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Synthesis, biodistribution and evaluation of ^{99m}Tc-sitafloxacin kit: a novel infection imaging agent

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Abstract Radiosynthesis of ^{99m}Tc-sitafloxacin (^{99m}Tc-STF) complex and its efficacy as a potential infection imaging agent was evaluated. Effect of sitafloxacin (STF) concentration, sodium pertechnetate ($Na^{99m}TcO_4$), stannous chloride dihydrate ($SnCl_2{\cdot}2H_2O$), and pH on the % radiochemical purity yield (RCP) of ^{99m}Tc-STF complex was studied. A stable ^{99m}Tc-STF complex up to 120 min with maximum %RCP yield was observed by mixing 2 mg of STF with 3 mCi of $Na^{99m}TcO_4$ and 150 μL of $SnCl_2{\cdot}2H_2O$ (1 μg/μL in 0.01 N HCl) at a pH 5.5. Artificially infected rats with *Staphylococcus aureus* were used for studying the biodistribution behavior of the ^{99m}Tc-STF complex. After 30 min of the intravenous (I.V.) administration of the ^{99m}Tc-STF complex, 7.50 ± 0.10% was absorbed in the infected thigh of the rats and the uptake gradually increased to 18.50 ± 0.20% within 90 min. Rabbits with artificially induced infection were used for evaluating the scintigraphic accuracy. Higher uptake in the infected thigh was observed after 2 h of I.V. administration of the ^{99m}Tc-STF complex. Target to non-target organ ratio of the % absorbed dose incase of infected/normal muscle was 6.82 ± 0.40, 17.11 ± 0.60, and 23.13 ± 1.00% at 30, 60 and 90 min of administration. Stable and higher %RCP, higher uptake in the infected thigh, and spectral studies, recommend the ^{99m}Tc-STF for routine infection imaging.

Keywords Sitafloxacin · Technetium-99m · Labeling · Infection

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Introduction

Diagnosis of infection and its discrimination from inflammation at early stage is quite important for clinicians for its rapid treatment with appropriate drug to reduce the rate of morbidity and mortality [1]. Sophisticated technologies like computerized tomography (CT) and magnetic resonance imaging (MRI) are failed to localize the infectious foci in preliminary phase [2, 3].

^{67}Gallium citrate and ^{111}Indium labeled leucocytes were used for infection imaging but their high radiation dose and other disadvantages as discussed in literature and insignificant clinical results abandoned their use as infection imaging agent [4, 5]. Recently, technetium-99m (^{99m}Tc) labeled antibiotics like ciprofloxacin [6, 7], ciprofloxacin dithiocarbamate [8], cefoperazone [9], lomefloxacin, ofloxacin [10], cefuroxine [11], pefloxacin [12], kanamycin [13], sparafloxacin [14], and moxifloxacin [15], are proposed for infection localization because of their higher %RCP yields, better biodistribution behavior and easy preparation then the ^{111}In and ^{99m}Tc labeled leukocytes [16].

Sitafloxacin (STF), {(−)-7-[(7*S*)-7-amino-5-azaspiro[2.4]heptan-5-yl]-8-chloro-6-fluoro-1-[(1*R*,2*S*)-2-fluoro-1-cyclopropyl]-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid} is another new antimicrobial agent, having similar 4-fluroquinolone skeleton (Fig. 1) that inhibit the growth of various aerobic and anaerobic gram-positive (G+) and gram-negative (G−) bacteria [17]. The antimicrobial activity of STF,

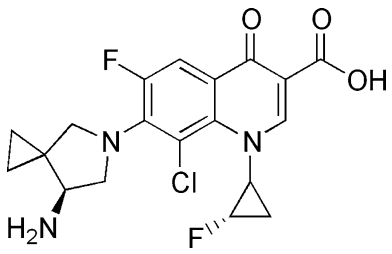

Fig. 1 Structure of sitafloxacin (STF)

against G+ and G− bacteria, is found better than the available reported range [18].

