J Radioanal Nucl Chem (2011) 288:297–302
DOI 10.1007/s10967-010-0914-9

Synthesis of the $^{99m}Tc(CO)_3$–trovafloxacin dithiocarbamate complex and biological characterization in artificially methicillin-resistant *Staphylococcus aureus* infected rats model

Syed Qaiser Shah · Muhammad Rafiullah Khan

Received: 28 October 2010 / Published online: 23 December 2010

Abstract Synthesis of the $^{99m}Tc(CO)_3$–trovafloxacin dithiocarbamate ($^{99m}Tc(CO)_3$–TVND) complex and biological characterization in artificially *Staphylococcus aureus* (*S. aureus*) infected rats model was assessed. The suitability of the complex was evaluated and compared with ^{99m}TcN–TVND, in terms of radiochemical immovability in saline, in vitro permanence in serum, in vitro binding with *S. aureus* and biodistribution in Male Sprague-Dawley rats (MSDR). After 30 min of the reconstitution both the complexes showed maximum radiochemical stabilities in saline and remain more than 90% stable up to 120 min. However the $^{99m}Tc(CO)_3$–TVND showed to some extent higher stability than ^{99m}TcN–TVND complex. In serum 1.75% less de-tagging was observed than ^{99m}TcN–TVND complex. Both the complexes showed saturated in vitro binding with *S. aureus* and no significant difference were observed between the uptakes. Six fold uptakes were noted in the infected muscle as compared to the inflamed and normal muscles of the MDSR. The uptake of the $^{99m}Tc(CO)_3$–TVND in infected muscle of the MSDR was 2.25% high as compared to the ^{99m}TcN–TVND complex. Based on radiochemical stabilities in saline, serum, in vitro binding with MRSA and significantly higher uptake in the infected muscle, we recommend both the complexes for in vivo investigation of the MRSA infection in human.

Keywords Trovafloxacin dithiocarbamate (TVND) · $^{99m}Tc(CO)_3$–TVND complex · MRSA

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

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

Introduction

Scintigraphic localization of infection and its discrimination from inflammation at early stages through technetium-99m (^{99m}Tc) labeled radiopharmaceuticals has solved the serious problem of the clinicians [1, 2]. The existing [3–15] and our reported [16–23] radiopharmaceuticals for infection localization had shown promising results in the detection of bacterial infections caused by various pathogens. The encouraging results support our effort to search for more specific radiopharmaceuticals for infection imaging.

Recently it has been reported that quinolones have shown strong antibiotic activity against a variety of pathogens e.g. *Staphylococcus aureus* (*S. aureus*), *Mycobacterium*, *Legionella pneumophila* (*L. pneumophila*), *Enterococcus faecium* (*E. faecium*) and *Escherichia coli* (*E. coli*). Trovafloxacin (TVN) (Fig. 1a), {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-carboyilic acid} is one of the novel antibiotic of quinolones group that exhibit broad spectrum antibacterial activity against *S. aureus*, *E. faecium* and *L. pneumophila* [24, 25].

The complexation of biomolecules with ^{99m}Tc using $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor have been recently explored and found it as a suitable radiolabeling procedure [22, 23]. In continuation to our ongoing investigations, the aim of the present study was to exploit and compare the radiolabeling of trovafloxacin dithiocarbamate (TVND) with ^{99m}Tc using the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor and $[^{99m}Tc\equiv N]^+$ core. The possibility of the $^{99m}Tc(CO)_3$–TVND complex as potential *S. aureus* infection radiotracer was assessed in Male Sprague-Dawley rats (MSDR) and compared with ^{99m}TcN–TVND complex. The effectiveness of the complex was evaluated in terms of in vitro

