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Radiochemical and biological characteristics of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin for detecting sites of infection

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The optimization of the radiolabeling yield of ciprofloxacin analogous, difloxacin and pefloxacin, with ^{99m}Tc was described. At pH 4, difloxacin was labeled with ^{99m}Tc with a labeling yield of 95.6% by adding ^{99m}Tc to 5 mg difloxacin in the presence of 100 μg $SnCl_2 \cdot 2H_2O$ whereas ^{99m}Tc-pefloxacin was labeled (98.1%) by adding ^{99m}Tc to 4 mg pefloxacin in the presence of 50 μg $SnCl_2 \cdot 2H_2O$. The radiochemical purity for both labeled compounds was evaluated with ITLC and HPLC system. Biological distribution of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin was carried out in experimentally induced infection rats, in the left thigh, using *Staphylococcus aureus*. Both thighs of the rats were dissected and counted and the ratio of bacterial infected thigh/contralateral thigh was then evaluated. T/NT for both ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin was found to be 5.5 ± 0.5 and 4.9 ± 0.3, respectively, which was higher than that of the commercially available ^{99m}Tc-ciprofloxacin.

Keywords: difloxacin; pefloxacin; ciprofloxacin; technetium-99m; infection

Introduction

Inflammation diseases (infections and non-infections) are a major problem in the most recent decades. Infection is a major cause of mortality and morbidity in the world. Internal infections resulting in delayed diagnosis, treatment and sometimes death, were difficult to detect in the early stages. Depending on the disease, the sensitivity and specificity of different diagnostic techniques may also vary and according to the pathophysiology operating in the individual disease, different imaging techniques will show different diagnostic accuracies. Thus, the diagnosis of inflammatory processes often relies on the detection of anatomical/structural changes of the affected organ depending on the nature of the inflammation under investigation. One goal of different imaging techniques is to integrate the diagnostic information combining anatomical with functional data in order to describe and characterize site, extent and activity of the disease under investigation. The anatomical description and the spatial relationships of a lesion are well investigated by using radiological imaging procedures such as X-ray, Ultrasonography (US), Computed Tomography (CT) and Magnetic Resonance Imaging (MRI), whereas Nuclear Medicine provides ideal imaging techniques for the study of functional and histological changes in inflammatory processes. But it is well known that these are not the best of the methods for the localization of infection at the early stages. The early detection of the infectious focus by radionuclide imaging helps both patient and physician and reduces the length and cost of hospitalization. The radiopharmaceuticals routinely used for scintigraphic detection include ^{67}Ga-citrate[1,2], ^{99m}Tc- or ^{111}In-labeled leukocytes[3], ^{99m}Tc-nano-colloid[4], ^{99m}Tc- or ^{111}In-labeled human polyclonal immunoglobulin (HIG)[5,6] and ^{99m}Tc-ubiquicidin 29–41.[7–9] Ciprofloxacin is a fluoroquinolone-like antibiotic; which binds to the DNA gyrase present in all dividing bacteria, even to those resistant. Since it binds only to living bacteria, it allows distinguishing among sterile inflammation and infection. ^{99m}Tc-labelled ciprofloxacin, registered as 'Infecton', has been successfully used in different infective diseases.[10] It has a favorable biodistribution: it is mainly excreted by the kidneys and shows low liver metabolism, consequently bowel uptake is seldom a problem.[11] The lack of bone marrow uptake is particularly useful for the detection of bone infections.[12] The major drawbacks of radiolabeled ciprofloxacin are related to radiochemical purity,[13–17] stability, its non-specific binding to DNA of eukaryotic cells[18] and the lack of bacterial uptake due to a rare mechanism of bacterial resistance.[19]

On the other hand, a non-specific ^{99m}Tc-human immunoglobulin (HIG)[20] and liposomes[21,22] accumulate at an infection/inflammatory site without distinction between bacterial infection and non-bacterial inflammation. The mechanism of non-specific localization is facilitated by locally enhanced vascular permeability.[20] Recently, several radiopharmaceuticals have been developed to differentiate between infection and sterile inflammation.[23–26] However, none of these are infection specific because the sensitivity and specificity can differ according to the type of micro-organism, infection, infection site and clinical conditions/response.

Difloxacin and pefloxacin are a fluoroquinolone derivatives with a broad spectrum of activity against the majority of aerobic

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and anaerobic gram positive and gram negative pathogenic bacteria.[27] The structure of difloxacin and pefloxacin were shown in Figure 1.

