °C (pH 5). Thereafter, the mixture was centrifuged at 2,000 rpm for 10 min followed by removal of the supernatant. The pellets were resuspended in 4 mL Na-PB and repeated the incubation and centrifugation process again as described above. For percent uptake calculation the bacterial pellets were counted for activity using WCSR.

Biodistribution in *S. aureus* infected MWR

Biologically the percent absorption of the ^{99m}Tc–RUN complex in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle was studied in artificially infected (*S. aureus*) MWR (weight, 140–180 g). Ten MWR in good physical condition and health were chosen and segregated into two groups of five each (**A** and **B**). Then 0.2 mL of sterile turpentine oil was infused intramuscularly (I.M.) (left thigh) to the healthy rats of group **A** and **B** for inducing artificial inflammation.

Subsequently, 0.2 mL of living *S. aureus* in normal saline was I.M. infused (right thigh) to the group **A** and heat killed *S. aureus* to the group **B** (MWR) for induction of artificial infection. After 20 h, 0.5 mCi of the ^{99m}Tc–RUN complex was intravenously injected to the group **A** and **B** (MWR) followed by execution in accordance with the permitted regulations of the Nuclear Medicine Research Laboratory (NMRL), University of Peshawar. The percent uptake of the ^{99m}Tc–RUN complex per gram of the blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle was measured WCSR.

Results and discussion

Chemistry and characterization of the ^{99m}Tc–RUN complex

The role of acidic stannous chloride dihydrate (25–250 μL with 25 μL rise) (μg/μL 0.01 N HCL) was to reduce the ^{99m}Tc radioactive metal (required for complexation with ligand) and to suppress the formation of radio-colloids. The reduced ^{99m}Tc radioactive metal easily reacts with the two sulfur atoms, a carboxyl and hydroxyl groups of the bidentate rufloxacin (RUN) (Fig. 1a under substitution reaction gave in high yield the ^{99m}Tc–RUN complex as shown in Fig. 1b.

High radiochemical yield ($98.10 \pm 0.18\%$) of the ^{99m}Tc–RUN complex was observed by mixing 2.5 mCi of sodium pertechnetate with 125 μg/μL stannous chloride (μg/μL 0.01 N HCL) and 2 mg of RUN at pH 5.5. The radiochemical yield of the complex decreased to 91.00 ± 0.16 from $98.10 \pm 0.18\%$ within 240 min. The radiochemical yield went down in either condition whether we increase or decrease the amount of the reacting species
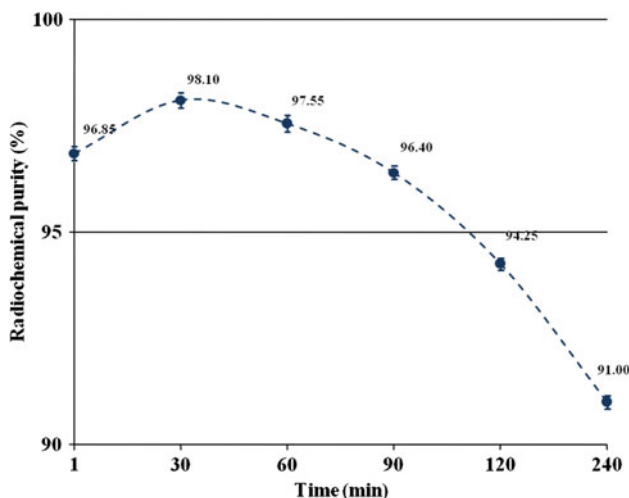
from 2.5 mCi of the sodium pertechnetate, 125 μg/μL stannous chloride (μg/μL 0.01 N HCL), 2 mg of RUN and pH 5.5. The radiochemical yield of the ^{99m}Tc–RUN complex determined at 30, 60, 90, 120 and 240 min after reconstitution is given in Fig. 2.