The aim of the present investigation is to synthesize ^{99m}Tc-STF complex and to determine its stability and efficacy as a potential infection imaging agent in terms of percent radiochemical purity yield (%RCP), biodistribution and animal scintigraphy, using different concentration of the STF, $Na^{99m}TcO_4$, $SnCl_2{\cdot}2H_2O$, at pH 5–6.

Experimental

Materials and methods

Sitafloxacin (STF) from Daiichi Sanko, Japan, analytical grade stannous chloride dihydrate, from Sigma, thin layer chromatography (TLC) aluminum strip and insulin syringes from Merck, were used in this investigation.

Equipment

Microwave oven from Panasonic, Matsushita Electric Industrial Co. model MN-S674 MF, single well counter interface with scalar count rate meter from Ludlum Measurements, Inc., USA, (Model 243 & 2000), and Gamma camera from GEADE Nuclearmedizine, Germany (model GKS-1000: SPECT system), were used.

Animal and pathogen

Rats, rabbit, and pathogen (*Staphylococcus aureus)* from Veterinary Research Institute, Peshawar, Pakistan.

Preparation of ^{99m}Tc-STF complex

Five sets of 50, 100, 150 and 200 μL of $SnCl_2{\cdot}2H_2O$ (1 μg/μL in 0.01 N HCl) solutions were prepared in different vials at pH 5.0, 5.2, 5.5, 5.7 and 6. 1–5 mg of STF and 1–5 mCi of $Na^{99m}TcO_4$ were added through sterilized syringe and heated in a microwave oven up to 100 °C for 15 s. All the solutions were then filtered through 0.22 μm cellulose ester membrane and the products were analyzed for %RCP yield.

Determination of RCP yield

TLC was used to check the %RCP yield of the ^{99m}Tc-STF complex. TLC strip was used as stationary and acetone as the mobile phase to separate the tagged ^{99m}Tc-STF and reduced hydrolyzed technetium ($^{99m}TcO_2$) from the free technetium ($^{99m}TcO_4^{-}$). In this system, the tagged ^{99m}Tc-STF and $^{99m}TcO_2$ stayed at the origin (Rf 2–3), while the $^{99m}TcO_4^{-}$ component moved with the solvent front (Rf 7–9). To separate ^{99m}Tc-STF from $^{99m}TcO_2$, the TLC strip was impregnated with human serum albumin (HSA), using ethanol:ammonia:water (2:1:5) as the mobile phase. In this system ^{99m}Tc-STF moved with the solvent front (Rf 7–9) leaving $^{99m}TcO_2$ at the bottom (Rf 2–3). The developed TLC strip was divided into two equal parts (Rf 5) and each part was then counted for activity, using single well interface with scalar count rate meter.

Animal model and biodistribution

Male rats (weight range, 20–25 g) were sedated with intraperitonial injection of 0.1 mL in saline containing 1 mg fluanisone and 0.03 mg fentanyl citrate. 0.05 mL broth containing 2×10^8 organisms of *Staphylococcus aureus* was injected into the right thigh for inducing artificial infection and after 24 h, 0.1 mL of the sterile turpentine oil was injected in the left thigh of the rat. 37 MBq of the ^{99m}Tc-STF was injected and after 30 min, 1 and 2 h, the animals were sacrificed. Blood sample was directly obtained from the heart through needle aspiration and samples of the infected, inflamed, normal muscle, liver, spleen, lungs and kidney were weighed and % injected dose absorbed per gram was calculated using single well counter interface with scalar count rate meter.

Animal scintigraphy

Broth, 0.1 mL containing 2×10^8 organisms of *Staphylococcus aureus* injected into the right thigh of a pathogen free rabbit before 12 h of scintigraphy. 0.1 mL of the sterile turpentine oil was I.M. injected in left thigh and 74 MBq of the ^{99m}Tc-STF was I.V. injected to the same rabbit. Whole body images were obtained using Gamma camera at 30, 60, and 90 min.