This work aims at studying the labeling conditions and biological distribution in inflammation bearing animals for ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin.

Results and discussion

Labeling of fluoroquinolone derivatives was not easy because the percent of colloid was relatively high (>20% at the optimum condition). Many of the previous works failed to calculate the percentage of colloid during quality control (study the labeling condition) because they use paper chromatography or TLC with only acetone or saline as a developing solvent which cannot separate labeled complex from colloid[28–31] so they use millipore filter or C_{18} cartridge for purification. In the present work, Reduced hydrolyzed technetium was determined by using ethanol: water: ammonium hydroxide mixture (2:5:1) as a developing solvent and labeling conditions were studied in details and the percent of $^{99m}TcO_4^-$, colloid and labeled complex were calculated to attain the optimum condition that give maximum yield.

Radiochemical purity and stability of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin complexes 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 ($R_f = 1$), whereas ^{99m}Tc-difloxacin or ^{99m}Tc-pefloxacin and reduced hydrolyzed technetium remained at the origin. Reduced hydrolyzed technetium was determined by using ethanol: water: ammonium hydroxide mixture (2:5:1) 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 sum of the percent of colloid and free pertechnetate from 100%. The radiochemical yield is the mean value of three experiments.

An HPLC radiochromatogram was 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.14.2 for ^{99m}Tc-pefloxacin and fraction No.16.3 for ^{99m}Tc-difloxacin which was found to coincide with the UV signal.

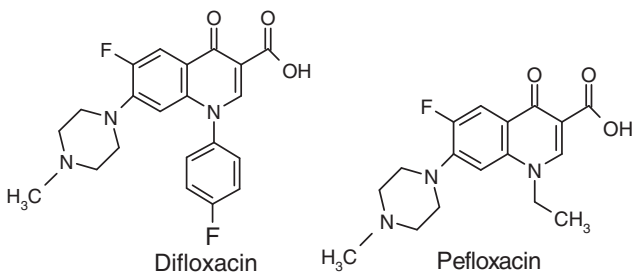

Figure 1. The chemical structure of difloxacin and pefloxacin.

Effect of ligand concentration

As shown in Figure 3, the labeling yield of ^{99m}Tc-difloxacin (35.3%) and ^{99m}Tc-pefloxacin (64.1%) was low at low ligand concentration (1 mg) and these low labeling yields were due to the ligand concentrations being insufficient to form the complex with all of the reduced technetium-99 m while the percent of colloid was high (63.5 and 33.2%, respectively). Increasing the ligand concentration led to higher labeling yield and the maximum yield was achieved at 5 and 4 mg, respectively. By increasing the ligand concentration over the optimum values, the labeling yield was remained stable.

Effect of $SnCl_2 \cdot 2H_2O$ concentration

The effect of the amount of stannous chloride was summarized in Figure 4. The data show that the radiochemical yield was dependent on the amount of $SnCl_2 \cdot 2H_2O$ present in the reaction mixture. At 10 μg $SnCl_2 \cdot 2H_2O$, the labeling yield of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin was 73.4 and 86.5%, respectively, due to $SnCl_2 \cdot 2H_2O$ concentration was insufficient to reduce all pertechnetate so the percentage of $^{99m}TcO_4^-$ was relatively high (14.9 and 4.3%, respectively). The labeling yield was significantly increased by increasing the amount of $SnCl_2 \cdot 2H_2O$ from 10 to 100 and 50 μg (optimum content), at which maximum labeling yield of 95.6 and 98.1%, respectively was obtained. By increasing the amount of $SnCl_2 \cdot 2H_2O$ above the optimum concentration value, the labeling yield decreased because excess $SnCl_2 \cdot 2H_2O$ was converted to colloid (89.6 and 79.4% at 150 μg $SnCl_2$).

