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Preparation, quality control and stability of ^{99m}Tc-sparafloxacin complex, a novel agent for detecting sites of infection

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Labeling of sparafloxacin with technetium-99m using stannous chloride as a reducing agent was investigated. Dependence of the yield of ^{99m}Tc-sparafloxacin complex on the concentration of sparafloxacin, reducing agent, pH and reaction time was studied. Under optimum conditions, the labeling yield of ^{99m}Tc-sparafloxacin complex (95%) was achieved by using 2.5 mg of sparafloxacin, 50 μg of Sn(II), pH 10 and 30-min reaction time. ^{99m}Tc-sparafloxacin complex was stable for 3 h after labeling, then the yield decreased gradually to 81.9% at 6 h. Biodistribution studies in rats were carried out in experimentally induced infection in the left thigh using *Staphylococcus aureus*. The ratios of bacterial infected thigh/contralateral thigh were then evaluated. The time for the maximum accumulation of ^{99m}Tc-sparafloxacin at the site of the infection was 30 min after the administration followed by gradual decline. The abscess-to-muscle ratio for ^{99m}Tc-sparafloxacin was 5.9 ± 0.7, while that for the commercially available ^{99m}Tc-ciprofloxacin was 3.8 ± 0.5 under the same experimental paradigm, indicating that ^{99m}Tc-sparafloxacin could be used for infection imaging.

Keywords: sparafloxacin; fluoroquinolone; technetium-99m; labeling; infection

Introduction

Bacterial infections are still one of the major causes of morbidity and mortality in developing countries. The most reliable method of establishing infection is to analyze microbiological samples of the lesion. Conventional anatomical imaging methods such as plain radiography, ultrasonography, computed tomography and magnetic resonance imaging are seen as effective means of infection localization. It is well known that these are not the best of methods for the localization of infection at early stages. These procedures detect the morphologic alterations of the tissues after abscess formation.[1]

The radiopharmaceuticals routinely used for scintigraphic inflammation detection include ^{67}Ga-citrate,[2,3] ^{99m}Tc or ^{111}In-labeled leukocytes,[4] ^{99m}Tc-nano-colloid,[5] ^{99m}Tc or ^{111}In-labeled human polyclonal immunoglobulin (HIG)[6,7] and ^{99m}Tc-ubiquicidin 29-41.[8–10] However, none of the preparations is capable of distinguishing between infections and inflammatory lesions in a clinically useful manner.[11]

The use of radiolabeled antibiotics presents a promising approach for the precise diagnosis and detection of infectious lesions, because they specifically bind to the bacterial component, making it possible to differentiate between infectious and sterile lesions.[1,12]

One of the most important radiopharmaceuticals that is now currently available for imaging infection is the antimicrobial agent ciprofloxacin labeled with ^{99m}Tc, which has probably shown the best results. However, previously reported data about the specificity of ^{99m}Tc-ciprofloxacin for infection are contradictory.[13–19] ^{99m}Tc-ciprofloxacin preparation has some disadvantages related to radiochemical purity (81% ± 4)[20] and stability, which are discussed in details in the literature.[1,13,20–24] Therefore, other antimicrobial agents such as levofloxacin,[25] pefloxacin,[26] lomefloxacin,[27] cefoprazone[28] and cefuroxime[29] were labeled with ^{99m}Tc to be used for imaging sites of infection and to overcome the drawback of ^{99m}Tc-ciprofloxacin.

Sparafloxacin is a fluoroquinolone derivative with a broad spectrum of activity against the majority of aerobic and anaerobic gram-positive and gram-negative pathogenic bacteria (Figure 1). In this paper, sparafloxacin was labeled with the most widely used imaging radionuclide, ^{99m}Tc. Factors affecting the labeling yield of ^{99m}Tc-sparafloxacin complex and biological distribution in inflammation bearing animals were studied in detail.