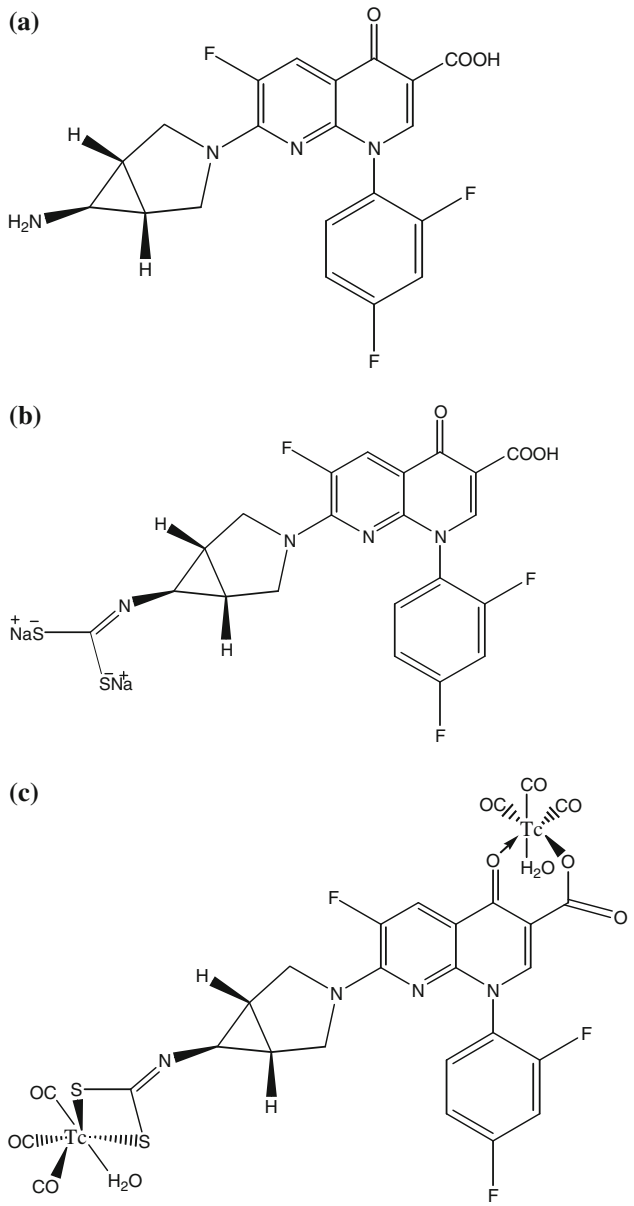

Fig. 1 **a** Chemical structure of the Trovafloxacin (TVN). **b** Trovafloxacin dithiocarbamate (TVND). **c** Proposed structure of the $^{99m}Tc(CO)_3$–TVND radiocomplex

immovability in normal saline and serum, in vitro binding with *S. aureus*, and biodistribution in MDSR.

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 radiocomplexation of trovafloxacin dithocarbamate

Trovafloxacin (TVN) was converted to trovafloxacin dithocarbamate (TVND) by reacting TVN with equimolar amount of carbon disulfide (CS_2) in the presence of sodium hydroxide and tetrahydrofuran (THF) using the reported procedure [23]. The radiocomplexation of the TVND with ^{99m}Tc using $[^{99m}Tc(OH_2)_3(CO)_3]^+$ precursor was investigated by adding 0.5 mL (1–2 mCi) of sodium pertechnetate ($Na^{99m}TcO_4^-$) and 0.1 mol/mL HCL solution to the Iso-link kit and incubated for 15 min. After that 2 mg liquefied TVND was added to the kit through a sterilized syringe followed by incubation at room temperature for 15 min.

Partition coefficient (P)

Partition coefficient of the $^{99m}Tc(CO)_3$–TVND complex was evaluated and compared with ^{99m}TcN–TVND complex adopting the process described earlier [23]. Briefly, the $^{99m}Tc(CO)_3$–TVND complex, octanol and phosphate buffer (PB) in equals amounts were vortexed for 5 min at room temperature. Then the reaction mixture was centrifuged at 5000 rpm/min for 15 min. Subsequently, aliquots (0.1 mL) were haggard at different intervals and counted using well counter interface with scalar count rate meter. The following formula was used for the calculation of the *P*. The same process was repeated for *P* calculation of the ^{99m}TcN–TVND complex.