Effect of pH

Radiochemical yield of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin was affected by changes in pH as graphically illustrated in Figure 5. The maximum yield was obtained at pH 4. At pH 2, the radiochemical yield was relatively low (73.7 and 80.2%,

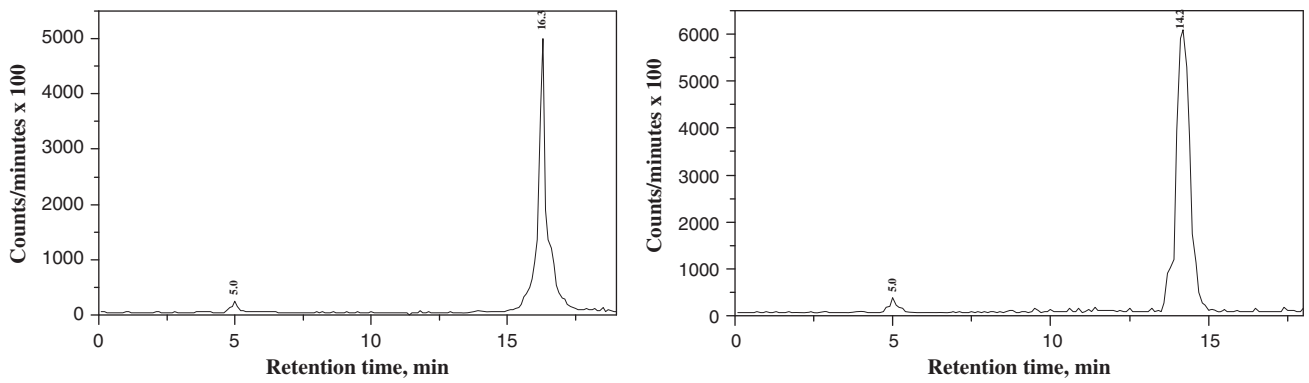

Figure 2. HPLC radiochromatogram of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin.

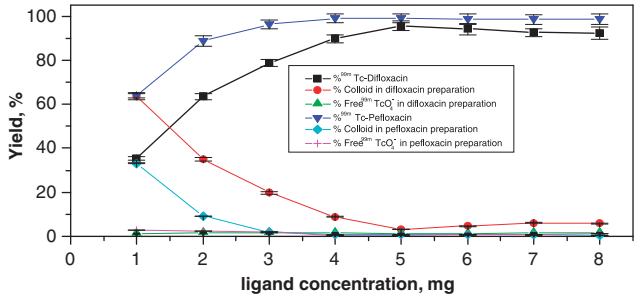

Figure 3. Percent labeling yield of difloxacin and pefloxacin with ^{99m}Tc as a function of ligand concentration; reaction conditions: *x* mg difloxacin and pefloxacin, 50 or 100 μg of $SnCl_2 \cdot 2H_2O$, 0.5 ml (~400 MBq) of $^{99m}TcO_4^-$ at pH 4, the reaction mixture was kept at room temperature for 30 min.

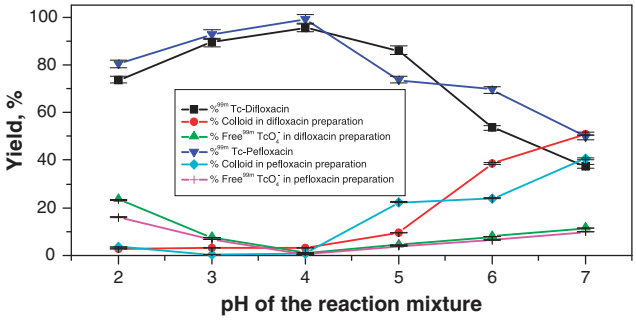

Figure 5. Effect of pH on the labeling yield of difloxacin and pefloxacin with ^{99m}Tc; reaction conditions: 5 mg difloxacin and 4 mg pefloxacin, 50 or 100 μg of $SnCl_2 \cdot 2H_2O$, 0.5 ml (~400 MBq) of $^{99m}TcO_4^-$ at pH = *x*, the reaction mixture was kept at room temperature for 30 min.

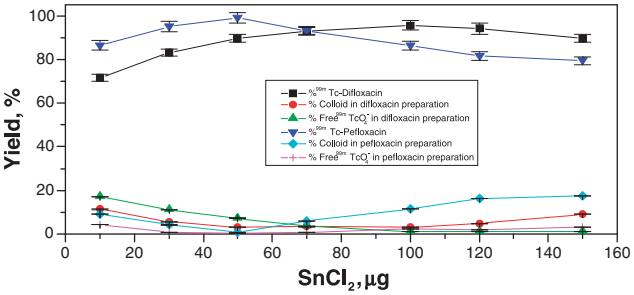

Figure 4. Effect of $SnCl_2 \cdot 2H_2O$ concentration on the labeling yield of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin; reaction conditions: 5 mg difloxacin and 4 mg pefloxacin, *x* μg of $SnCl_2 \cdot 2H_2O$, 0.5 ml (~400 MBq) of $^{99m}TcO_4^-$ at pH 4, the reaction mixture was kept at room temperature for 30 min.