Results and discussion

Radiochemical purity and stability of ^{99m}Tc-sparafloxacin complex were assessed by thin layer chromatographic method and reversed-phase high-performance liquid chromatography (HPLC). In thin layer chromatography using acetone as the solvent, free $^{99m}TcO_4^-$ moved with the solvent front ($R_f = 1$), while ^{99m}Tc-sparafloxacin and reduced hydrolyzed technetium remained at the point of spotting. Reduced hydrolyzed technetium was determined by using ethanol:water:ammonium hydroxide mixture (2:5:1) or 5 N NaOH as the mobile phase, where reduced hydrolyzed technetium remains at the origin ($R_f = 0$) while other species migrate with the solvent front ($R_f = 1$). The radiochemical purity was determined by subtracting the

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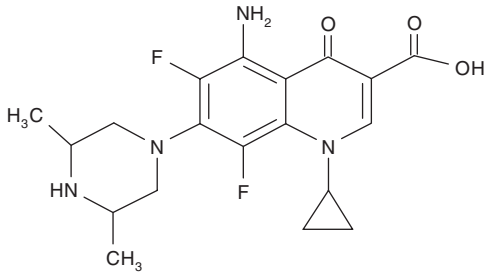

Figure 1. Chemical structure of sparafloxacin.

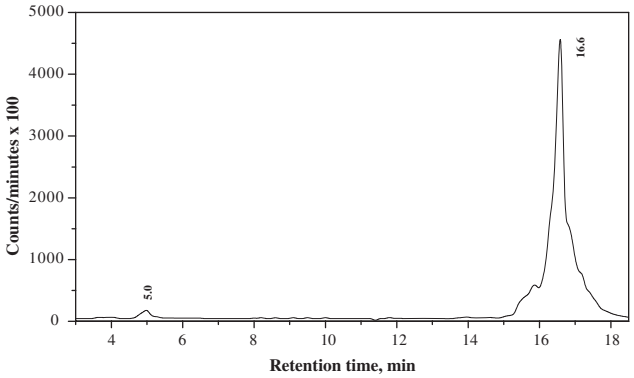

Figure 2. HPLC radiochromatogram of ^{99m}Tc-sparafloxacin complex.

sum of the percent of colloid and free pertechnetate from 100%. The radiochemical yield is the mean value of three experiments. An HPLC radiochromatogram is presented in Figure 2 and showed two peaks, one at fraction No. 5, which corresponds to $^{99m}TcO_4^-$, while the second peak was collected at fraction No. 16.6, which corresponds to ^{99m}Tc-sparafloxacin, which was found to coincide with the UV signal.

Effect of sparafloxacin amount

The labeling yield of ^{99m}Tc-sparafloxacin complex increased with increasing the amount of sparafloxacin from 33.4% at 1 mg of sparafloxacin till reaching the maximum value of 95% at 2.5 mg of sparafloxacin. When the sparafloxacin amount increased above 2.5 mg, the formed complex slightly decreased till it became 91.9% at 3.5 mg.

Effect of Sn(II) content

As shown in Figure 3, at a low amount of Sn(II), the radiochemical yield of ^{99m}Tc-sparafloxacin complex was low (75.5% at 25 μg) with the appearance of free pertechnetate (24.4%) due to insufficient Sn(II) to reduce all pertechnetate present in the reaction mixture. Increasing the amount of Sn(II) to 50 μg led to an increase in the labeling yield to 95%. By increasing the amount of reducing agent above 50 μg, the labeling yield decreased again to 47.6% due to colloid formation (50.9%).

Effect of pH of the reaction mixture

As shown in Figure 4, at pH 1, the labeling yield of ^{99m}Tc-sparafloxacin complex was 43% and the yield increased with increasing the pH of the reaction mixture to a maximum yield of 95% at pH 10. By increasing the pH greater than 10, the labeling yield decreased again to 66% at pH 11.

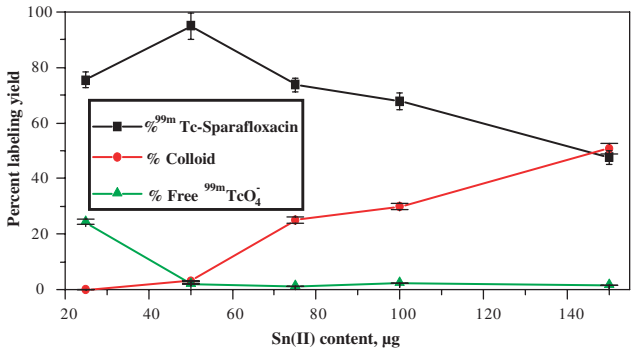

Figure 3. Effect of stannous chloride dihydrate amount on the percent labeling yield of ^{99m}Tc-sparafloxacin; reaction conditions: 2.5 mg of sparafloxacin, *x* μg of $SnCl_2 \cdot 2H_2O$, 0.5 ml (~500 MBq) of $^{99m}TcO_4^-$ at pH 10. This figure is available in color online at www.interscience.wiley.com/journal/jlcr.