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

Radiochemical purity (RCP) determination and characterization

In normal saline the RCP values of the $^{99m}Tc(CO)_3$–TVND complex was determined by using HPLC method as described earlier [23]. Briefly, 10 μL aliquots of the freshly prepared $^{99m}Tc(CO)_3$–TVND complex were withdrawn from vial and injected into 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 and C-18 (4.6 × 150 mm) column. Water:methanol (W:M) was employed as the mobile phase and a flow rate of 1 mL/min

was applied for 15 min. The mobile phase (W:M) used was; 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 (100:100). During the elution (1–15 min) the radio-fractions collected were measured separately using the single gamma rays detecting well counter interface with scalar count rate meter. The same process was repeated for the ^{99m}TcN–TVND complex.

In vitro stability in serum

In vitro stability of the $^{99m}Tc(CO)_3$–TVND complex was investigated in serum at 37 °C using the TLC procedure. 1.8 mL of the serum was incubated at 37 °C with $^{99m}Tc(CO)_3$–TVND complex. Thereafter, aliquots at 0, 2, 4, 6, 8, 10, 12, 14 and 16 h of incubation were withdrawn and executed for percent stability on TLC strip. The strips were than developed in saline and CH_2Cl_2:CH_3OH (9:1) (v/v). Subsequently, the developed strips were divided into two equal parts and measured for percent in vitro stability using single well gamma rays detecting well counter interface with scalar count rate meter. The same process was repeated for the earlier reported ^{99m}TcN–TVND complex [23].

In vitro binding with MRSA

In vitro binding of the $^{99m}Tc(CO)_3$–TVND complex with *S. aureus* was assessed using the reported procedure [26]. Briefly, the $^{99m}Tc(CO)_3$–TVND complex (10 MBq) was poured into a sterilized test tube having 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 *S. aureus* and incubation at 4 °C for 1 h with a final pH 5. The reaction mixture was centrifuged at 2000 rpm for 10 min. Thereafter, the supernatant was removed and the bacterial pellets were resuspended in 2 mL Na-BP. The blend was again centrifuged at 2000 rpm for 10 min. Thereafter, the pellets were measured for percent in vitro uptake.

In vivo percent uptake in MRSA infected MSDR

Healthy Male Sprague-Dawley Rats (MSDR) (weight, 180–220 g) were randomly selected and divided into two groups (**A** and **B**) with seven rats in each group for the determination of biodistribution of the $^{99m}Tc(CO)_3$–TVND complex. Rats of group **A** and **B** were intramuscularly (I.M.) injected with 0.2 mL sterile turpentine oil into the right thigh and 0.2 mL of living strain of *S. aureus* (containing approximately 1×10^8 CFU) to the left thigh. After 18 h, 0.5 mL (18.5 MBq) of the $^{99m}Tc(CO)_3$–TVND complex through ear vein was injected to the rats of group **A** and **B**. Thereafter, all the rats were killed as per rule stipulated in the manual of Nuclear Medicine Research Laboratory (NMRL) University of Peshawar Part-I and II. The percent absorbed dose per gram in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle was measured with single well gamma rays detecting counter interface with scalar count rate meter. The biodistribution profile of the $^{99m}Tc(CO)_3$–TVND complex was compared with the ^{99m}TcN–TVND complex.

Results and discussion

Chemistry and geometry

Trovafloxacin dithiocarbamate (TVND) Fig. 1b was obtained in good yield using trovafloxacin (Fig. 1a) as the starting material and its radiocomplexation with ^{99m}Tc using the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor to give the $^{99m}Tc(CO)_3$–TVND complex as shown in Fig. 1c. The water molecule in the radiocomplexation was rapidly displaced from the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor by the TVND. The $^{99m}Tc(CO)_3$–TVND radiocomplex in a high yield was recovered with a 98.75 ± 0.15% radiochemical purity. The proposed structure of the $^{99m}Tc(CO)_3$–TVND radiocomplex (Fig. 1c) will have a square planner bipyramidal geometry with metal:ligand ratio of 2:1.