Results and discussion

%RCP yield and effect of stannous chloride dihydrate

The %RCP yield was determined at 15 s, 30, 60, 90, 120 and 240 min after labeling and the results are shown in Table. 1. A stable ^{99m}Tc-STF complex up to 120 min with

maximum %RCP yield (98.96 ± 0.10 %) was observed by mixing 2 mg of STF with 3 mCi of $Na^{99m}TcO_4$ and 150 µL of $SnCl_2{\cdot}2H_2O$ (1 µg/µL in 0.01 N HCl) at a pH 5.5. Decrease in %RCP yield and tagging time was observed at the lower and the higher concentration of the stannous chloride than 150 µL as shown in Fig. 2.

Effect of pH, concentration of STF and sodium pertechnetate

The effect of pH on %RCP values was studied from pH 5 to 6 and the results are shown in Fig. 3. Maximum %RCP yield was observed at pH 5.5 and minimum at pH 6. The effect of concentration of STF on ^{99m}Tc-STF complex was checked between 1 to 5 mg and the results are shown in Fig. 4. Higher RCP yield was observed using 2 mg of STF. Sodium pertechnetate amount was characterized over a range of 5 mCi. It was observed that the labeling shows maximum RCP yield at 3 mCi and the values gradually decreases as the amount of sodium pertechnetate, either increases or decreases from 3 mCi, as shown in Fig. 5.

Biodistribution in various organs of rat

Biodistribution behavior of the labeled preparation was investigated in artificially infected rats after 30, 60 and 90 min of I.V. administration of the ^{99m}Tc-STF complex and the results are shown in Table 2. The % adsorbed dose in infected muscle was 7.50 ± 0.10, 16.25 ± 0.15 and 18.50 ± 0.20%. In artificially inflamed and normal muscles a decrease in the % absorbed dose was observed and the value of % absorbed dose goes down from 1.25 ± 0.15 to 0.90 ± 0.10% and 1.00 ± 0.25 to 0.80 ± 0.20%. Target to non-target organ ratio of the % absorbed dose incase of infected/normal muscle was 6.82 ± 0.40, 17.11 ± 0.60, and 23.13 ± 1.00% at 30, 60 and 90 min of administration. The same trailing pattern was observed for liver and the % absorbed activity was gradually decreased from 20.50 ± 0.50 to 5.50 ± 0.00%. In kidney the % absorbed activity was 10.50 ± 0.50 and gradually the uptake goes higher up to 27.25 ± 0.22% within 90 min. This increases in the uptake