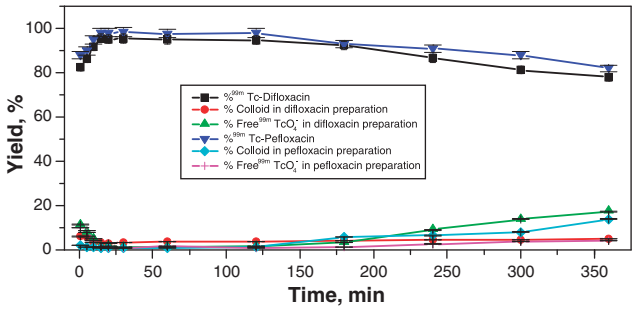

Figure 6. ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin yields vs reaction time; reaction conditions: 5 mg difloxacin and 4 mg pefloxacin, 50 or 100 μg of $SnCl_2 \cdot 2H_2O$, 0.5 ml (~400 MBq) of $^{99m}TcO_4^-$ at pH 4, the reaction mixture was kept at room temperature for different intervals of time.

respectively) with the appearance of free pertechnetate as predominant species compared to 37.5 and 49.7%, respectively, at pH 7 with the formation of colloids.

Effect of reaction time

Figure 6 describe the effect of incubation time on the radiochemical purity of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin complexes. At 1 min post-labeling, the radiochemical purity was low and equal to 82.5 and 88%, respectively, which increased with time till reaching its maximum value of 95.6 and 98.1%, respectively, at 15 min. The radiochemical purity remains stable for time up to 2 h after that the yield decreased again.

Stability test

As shown in Figure 7, incubation of the preparation containing ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin in normal serum for 24 h at 37°C resulted in a small release of radioactivity (13.7 ± 1.6% and 15.7 ± 1.3%, respectively; $n = 5$ experiments) from the ^{99m}Tc-complex, as determined by ITLC.

In vitro binding studies

In vitro binding studies revealed that, the binding of ^{99m}Tc-pefloxacin to *S. aureus* bacteria was slightly less than that of ^{99m}Tc-difloxacin (45–55%) whereas the binding of ^{99m}Tc-difloxacin to these bacteria was comparable to that of ^{99m}Tc-ciprofloxacin, a promising agent for the diagnosis of bacterial infection,[17,32] ranged from 47 to 59% (Figure 8). Also, no significant differences

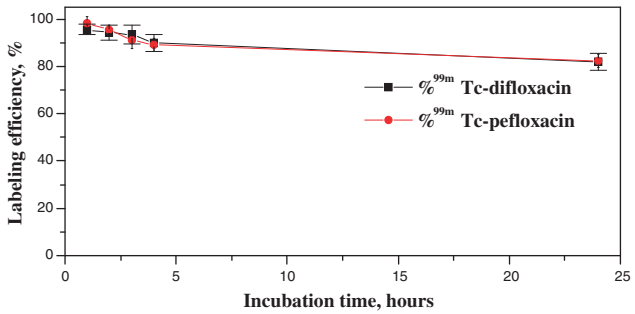

Figure 7. *In vitro* stability of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin in normal serum.

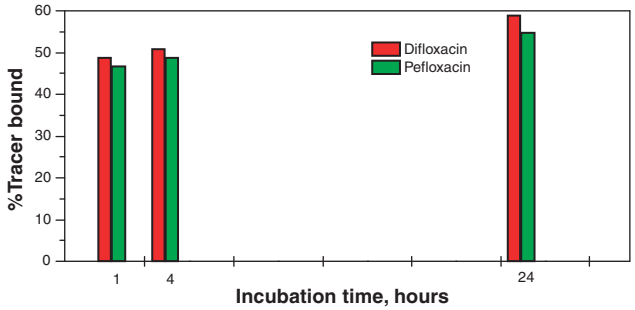

Figure 8. *In vitro* binding of the ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin to *Staphylococcus aureus* ($n = 4$).

were found for binding to *S. aureus* and heat-killed *S. aureus* bacteria with either compounds. Binding percentage was not affected by incubation of the microorganisms with 50 fold excess unlabeled difloxacin and pefloxacin indicating that ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin are not bound specifically to *S. aureus*.