Partition coefficient (*P*)

The participation coefficient (*P*) values of the $^{99m}Tc(CO)_3$–TVND and ^{99m}TcN–TVND complexes observed were 0.44 ± 0.01 and 1.08 ± 0.02 respectively. Both the *P* values of radiocomplexes suggested lipophilic nature.

HPLC radio-characterization

Two radio-peaks at retention time (RT) 3.1 and at 11.2 min were observed in the HPLC radio-chromatogram of the $^{99m}Tc(CO)_3$–TVND complex. The radio-peak at 11.2 represents the yield of the $^{99m}Tc(CO)_3$–TVND complex. A similar HPLC radio-chromatogram was shown by the ^{99m}TcN–TVND complex with two radio-peaks at RT 3.9 and 12.6 min [R]. The radio-peak at RT 3.9 min correspond to $[^{99m}TcN]^{2+}$ intermediate and 12.6 min to the ^{99m}TcN–TVND complex Fig. 2.

Radiochemical purity (RCP)

A stable in vitro radiochemical purity yields were observed for both ($^{99m}Tc(CO)_3$–TVND and ^{99m}TcN–TVND) radiocomplexes at different intervals after reconstitution as given in Fig. 3. It was observed that the RCP values of both

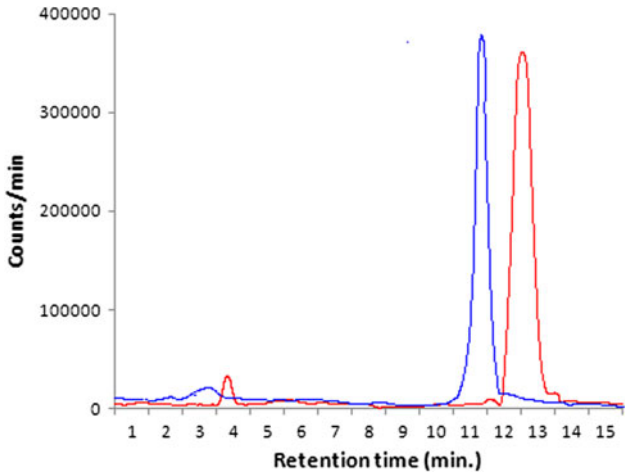

Fig. 2 $^{99m}Tc(CO)_3$–TVND complex HPLC radiochromatogram

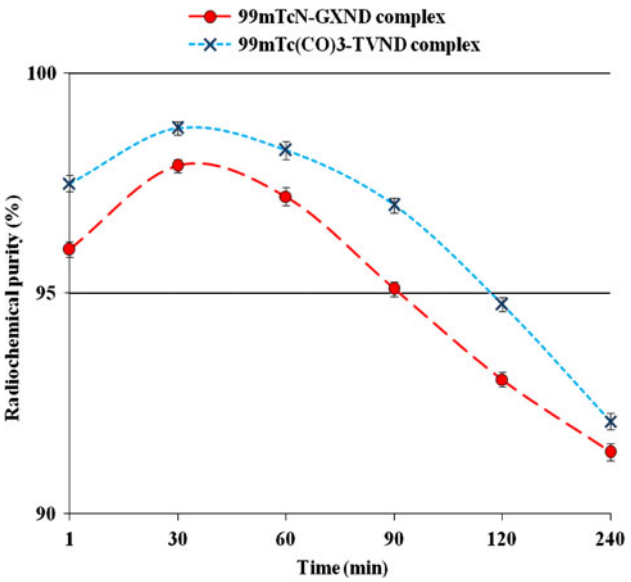

Fig. 3 Stability of the ^{99m}TcN–TVND and $^{99m}Tc(CO)_3$–TVND radiocomplexes in normal saline

the radiocomplexes decreased with time. The RCP values determined after 30 min of the preparation were 97.90 ± 0.22% ($n = 9$) and 98.75 ± 0.15% ($n = 9$) respectively. After 240 min the RCP values went down to 91.40 ± 0.25% ($n = 9$) and 92.10 ± 0.19% ($n = 9$) respectively. Insignificantly higher values of the RCP were observed for the complex synthesized through $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor. After 240 min no significant difference was observed between the RCP values of both the radiocomplexes.