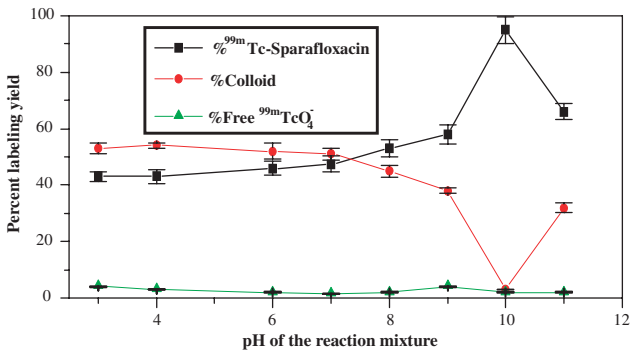

Figure 4. Effect of pH on the labeling yield of ^{99m}Tc-sparafloxacin; reaction conditions: 2.5 mg of sparafloxacin, 50 μg of $SnCl_2 \cdot 2H_2O$, 0.5 ml (~500 MBq) of $^{99m}TcO_4^-$ at pH = *x*. This figure is available in color online at www.interscience.wiley.com/journal/jlcr.

Effect of reaction time

The labeling of sparafloxacin with technetium-99m was done at room temperature and carried out at different intervals of time. The labeling reaction was completed after 30 min with a radiochemical yield of 95%. The formed complex was stable for a time up to 3 h, after that the yield decreased again till it reached 81.9% at 6 h.

Stability test

Incubation of the preparation containing ^{99m}Tc-sparafloxacin in normal serum for 24 h at 37°C resulted in a small release of radioactivity (20.5 ± 2.7%, $n = 5$ experiments) from the ^{99m}Tc-sparafloxacin, as determined by ITLC.

In vitro binding studies

In vitro binding of ^{99m}Tc-sparafloxacin to bacteria was similar to ^{99m}Tc-ciprofloxacin[22] where the binding of ^{99m}Tc-sparafloxacin was in the range of 40–50% ($n = 4$), while the binding of ^{99m}Tc-ciprofloxacin ranged from 40 to 65% ($n = 4$). Varying amounts of sparafloxacin added (10–100-fold) showed similar efficiency with bacteria.

Biodistribution in animals

Table 1 gives the biodistribution of ^{99m}Tc-sparafloxacin in important body organs and fluids. ^{99m}Tc-sparafloxacin was removed from the circulation mainly through the kidneys and urine (approximately 37.8% injected dose, ID, at 24 h after injection of the tracer). The liver

Table 1. Biodistribution of ^{99m}Tc-sparafloxacin complex

Organs and body fluid	% ID/organ and body fluid at different postinjection times				
	15 min	30 min	120 min	240 min	24 h
Liver	16.1 ± 0.6	20.9 ± 2.1	19.2 ± 1.1	18.3 ± 2.3	7.60 ± 1.0
Urine	5.10 ± 0.2	8.60 ± 2.1	18.1 ± 1.1	23.0 ± 2.3	31.5 ± 3.0
Kidneys	4.50 ± 0.1	5.20 ± 0.1	12.2 ± 1.1	10.2 ± 0.3	6.30 ± 1.0
Blood	26.1 ± 0.9	25.9 ± 1.1	5.20 ± 0.1	4.30 ± 0.3	1.60 ± 1.0
Heart	0.3	0.40 ± 0.1	0.3	0.09	0.2
Lung	0.70 ± 0.1	0.90 ± 0.1	1.20 ± 0.1	0.6	0.4
Intestine and stomach	6.6 ± 0.6	8.20 ± 0.6	13.2 ± 1.9	5.3 ± 0.3	3.70 ± 1.0
Spleen	0.5	0.90 ± 0.1	2.20 ± 0.1	1.30 ± 2.3	0.6
Bone	1.10 ± 0.1	0.9	1.0	0.6	0.3
Inflamed muscle	1.9 ± 0.2	2.72 ± 0.6	2.30 ± 1.1	1.7 ± 0.6	1.10 ± 0.3
Control muscle	0.44 ± 0.1	0.46 ± 0.1	0.45 ± 0.2	0.43 ± 0.1	0.32
T/NT	4.30 ± 0.5	5.90 ± 0.7	5.10 ± 0.4	4.00 ± 0.6	3.4 ± 0.4

Mean ± SD (mean of five experiments).