Biodistribution

Data in Tables 1 and 2 revealed that, after 24 h of tracer administration the major part of activity of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin was found in both kidneys (7.6 ± 0.4% and 6.7 ± 0.3% ID, respectively) and urinary bladder (30.9 ± 3.3% and 28.3 ± 3.1% ID, respectively). In contrast, we found significant large amount of ^{99m}Tc-pefloxacin activity in the liver (13.6 ± 1.3% ID) compared to the amount of ^{99m}Tc-difloxacin (6.4 ± 0.3% ID). Rats with infectious lesions injected with ^{99m}Tc-difloxacin showed a mean abscess-to-muscle (target to non-target, T/NT) ratio equal to 5.5 ± 0.5 but for ^{99m}Tc-pefloxacin was 4.9 ± 0.3 where these two ^{99m}Tc-fluoroquinolones showed higher uptake in infected tissue than the commercially available ^{99m}Tc-ciprofloxacin (T/NT = 3.8 ± 0.8).[16] The above mentioned results showed that, no significant difference in the percent uptake of the two ^{99m}Tc-fluoroquinolones in the *S. aureus*, heat-killed *S. aureus* and turpentine oil inflamed muscle (Figure 9), which indicated that both ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin are non-specific agents and could not distinguish bacterial infection from sterile inflammation.

Experimental

Difloxacin was purchased from Abbott Laboratories (North Chicago, USA) and pefloxacin was purchased from Rhone-Poulenc Rorer (Neuilly/Seine, France) and all other chemicals were purchased from Merck and they were reactive grade.

Labeling procedure

Accurately weighed 5 μg difloxacin or 4 mg pefloxacin was transferred to a penicillin vial, then the vial was evacuated. Exactly 100 and 50 μg $SnCl_2 \cdot 2H_2O$ solution, respectively, were added and the pH of the mixture was adjusted to 4 using 0.1 mol/L HCl, then the volume of the mixture was completed to 1 ml by N_2-purged distilled water. One millilitre 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 required to complete the reaction.

Analysis

Radiochemical purity of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin was performed 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) as the mobile phase. It was further confirmed by a Shimadzu HPLC system, which consists of pumps LC-9A, Rheodyne injector and UV spectrophotometer detector (SPD-6A) operated at a wavelength of 320 nm. Chromatographic analysis was performed by injection of 10 μl from the reaction mixture of difloxacin and pefloxacin into a reversed-phase column (Lichrosorb RP18, 4 mm × 250 μm; 5 mm). The column

Table 1. Biodistribution of ^{99m}Tc-difloxacin in *Staphylococcus aureus*, heat-killed *Staphylococcus aureus* and turpentine oil inflamed rats at different time intervals

Organs and body fluids	% injected dose/organs at different time intervals (h)									
	S. aureus			Heat-killed *S. aureus*			Turpentine oil			
	2	4	24	2	4	24	2	4	24
Inflamed muscle	0.99 ± 0.2	0.71 ± 0.11	0.26 ± 0.06	1.2 ± 0.1	0.78 ± 0.2	0.34 ± 0.1	1.1 ± 0.0	0.69 ± 0.2	0.31 ± 0.1
Control muscle	0.18 ± 0.1	0.19 ± 0.2	0.09 ± 0.0	0.23 ± 0.0	0.25 ± 0.1	0.16 ± 0.0	0.22 ± 0.0	0.21 ± 0.0	0.15 ± 0.0
Liver	18.1 ± 3.2	13.9 ± 2.0	6.4 ± 0.3	19.6 ± 2.4	14.2 ± 2.2	6.9 ± 0.5	18.5 ± 3.0	14.1 ± 2.2	7.1 ± 0.6
Urine	19.5 ± 3.5	25.9 ± 4.2	30.9 ± 3.3	19.1 ± 1.4	26.3 ± 2.1	31.4 ± 2.7	19.0 ± 0.9	26.4 ± 1.8	30.8 ± 2.4
Kidneys	12.2 ± 1.2	11.7 ± 1.3	7.6 ± 0.4	12.9 ± 1.3	12.2 ± 2.3	7.5 ± 0.7	12.3 ± 2.5	12.0 ± 1.2	6.9 ± 0.3
Blood	6.3 ± 0.4	4.1 ± 0.2	1.00 ± 0.0	7.10 ± 0.2	5.0 ± 0.2	1.3 ± 0.1	6.5 ± 0.2	4.3 ± 0.2	1.0 ± 0.0
Heart	0.4 ± 0.1	0.1 ± 0.0	0.09 ± 0.0	0.3 ± 0.09	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.08	0.2 ± 0.0	0.1 ± 0.0
Lung	1.3 ± 0.2	0.3 ± 0.0	0.1 ± 0.0	1.1 ± 0.09	0.2 ± 0.0	0.1 ± 0.0	1.4 ± 0.09	0.4 ± 0.1	0.2 ± 0.0
Intestine and stomach	20.9 ± 2.5	4.90 ± 0.5	3.10 ± 0.3	21.1 ± 3.4	5.7 ± 0.4	4.2 ± 0.7	19.9 ± 1.9	5.2 ± 0.8	3.4 ± 0.4
Spleen	2.20 ± 0.1	1.10 ± 0.2	0.3 ± 0.1	2.3 ± 0.3	1.3 ± 0.0	0.5 ± 0.1	2.0 ± 0.1	1.3 ± 0.0	0.3 ± 0.0
Bone	0.90 ± 0.1	0.50 ± 0.1	0.20 ± 0.0	1.1 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	1.1 ± 0.2	0.5 ± 0.1	0.1 ± 0.0