In vitro stability in serum

Both the radiocomplexes showed in vitro stability in serum at 37 °C as shown in Fig. 4. The stability of both the

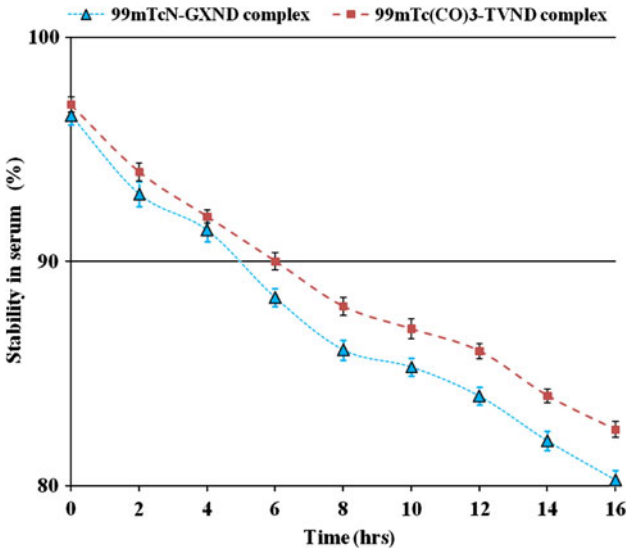

Fig. 4 In vitro stability of the ^{99m}TcN–TVND and $^{99m}Tc(CO)_3$–TVND radiocomplexes in serum at 37 °C

radiocomplexes marginally went down from 96.50 ± 0.40 to 80.25 ± 0.42% and 97.00 ± 0.35 to 82.50 ± 0.30% respectively, after 16 h of incubation.

In vitro binding with MRSA

Both the radiocomplexes showed saturated in vitro binding with *S. aureus* as shown in Fig. 5. An insignificantly higher uptake was noted with $^{99m}Tc(CO)_3$–TVND as compared to the ^{99m}TcN–TVND radiocomplex.

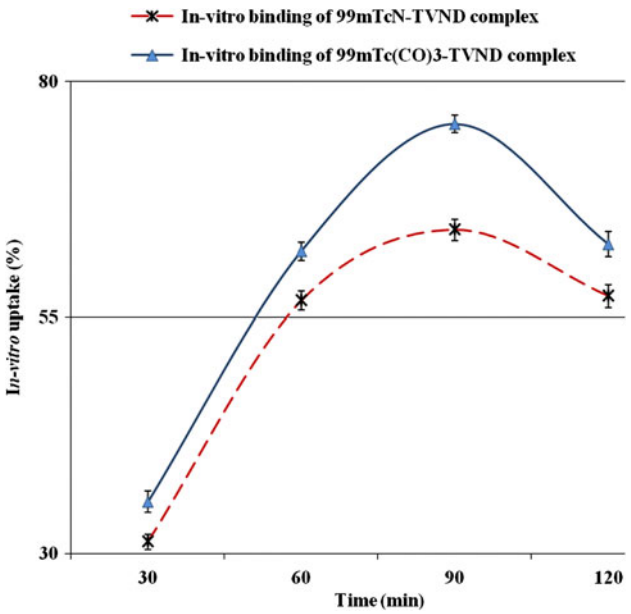

Fig. 5 Comparative in vitro binding of the ^{99m}TcN–TVND and $^{99m}Tc(CO)_3$–TVND radiocomplexes with *Staphylococcus aureus*

Table 1 Biodistribution of the $^{99m}Tc(CO)_3$–TVND complex in various organs of *Staphylococcus aureus* infected Male Sprague-Dawley rats