Table 2. Biodistribution of ^{99m}Tc-ciprofloxacin complex

Organs and body fluid	% ID/organ and body fluid at different postinjection times				
	15 min	30 min	120 min	240 min	24 h
Liver	5.20 ± 0.2	10.1 ± 0.7	7.60 ± 0.8	6.6 ± 1.4	4.6 ± 0.5
Urine	9.90 ± 0.6	17.3 ± 2.4	21.7 ± 1.2	28.3 ± 3.1	36.4 ± 3.9
Kidneys	10.3 ± 1.2	15.4 ± 0.9	19.6 ± 1.1	16.10 ± 0.7	11.3 ± 0.8
Blood	21.3 ± 0.9	4.90 ± 1.2	4.10 ± 0.2	0.90 ± 0.3	0.8
Heart	0.3	0.30 ± 0.1	0.11	0.23 ± 0.1	0.21 ± 0.1
Lung	1.10 ± 0.1	1.80 ± 0.2	0.90 ± 0.2	0.30	0.30 ± 0.05
Intestine and stomach	7.50 ± 0.5	11.0 ± 1.8	5.60 ± 1.0	3.70 ± 0.3	7.50 ± 0.5
Spleen	0.50 ± 0.1	2.60 ± 0.1	1.30 ± 0.1	0.70 ± 0.09	0.5
Bone	1.40 ± 0.1	2.10 ± 0.3	2.00 ± 0.2	1.0	0.9 ± 0.2
Inflamed muscle	1.54 ± 0.3	1.71 ± 0.3	1.44 ± 0.1	1.18 ± 0.3	0.53 ± 0.1
Control muscle	0.44 ± 0.1	0.45 ± 0.1	0.40 ± 0.1	0.38 ± 0.1	0.28
T/NT	3.50 ± 0.1	3.8 ± 0.5	3.60 ± 0.4	3.1 ± 0.4	1.9 ± 0.2

Mean ± SD (mean of five experiments).

uptake decreased markedly with time, which decreased from 20.9% at 30 min till it reached 7.6 at 24 h. Rats with infectious lesions injected with ^{99m}Tc-sparafloxacin showed a mean abscess-to-muscle (target-to-non-target, *T*/NT) ratio equal to 5.9 ± 0.7, which is greater than that of ^{99m}Tc-ciprofloxacin (*T*/NT = 3.8 ± 0.5, Table 2) under the same experimental conditions. The accumulation of activity at the site of infection was maximized at 30 min after intravenous injection, then slightly decreased with time until *T*/NT equal to 4.0 ± 0.6 at 4-h postinjection.

Experimental

Sparafloxacin was purchased from Sigma-Aldrich Chemical Company, USA, and all other chemicals were purchased from Merck and they were of analytical grade.

Method

Labeling of sparafloxacin

Accurately weighed 2.5 mg of sparafloxacin was transferred to an evacuated penicillin vial. Exactly 50 μg of $SnCl_2$ solution was added and the pH of the mixture was adjusted to 10 using 0.1 N HCl; then the volume of the mixture was adjusted to 1 ml by N_2-purged distilled water. One milliliter of freshly eluted $^{99m}TcO_4^-$ (400 MBq) was added to the above mixture. The reaction mixture was vigorously shaken and allowed to react at room temperature for sufficient time to complete the reaction.

Quality control

Radiochemical yield of ^{99m}Tc-sparafloxacin was checked by thin layer chromatographic method using strips of silica gel impregnated glass fiber sheets (ITLC-SG). Free $^{99m}TcO_4^-$ in the preparation was determined using acetone as the mobile phase. Reduced hydrolyzed technetium was determined by using ethanol:water:ammonium hydroxide mixture (2:5:1) or 5 N NaOH as the mobile phase.