Table 2. Biodistribution of ^{99m}Tc-pefloxacin in *Staphylococcus aureus*, heat-killed *Staphylococcus aureus* and turpentine oil inflamed rats at different time intervals organs and body fluids % injected dose/organs at different time intervals (h)

Organs and body fluids	% injected dose/organs at different time intervals (h)									
	S. aureus			Heat-killed *S. aureus*			Turpentine oil			
	2	4	24	2	4	24	2	4	24
Inflamed muscle	1.59 ± 0.3	1.20 ± 0.1	0.90 ± 0.1	1.67 ± 0.1	1.25 ± 0.2	1.00 ± 0.1	1.4 ± 0.3	1.00 ± 0.1	0.89 ± 0.0
Control muscle	0.32 ± 0.1	0.31 ± 0.0	0.30 ± 0.0	0.34 ± 0.0	0.34 ± 0.1	0.30 ± 0.0	0.27 ± 0.0	0.27 ± 0.0	0.26 ± 0.0
Liver	30.6 ± 3.1	15.9 ± 1.4	13.6 ± 1.3	29.6 ± 2.3	14.2 ± 1.2	11.4 ± 1.1	30.5 ± 3.0	15.9 ± 2.0	14 ± 1.6
Urine	14.2 ± 2.4	19.3 ± 1.2	28.3 ± 3.1	15.3 ± 1.9	19.9 ± 2.1	30.3 ± 2.8	14.4 ± 1.3	18.8 ± 2.8	29.9 ± 3.0
Kidneys	10.1 ± 0.3	7.6 ± 1.1	6.7 ± 0.3	10.9 ± 0.3	8.20 ± 1.3	6.50 ± 0.2	11.3 ± 0.5	8.00 ± 0.2	6.90 ± 0.3
Blood	4.90 ± 1.2	4.30 ± 0.2	0.90 ± 0.1	5.10 ± 0.2	4.00 ± 0.2	1.30 ± 0.1	6.5 ± 0.1	4.3 ± 0.2	1.0 ± 0.0
Heart	0.3 ± 0.1	0.11 ± 0.0	0.10 ± 0.0	0.41 ± 0.1	0.20 ± 0.0	0.21 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
Lung	1.90 ± 0.2	0.90 ± 0.0	0.20 ± 0.0	1.70 ± 0.1	1.0 ± 0.0	0.2 ± 0.0	1.9 ± 0.1	1.1 ± 0.1	0.2 ± 0.0
Intestine and stomach	22.0 ± 1.8	5.60 ± 0.9	3.70 ± 0.3	21.6 ± 2.4	5.09 ± 0.7	4.20 ± 0.7	23.9 ± 2.8	7.3 ± 0.3	4.40 ± 0.2
Spleen	2.60 ± 0.1	1.30 ± 0.1	0.7 ± 0.1	2.80 ± 0.1	1.7 ± 0.0	0.9 ± 0.1	2.50 ± 0.2	1.50 ± 0.0	0.70 ± 0.0
Bone	2.10 ± 0.2	1.00 ± 0.0	1.00 ± 0.0	2.4 ± 0.1	1.3 ± 0.1	0.9 ± 0.0	2.0 ± 0.2	1.1 ± 0.1	0.9 ± 0.0

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-difloxacin and ^{99m}Tc-pefloxacin in serum

Stability of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin was studied *in vitro* by mixing 1.8 ml of normal serum and 0.2 ml of ^{99m}Tc-complex 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-complex, reduced hydrolyzed technetium and free pertechnetate.