Organs/tissues (g)	Percent in vivo absorption at different intervals (in min)							
	Alive *S. aureus*				Heat killed *S. aureus*			
	30	60	90	120	30	60	90	120
Infected muscle	7.10 ± 0.35	12.40 ± 0.40	15.10 ± 0.30	12.55 ± 0.36	2.50 ± 0.30	3.00 ± 0.35	3.00 ± 0.33	2.80 ± 0.40
Inflamed muscle	3.00 ± 0.40	4.00 ± 0.34	3.00 ± 0.32	3.00 ± 0.37	3.00 ± 0.34	3.50 ± 0.37	3.00 ± 0.30	3.00 ± 0.35
Normal muscle	3.00 ± 0.33	2.50 ± 0.40	2.50 ± 0.36	2.50 ± 0.35	2.50 ± 0.40	2.50 ± 0.34	2.50 ± 0.36	2.50 ± 0.37
Blood	22.00 ± 0.35	10.50 ± 0.30	8.20 ± 0.34	3.85 ± 0.40	22.50 ± 0.40	9.80 ± 0.33	7.55 ± 0.35	3.40 ± 0.40
Liver	15.80 ± 0.40	11.00 ± 0.33	8.35 ± 0.37	6.00 ± 0.30	16.10 ± 0.32	12.00 ± 0.35	9.35 ± 0.40	5.50 ± 0.36
Spleen	9.50 ± 0.35	7.10 ± 0.40	6.00 ± 0.37	3.45 ± 0.33	9.25 ± 0.30	7.00 ± 0.35	4.75 ± 0.40	3.40 ± 0.33
Kidney	9.50 ± 0.37	16.85 ± 0.33	19.50 ± 0.37	22.85 ± 0.35	9.85 ± 0.33	17.10 ± 0.39	19.00 ± 0.40	22.25 ± 0.34
Stomach and intestines	7.95 ± 0.34	6.55 ± 0.39	5.90 ± 0.33	3.50 ± 0.40	8.10 ± 0.37	7.00 ± 0.30	5.85 ± 0.34	3.85 ± 0.38

Table 2 Biodistribution of the ^{99m}TcN–TVND complex in various organs of *Staphylococcus aureus* infected Male Sprague-Dawley rats [23]

Organs/tissues (g)	Percent in vivo absorption at different intervals (in 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

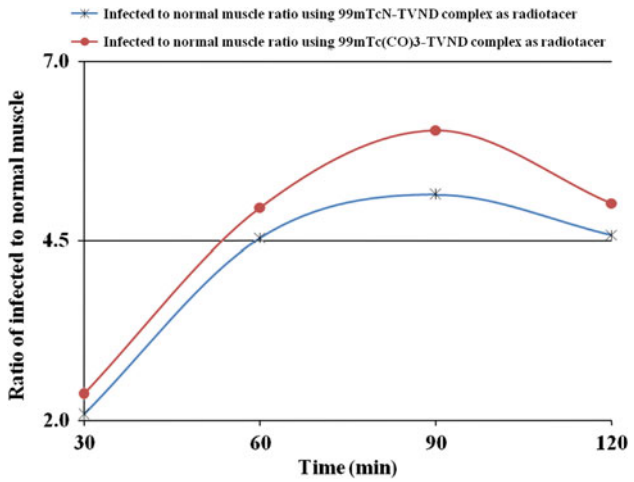

Fig. 6 Infected to normal muscle ratio profile of the ^{99m}TcN–TVND and $^{99m}Tc(CO)_3$–TVND radiocomplexes at different intervals

In vivo percent uptake in MRSA infected MSDR

Tables 1 and 2, gives the percent absorbed dose of the $^{99m}Tc(CO)_3$–TVND and ^{99m}TcN–TVND radiocomplex respectively, per gram in blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of the healthy Male Sprague-Dawley Rats (MSDR) artificially infected with living and heat killed *S. aureus*. The activity of both the complexes in blood (per gram) in the beginning was 20.50 ± 0.18 and 22.00 ± 0.35% respectively. These high activities markedly decreased to 3.50 ± 0.15 and 3.85 ± 0.40% within 120 min of the I.V. administration. In liver, spleen, stomach and intestines a similar trailing profile was noted. However in kidneys a reverse profile was noted where the activities in the beginning were 10.50 ± 0.16 and 9.50 ± 0.37% which increased to 23.25 ± 0.16 and 22.85 ± 0.35% within 120 min respectively. Both the complexes showed significantly higher uptake in the *S. aureus* infected muscle of the MSDR as compared to the inflamed and normal muscles. The appearance of activity of both the radiocomplex in urinary system and disappearance from circulatory system established the usual path of excretion.