An HPLC analysis of sparafloxacin solution was done by the injection of 10 μl from the reaction mixture into the column (RP-18-250 × 4 mm^2, 5 μm, Lischrosorb) built-in HPLC Shimadzu model, which consists of pumps LC-9A, Rheohydron injector and UV spectrophotometer detector (SPD-6A) adjusted to the 320 nm wavelength. The column was eluted with 10% ethanol

in 0.2 M phosphate buffer pH 7.2 and the flow rate was adjusted to 0.5 ml/min. Then fractions of 0.5 ml were collected separately using a fraction collector up to 19 ml and counted in a well-type γ-scintillation counter.

Stability of ^{99m}Tc-sparafloxacin in human serum

Stability of ^{99m}Tc-sparafloxacin was studied *in vitro* by mixing 1.8 ml of normal human serum and 0.2 ml of ^{99m}Tc-sparafloxacin and incubated at 37°C for 24 h. Exactly 0.2 ml aliquots were withdrawn during the incubation at different time intervals up to 24 h and subjected to ITLC for the determination of the percent of ^{99m}Tc-sparafloxacin, reduced hydrolyzed technetium and free pertechnetate.

In vitro binding of ^{99m}Tc-sparafloxacin to bacteria

Binding of ^{99m}Tc-sparafloxacin to *Staphylococcus aureus* bacteria was assessed by the method described elsewhere.[30] Briefly, 0.1 ml of sodium phosphate buffer containing about 5 MBq of ^{99m}Tc-sparafloxacin was transferred to a test tube. Exactly 0.8 ml of 50% (v/v) of 0.01 M acetic acid in phosphate buffer containing approximately 1×10^8 viable bacteria was added. The mixture was incubated for 1 h at 4°C and then centrifuged for 5 min at 2000 rpm at 4°C. Simultaneously, incubation was performed in the presence of an excess of unlabeled sparafloxacin (10-, 50-, 100-fold). The supernatant was removed and the bacterial pellet was gently resuspended in 1 ml of ice-cooled phosphate buffer and recentrifuged. The supernatant was removed and the radioactivity in the bacterial pellet was determined by a γ-counter. The radioactivity related to bacteria was expressed in percent of the added ^{99m}Tc activity bound to viable bacteria in regard to the total ^{99m}Tc activity.

Biodistribution studies in animals

The biodistribution of the ^{99m}Tc-sparafloxacin complex was evaluated in male Sprague–Dawley rats (body mass 130–160 g). To induce the inflammation, approximately 10^5–10^6 colony forming units of *S. aureus* suspended in 0.2 ml of saline were administrated into the left thigh. For the quantitative determination of organ distribution, five rats were used for each experiment and 0.1 ml of about 18 MBq of ^{99m}Tc-sparafloxacin and ^{99m}Tc-ciprofloxacin solution was injected into the tail vein of rats after 24 h of bacterial induction. Then the rats were killed and blood was obtained by cardiac puncture. Samples of fresh blood, bone and muscle were collected in pre-weighed vials and counted. The different organs were removed, counted and compared with a standard solution of the labeled sparafloxacin. The average percent values of the administrated dose/organ were calculated. Blood, bone and muscles were assumed to be 7, 10 and 40%, respectively, of the total body weight.[31] Corrections were made for background radiation and physical decay during experiment. Both target and non-target thighs were dissected and counted. Target and non-target thigh radioactivity ratio was also determined.

Conclusion

Sparafloxacin was labeled with ^{99m}Tc by direct labeling method at room temperature with a high labeling yield. A comparative biodistribution study for both ^{99m}Tc-sparafloxacin and ^{99m}Tc-ciprofloxacin, performed in infected rats under the same experimental conditions, demonstrated high and rapid accumulation of ^{99m}Tc-sparafloxacin at the site of infection compared with ^{99m}Tc-ciprofloxacin. The results indicated also that the new ^{99m}Tc-sparafloxacin is rapidly cleared from the body through the urinary pathway.

These findings, combined with the advantage of the high labeling yield of ^{99m}Tc-sparafloxacin, were promising enough to encourage clinical investigation of this new Tc-99m agent for detecting sites of infection.

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