In vitro binding of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin to bacteria

Binding of ^{99m}Tc-difloxacin and ^{99m}Tc-pefloxacin to both living and heat-killed *Staphylococcus aureus* bacteria was assessed by the method described elsewhere.[33] Briefly, 0.1 ml of sodium phosphate buffer containing about 5 MBq of ^{99m}Tc-complex was transferred to a test tube. Exactly, 0.8 ml of 50% (v/v) of 0.01 mol/L acetic acid in phosphate buffer containing approximately 1×10^8 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 difloxacin and pefloxacin (10, 20, 50 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 the percent of the added ^{99m}Tc activity bound to bacteria with regard to total ^{99m}Tc activity.

Biological evaluation

The study was approved by the animal ethics committee, Labeled Compound Department, and was in accordance with the guidelines set out by the Egyptian Atomic Energy Authority. Biodistribution of the two ^{99m}Tc-fluoroquinolones was evaluated in male Sprague-Dawley rats (body mass 140–180 g). To induce the inflammation, autoclaved turpentine oil, approximately 10^7–10^8 colony forming units of *S. aureus*, and heat killed *S. aureus* suspended in 0.2 ml of saline were administrated into the left thigh. For quantitative determination of organ distribution, five rats were used for each experiment and 0.1 ml of about 18 MBq of ^{99m}Tc-complex solution was injected into the tail vein of rats after 24 h of bacterial induction. Then the rats were sacrificed by the decapitation under chloroform anesthesia at 1/2, 2, 4 and 24 h post-injection. Blood sample was collected at the time of decapitation. Both thighs (left thigh muscle as target and right thigh muscle as control) and organs were dissected, weighed and their radioactivity was measured using a well-type NaI(Tl) detector connected with a single channel γ-counter (SR-7). The results were expressed as percent of the injected dose per organ or body fluid. Target and non-target thigh radioactivity ratio was also determined.[34] Differences in the data were evaluated with the Student *t* test. The Results for *p* using the 2-tailed test are reported and all the results are given as mean ± SEM. The level of significance was set at $p < 0.05$.

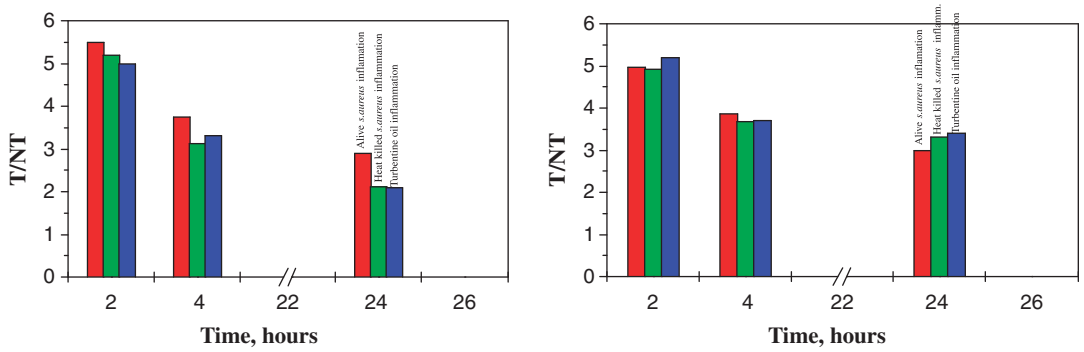

Figure 9. The ratio of target muscle (T) to non-target muscle (NT) of ^{99m}Tc-difloxacin (right) and ^{99m}Tc-pefloxacin (left) in different inflammation models at different post-injection times.

Conclusions

Difloxacin and pefloxacin are fluoroquinolone derivatives, which can be labeled easily with technetium-99 m using stannous chloride as a reducing agent. Both preparations give higher labeling yield than the commercially available ^{99m}Tc-ciprofloxacin. Biodistribution studies showed that, neither ^{99m}Tc-difloxacin nor ^{99m}Tc-pefloxacin was able to distinguish infection from sterile inflammation. This result was supported by data from *in vitro* binding, however, did not show preferential binding to live or heat killed *S. aureus*.

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