The HPLC radiochromatogram of the ^{99m}Tc–RUN complex is given in Fig. 3. Two distinctly variable radiopeaks at 3.8 and 11.2 min of retention was observed. The radiopeak at 3.8 min of retention characterize the free pertechnetate and that at 11.2 min the succumb of the ^{99m}Tc–RUN complex.
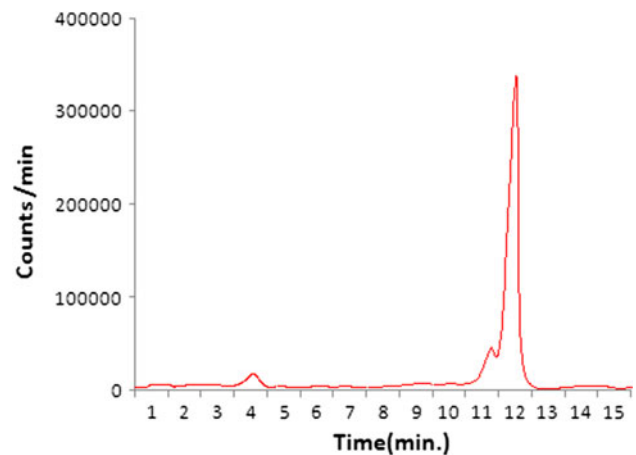

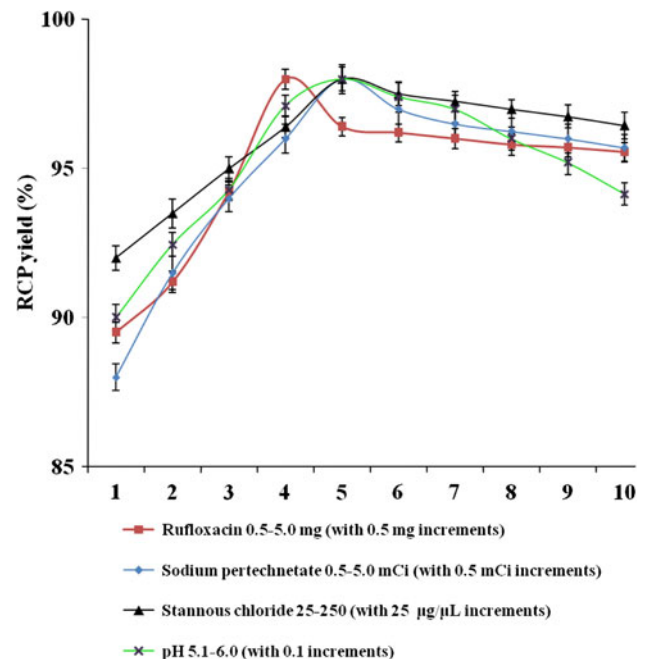**Fig. 3** HPLC radiochromatogram of the ^{99m}Tc–RUN complex

Fig. 4 Effect of the ingredients on the radiochemical purity yield of the ^{99m}Tc–RUN complex

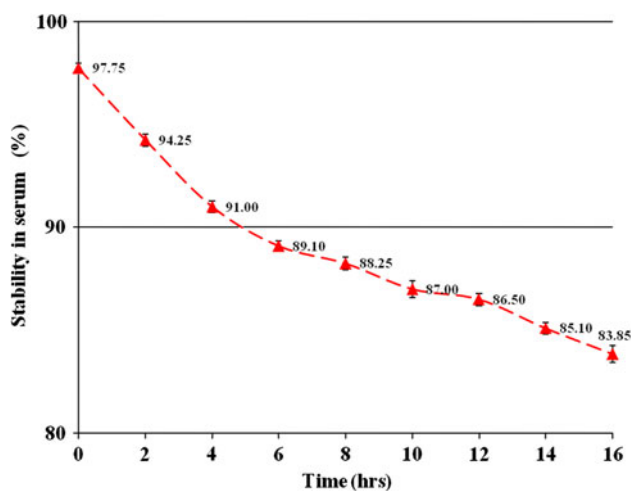**Fig. 2** Radiochemical stability of the ^{99m}Tc–RUN complex