The ratios of the uptake of both the complexes (infected to normal muscle, infected to inflamed muscle and inflamed to normal muscle) are shown in Fig. 6. In both the

complexes almost six and five fold uptake was noted in the infected (target organ) to inflamed or normal muscles (non-target organ) respectively.

Conclusion

Synthesis of the $^{99m}Tc(CO)_3$–TVND radiocomplex and biological characterization in *S. aureus* infected MSDR was assessed. The aptness of the complex was evaluated in terms of radiochemical immovability in saline, in vitro permanence in serum, in vitro binding with MRSA and biodistribution and compared with ^{99m}TcN–TVND. Both the radiocomplexes shown comparably similar results but the high target to non-target ratios posed the $^{99m}Tc(CO)_3$–TVND radiocomplex as a better *S. aureus* infection radiotracer than ^{99m}TcN–TVND radiocomplex.

References

1. Gallagher H, Ramsay SC, Barnes J, Maggs J, Cassidy N, Ketheesan N (2006) Neutrophil labeling with [^{99m}Tc]-technetium stannous colloid is complement receptor 3-mediated and increases the neutrophil priming response to lipopolysaccharide. Nucl Med Biol 33:433
2. Basu S, Chryssikos T, Moghadam-Kia S, Zhuang H, Torigian DA, Alvai A (2009) Positron Emission Tomography as a diagnostic tool in infection: present role and future possibilities. Semin Nucl Med 39:36
3. Chattopadhyay S, Das SS, Chandra S, De K, Mishra M, Sarkar BR, Sinha S, Ganguly S (2010) Synthesis and evaluation of ^{99m}Tc-moxifloxacin, a potential infection specific imaging agent. Appl Radiat Isotopes 68:314
4. Motaleb MA (2007) Preparation of ^{99m}Tc-cefoperazone complex, a novel agent for detecting sites of infection. J Radioanal Nucl Chem 272:167
5. Motaleb MA (2007) Preparation and biodistribution of ^{99m}Tc-lomefloxacin and ^{99m}Tc-olfloxacin complex. J Radioanal Nucl Chem 272:95
6. Zhang J, Guo H, Zhang S, Lin Y, Wang X (2008) Synthesis and biodistribution of a novel ^{99m}TcN complex of ciprofloxacin dithiocarbamate as a potential agent for infection imaging. Bioorg Med Chem Lett 18:51
7. Roohi S, Mushtaq A, Jehangir M, Ashfaq MS (2006) Synthesis, quality control and biodistribution of ^{99m}Tc-Kanamycin. J Radioanal Nucl Chem 267:561
8. Oh SJ, Ryu J, Shin JW, Yoon EJ, Ha H, Cheon JH, Lee HK (2002) Synthesis of ^{99m}Tc-ciprofloxacin by different methods and its biodistribution. Appl Radiat Isot 57:193
9. El-Gany EA, El-Kolaly MT, Amine AM, El-Sayed AS, Abdel-Gelil F (2005) Synthesis of ^{99m}Tc-pefloxacin: a new targeting agent for infectious foci. J Radioanal Nucl Chem 266:131
10. Motaleb MA (2009) Preparation, quality control and stability of ^{99m}Tc-sparafloxacin complex, a novel agent for detecting sites of infection. J Labelled Comp Radiopharm 52:415
11. Xia J, Wang Y, Yu J, Li S, Tang L, Zheng M, Liu X, Li G, Cheng D, Liang S, Yin D (2008) Synthesis, in vitro and in vivo behavior of ^{188}Re(I)-tricarbonyl complexes for the future functionalization of biomolecules. J Radioanal Nucl Chem 275:325