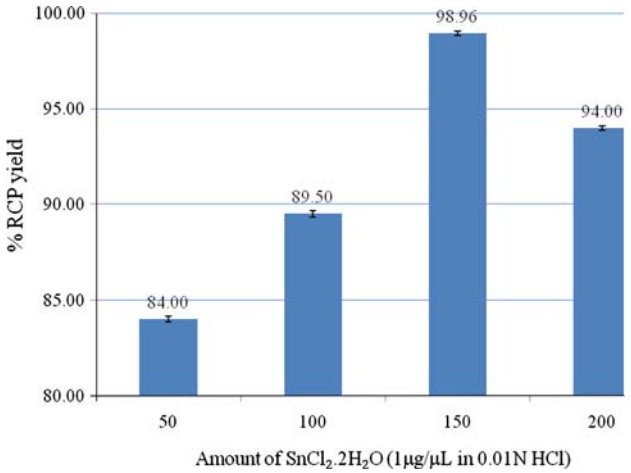

Fig. 2 Effect of stannous chloride amount on %RCP yield

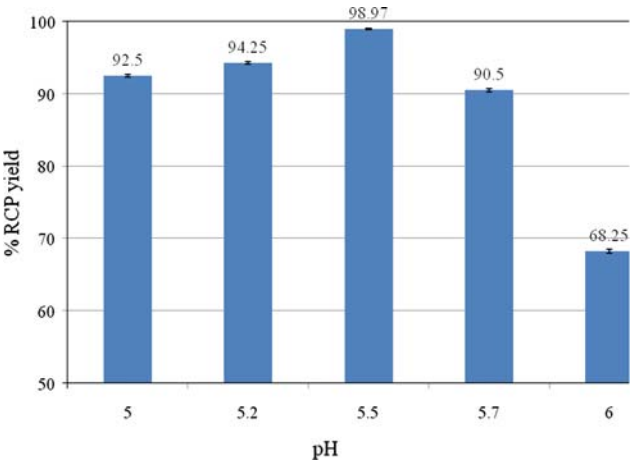

Fig. 3 Effect of pH on %RCP yield

values for kidney suggested that drug is biodegradable and its route of excretion is kidney. The values of the activity in blood gradually deceased from 22.60 ± 0.25 to 2.50 ± 0.20%. This decrease in the % absorbed dose suggested that the labeled drug shows normal biodistribution of tagged STF.

Animal scintigraphy

The efficacy of the ^{99m}Tc-STF was finally evaluated using rabbits, having artificially induced infection and

Table 1 %RCP of ^{99m}Tc-STF complex at different intervals (mean ± SD)

Reducing agent[a]	%RCP yield					
	15 s	30 min	60 min	90 min	120 min	240 min
50	84.00 ± 0.15	84.50 ± 0.10	84.10 ± 0.15	82.81 ± 0.18	78.84 ± 0.13	72.50 ± 0.18
100	89.50 ± 0.18	89.35 ± 0.20	89.30 ± 0.16	88.25 ± 0.14	84.00 ± 0.10	77.75 ± 0.20
150	98.96 ± 0.10	98.95 ± 0.00	98.91 ± 0.09	97.58 ± 0.15	93.59 ± 0.20	87.28 ± 0.10
200	94.00 ± 0.10	94.00 ± 0.00	93.95 ± 0.14	92.60 ± 0.00	88.60 ± 0.10	82.35 ± 0.18

[a] ($SnCl_2{\cdot}2H_2O$) (1 µg/µL in 0.01 N HCl)