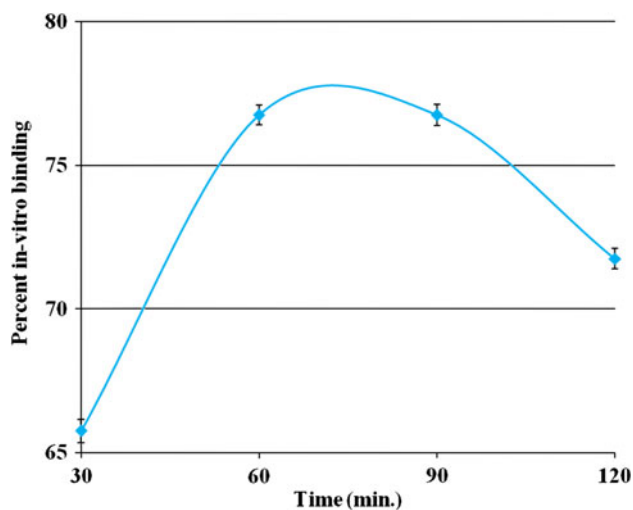**Fig. 5** Radiochemical stability of the ^{99m}Tc–RUN complex in serum

Fig. 6 In-vitro binding *S. aureus*

The proposed structure of ^{99m}Tc–RUN complex (Fig. 1b) will have a square planner pyramidal geometry and ^{99m}TcO:RUN ratio of 1:2 with the bidentate ligand.

Effects of the amount of RUN, sodium pertechnetate, reducing agent and pH on the percent radiochemical purity yield are given in Fig. 4. The ^{99m}Tc–RUN complex prepared by mixing amount other than 2.5 mCi of the sodium pertechnetate, 125 μg/μL stannous chloride (μg/μL 0.01 N HCL), 2 mg of RUN and pH 5.5, ultimately lowering the radiochemical purity yield.

Radiochemical stability in serum

The radiochemical permanence of the ^{99m}Tc–RUN complex in serum at 37 °C determined at different interval of incubation is shown in Fig. 5. A stable behaviour was noted with an unwanted 13.90% free radioactivity at 16 h after incubation. However, 90% stability was seen up to 4 h.

In vitro binding with *S. aureus*

The complex shows saturated in vitro binding with the ^{99m}Tc–RUN complex at 30, 60, 90 and 120 min. The in vitro binding of the ^{99m}Tc–RUN complex with *S. aureus* determined at 30, 60, 90 and 120 min is shown in Fig. 6. The maximum and saturated binding of 65.75–76.75% was observed between 30 to 120 min.

Biodistribution in *S. aureus* infected MWR

The in vivo absorption (%) of the ^{99m}Tc–RUN complex in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of MWR measured at 30, 60, 90 and 120 min of the I.V. administration is given in Table 1. The absorption (%) of the complex per gram of

Table 1 The in vivo absorption (%) of the ^{99m}Tc–RUN complex in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of MWR