12. Zhang J, Wang X, Jin C (2007) Synthesis and biodistribution of the $^{99m}Tc(CO)_3$-DEDT complex as a potential new radiopharmaceutical for brain imaging. J Radioanal Nucl Chem 272:91
13. Djokic DD, Jankovic DL, Stamenkovic LL, Pirmettis I (2004) Chemical and biological evaluation of $^{99m}Tc(CO)_3$ and ^{99m}Tc complexes of some IDA derivatives. J Radioanal Nucl Chem 260:471
14. Xia J, Long S, Yu J, Wang Y, Cao Z (2009) Pyridyl derivatives provide new pathways for labeling protein with *fac*-$[^{188}Re(CO)_3(H_2O)_3]^+$. J Radioanal Nucl Chem 281:493
15. Zhang JB, Wang XB, Jin C (2006) Synthesis of $^{99m}Tc(CO)_3$-NOET via $[^{99m}Tc(OH_2)_3(CO)_3]^+$ precursor and comparative biological studies with ^{99m}TcN-NOET. J Radioanal Nucl Chem 269:227
16. Qaiser SS, Khan AU, Khan MR (2010) Synthesis, biodistribution and evaluation of ^{99m}Tc-Sitafloxacin kit: a novel infection imaging agent. J Radioanal Nucl Chem 284:189
17. Shah SQ, Khan AU, Khan MR (2010) Radiosynthesis of ^{99m}Tc-nitrifuratonin a novel radiotracer for in vivo imaging of *Escherichia coli* infection. J Radioanal Nucl Chem. doi:10.1007/s10967-010-0697-z
18. Shah SQ, Khan AU, Khan MR (2010) Radiosynthesis and biodistribution of ^{99m}Tc-rifampicin: a novel radiotracer for in vivo infection imaging. Appl Radiat Isot 68:2255
19. Shah SQ, Khan AU, Khan MR (2010) ^{99m}Tc-Novobiocin: a novel radiotracer for infection imaging. Radiochim Acta (in press)
20. Shah SQ, Khan AU, Khan MR (2010) Radiosynthesis, biodistribution and scintigraphy of the ^{99m}Tc-Teicoplanin complex in artificially infected animal models. J Labelled Comp Radiopharm. doi:10.1002/jlcr.1834
21. Shah SQ, Khan AU, Khan MR (2010) Radiosynthesis and biological evaluation of ^{99m}TcN-sitafloxacin dithiocarbamate as potential radiotracer for *Staphylococcus aureus* infection. J Radioanal Nucl Chem. doi:10.1007/s10967-010-0833-9
22. Shah SQ, Khan AU, Khan MR (2010) Radiosynthesis and biological evolution of $^{99m}Tc(CO)_3$-sitafloxacin dithiocarbamate complex: a promising *Staphylococcus aureus* infection radiotracer. J Radioanal Nucl Chem. doi:10.1007/s10967-010-0880-2
23. Shah SQ, Khan MR (2010) Radiocomplexation and biological characterization of the ^{99m}TcN-Trovafloxacin dithiocarbamate: a novel methicillin-resistant *Staphylococcus aureus* infection imaging agent. J Radioanal Nucl Chem. doi:10.1007/s10967-010-0903-z
24. Ocaña JA, Callejón M, Barragán FJ (2001) Determination of trovafloxacin in human serum by time resolved terbium-sensitised luminescence. Eur J Pharm Sci 13:297
25. Hsiao CJ, Younis H, Boelsterli UA (2010) Trovafloxacin, a fluoroquinolone antibiotic with hepatotoxic potential, causes mitochondrial peroxynitrite stress in a mouse model of underlying mitochondrial dysfunction. Chem Biol Interact 188:204
26. Welling MM, Paulusma-Annema A, Batler HS, Pauwels EKJ, Nibbering PH (2000) Technetium-99m labelled antimicrobial peptides discriminate between bacterial infections and sterile inflammations. Eur J Nucl Med 27:292