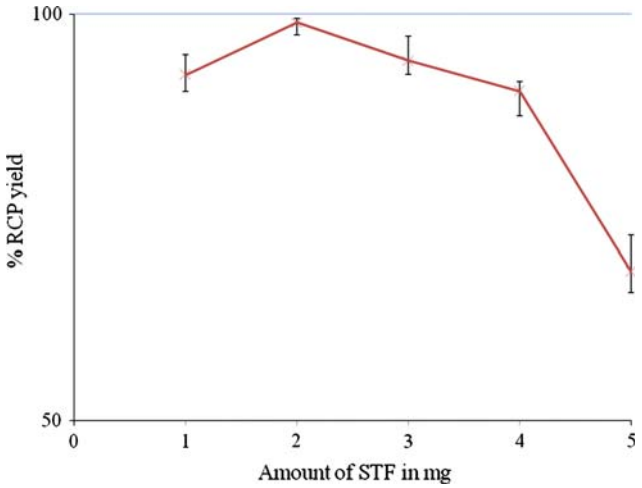

Fig. 4 Effect of STF amount on %RCP

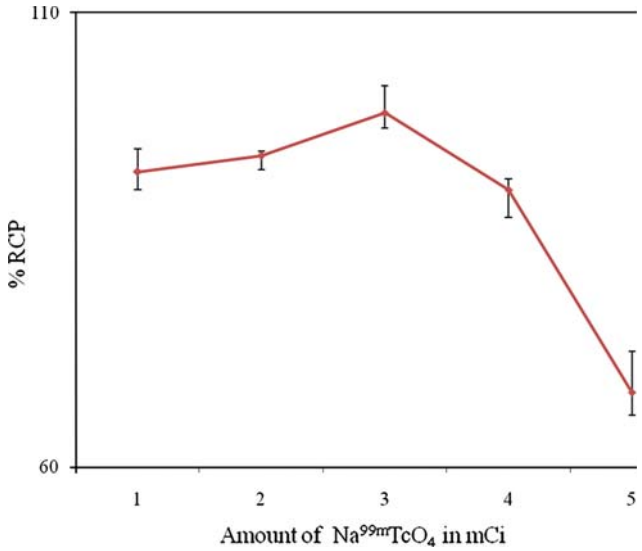

Fig. 5 Effect of $Na^{99m}TcO_4$ on %RCP

inflammation in contra-lateral thighs. Whole body static images of the infected rabbits were obtained at 30, 60 and 90 min after I.V. administration of the ^{99m}Tc-STF. The images are shown in Fig. 6a–c. Initially the activity was higher in the heart, liver and spleen of the rabbit as shown in Fig. 6a, and uniform distribution in the infected, inflamed, normal muscles and other organs were observed. Higher uptake in the infected muscle than inflamed and normal muscle was observed after 60 min as shown in Fig. 6b. However the scan after 60 min of the I.V. administration was blurred giving insignificant diagnostic information. The uptake in the infected muscle significantly increases after 90 min giving clear visibility of the infected region of the rabbit than the normal and inflamed portions as shown in Fig. 6c.

Table 2 % Absorbed dose of ^{99m}Tc-STF complex in various organ of rat (mean ± SD)

Organ/tissue	% ID/g after reconstitution[a]		
	30 min	1.00 h	2.00 h
Infected muscle	7.50 ± 0.10	16.25 ± 0.15	18.50 ± 0.00
Inflamed muscle	1.25 ± 0.15	1.20 ± 0.20	0.90 ± 0.10
Normal muscle	1.10 ± 0.25	0.95 ± 0.25	0.80 ± 0.20
Liver	20.50 ± 0.50	13.50 ± 0.15	5.50 ± 0.00
Kidney	10.50 ± 0.50	22.50 ± 0.25	27.25 ± 0.22
Blood	22.60 ± 0.25	6.50 ± 0.15	2.50 ± 0.20
T/NT[b]	1.14 ± 0.60	1.26 ± 0.80	1.13 ± 0.50
T/NT[c]	6.82 ± 0.40	17.11 ± 0.60	23.13 ± .100

[a] Percent absorbed dose per gram

[b] Inflamed/normal muscle ratio

[c] Infected/normal muscle ratio

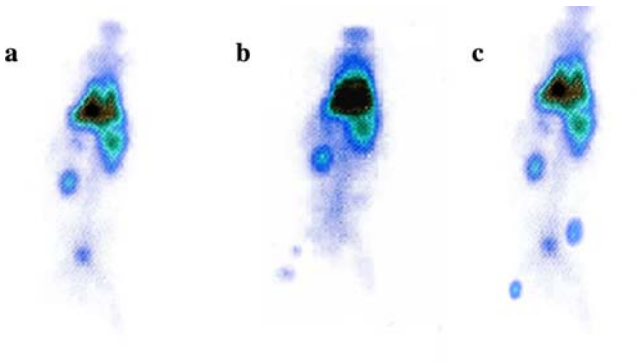

Fig. 6 **a–c** Whole body static images of the infected rabbit at 30, 60, and 90 min of I.V. administration of 74 MBq of ^{99m}Tc-STF complex

Conclusion

The ^{99m}Tc-STF complex showed stability up to 98.96 ± 0.10% and remained >90% tagged up to 2 h using 2 mg of STF, 3 mCi $Na^{99m}TcO_4$, 150 µL $SnCl_2{\cdot}2H_2O$ at pH 5.5. The labeled complex under similar conditions showed maximum uptake in the infected muscle from 7.50 ± 0.10, 16.25 ± 0.15 to 18.50 ± 0.20 within 2 h after I.V. administration to the rats. Target to non-target organ ratio of the absorbed dose incase of infected/normal muscle was 6.82 ± 0.40, 17.11 ± 0.60, and 23.13 ± 1.00 at 30, 60 and 90 min of administration. The scintigraphic accuracy of the ^{99m}Tc-STF complex is significantly high, clearly showing the uptake in the infected region. The ^{99m}Tc-STF complex is recommended for imaging the infectious foci at early stage.

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