| Organs/tissues (g)	Absorption (%) of the ^{99m}Tc–RUN at different time							
	Group A (living *S. aureus*)				Group B (heat killed *S. aureus*)			
	30	60	90	120	30	60	90	120
Infected muscle	6.10 ± 0.32	10.55 ± 0.34	15.10 ± 0.30	13.25 ± 0.33	2.50 ± 0.30	3.00 ± 0.32	3.50 ± 0.33	3.00 ± 0.32
Inflamed muscle	3.50 ± 0.30	3.50 ± 0.32	3.50 ± 0.20	3.00 ± 0.34	4.00 ± 0.32	3.50 ± 0.36	3.50 ± 0.30	3.00 ± 0.34
Normal muscle	3.00 ± 0.36	3.00 ± 0.30	2.50 ± 0.34	2.50 ± 0.30	2.50 ± 0.34	3.00 ± 0.30	2.50 ± 0.36	2.50 ± 0.32
Blood	20.15 ± 0.32	12.45 ± 0.36	10.40 ± 0.34	4.50 ± 0.30	20.40 ± 0.36	11.65 ± 0.33	10.00 ± 0.36	5.00 ± 0.30
Liver	18.85 ± 0.36	12.25 ± 0.30	10.20 ± 0.30	5.70 ± 0.34	18.20 ± 0.34	12.45 ± 0.30	10.00 ± 0.34	6.10 ± 0.30
Spleen	10.10 ± 0.30	8.55 ± 0.34	7.00 ± 0.33	4.35 ± 0.32	9.95 ± 0.36	8.35 ± 0.30	6.75 ± 0.32	4.00 ± 0.36
Kidney	9.25 ± 0.34	17.70 ± 0.30	20.50 ± 0.34	23.50 ± 0.36	9.10 ± 0.36	18.10 ± 0.34	20.25 ± 0.30	24.00 ± 0.30
Stomach and intestines	8.50 ± 0.30	7.75 ± 0.34	6.90 ± 0.36	4.35 ± 0.36	8.75 ± 0.00	7.60 ± 0.34	6.50 ± 0.30	4.50 ± 0.36
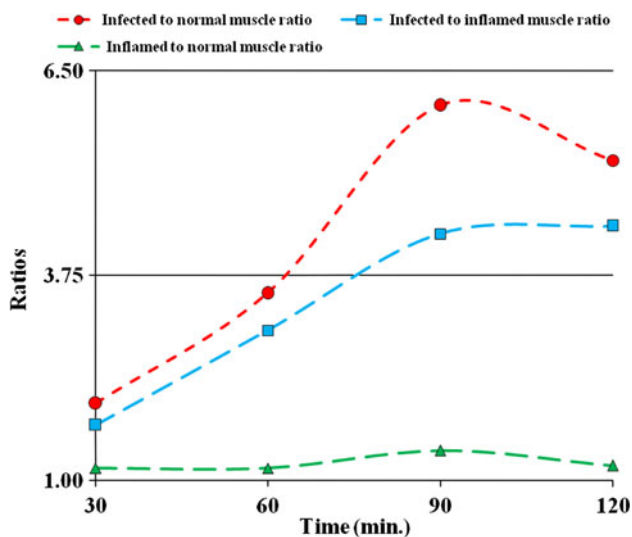
Fig. 7 Uptake ratios of the ^{99m}Tc–RUN complex at different intervals

the blood was initially high and decreased to 4.50 ± 0.30 from $22.15 \pm 0.32\%$ within 120 min in group **A** (MWR). Almost similar profile was noted in MWR of group **B**. Similar trailing patron was seen in liver, spleen, stomach and intestine where the uptake gradually decreased from 18.85 ± 0.36 to $5.70 \pm 0.34\%$, 10.10 ± 0.30 to $4.35 \pm 0.32\%$ and 8.50 ± 0.30 to $4.35 \pm 0.36\%$ respectively. However, in kidney the activity was gradually increased from 9.25 ± 0.34 to $23.50 \pm 0.36\%$ in 120 min. In infected muscle high activity was seen as compared to the inflamed and normal muscle. Figure 7 gives the activity uptake ratios of infected to normal muscle, infected to inflamed muscle and inflamed to normal muscle.

Conclusion

A novel ^{99m}Tc–rufloxacin complex was prepared for the in vivo scintigraphic localization of the *S. aureus* infectious foci in male Wister rats' model. The permanence of the complex in saline, serum, in vitro binding with *S. aureus* and biodistribution results we recommend the ^{99m}Tc–rufloxacin complex for the in vivo scintigraphic localization of the *S. aureus* infectious foci in human.

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