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Preparation and biodistribution of ^{99m}Tc-lomefloxacin and ^{99m}Tc-ofloxacin complexes

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This paper addresses the development of two new radiopharmaceuticals for infection imaging. The optimization of the labeling yield of ciprofloxacin analogous, lomefloxacin and ofloxacin, with ^{99m}Tc is described. ^{99m}Tc-lomefloxacin was obtained with a radiochemical yield of 93.6% by adding ^{99m}Tc to 2.5 mg lomefloxacin in the presence of 50 μg $SnCl_2$ while ^{99m}Tc-ofloxacin was obtained (96.6%) by adding ^{99m}Tc to 2 mg ofloxacin in the presence of 50 μg $SnCl_2$. Biodistribution studies in rats were carried out in experimentally induced infection 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. ^{99m}Tc-lomefloxacin showed higher uptake ($T/NT = 6.5\pm0.5$) in the infectious lesion than ^{99m}Tc-ofloxacin ($T/NT = 4.3\pm0.6$) and abscess-to-muscle ratios for both preparations were higher than that of ^{99m}Tc-ciprofloxacin ($T/NT = 3.8\pm0.8$), indicating that ^{99m}Tc-lomefloxacin could be used for infection imaging.

Introduction

Even in most recent decades, infection and inflammation remain a major cause of mortality and morbidity globally. Infections, especially internal infections, resulting in delayed diagnosis, treatment, and sometimes death, were difficult to detect in the early stages. Clinicians use a variety of clues, e.g., clinical, laboratory, and radiological tests, to give a good diagnosis of infection as early as possible. Several imaging methods like ultrasonography (US), computer tomography (CT) and magnetic resonance imaging (MRI) are available and have been used for the past several decades for the localization of infection. 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] These may take some time to become visible, may not always be present, and their resolution lags behind the cure of the infection. In addition, they are neither infection nor inflammation specific. The introduction of radiopharmaceuticals in nuclear medicine has enhanced infection imaging, because it depends on the demonstration of pathophysiological and pathobiological changes, which occur earlier in the infection process and also resolve quicker after cure of the infection compared with gross changes in structure. Clearly, radiopharmaceuticals that bind to a variety of bacteria would be better candidates for specific infection imaging.[2] The first to be proposed was ciprofloxacin radiolabeled with ^{99m}Tc, which is supposed to bind to DNA-gyrase and topoisomerase IV of bacteria, as does unlabeled ciprofloxacin.[3,4] However, previously reported data about the specificity of ^{99m}Tc-ciprofloxacin for infection are contradictory.[5–12] ^{99m}Tc-ciprofloxacin preparation has some disadvantages related to radiochemical purity (81%±4)[13] and stability which are discussed in details in the literature.[1,5,13–17] The structure of ciprofloxacin, lomefloxacin and ofloxacin are similar (Fig. 1). These compounds demonstrate a significant antibiotic effect for both gram-positive and gram-negative bacteria. Because of their structural similarity, we hypothesized that lomefloxacin and ofloxacin can be labeled with ^{99m}Tc like ciprofloxacin and may have a better characteristics than ^{99m}Tc-ciprofloxacin.

In the present study, the labeling conditions for ^{99m}Tc-lomefloxacin and ^{99m}Tc-ofloxacin were studied in detail and their biological distribution in inflammation bearing animals was studied.

Experimental

Lomefloxacin and ofloxacin were purchased from Sigma-Aldrich Chemical Company, USA, and all other chemicals were purchased from Merck and they were reactive grade.

Method

Labeling procedure: Accurately weighed 2.5 mg lomefloxacin or 2 mg ofloxacin was transferred to an evacuated penicillin vial. Exactly 50 μg $SnCl_2$ solution was added and the pH of the mixture was adjusted to 3.5–5 using 0.1N HCl, then the volume of the mixture was adjusted to one ml by N_2-purged distilled water. One ml 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.

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Analysis: The percent labeling yield was determined by using ascending paper chromatography. Paper strips of silica gel impregnated glass fiber sheets (ITLC-SG), 10×1.5 cm^2, were marked gently with a pencil at a distance of 2 cm from the lower end. A spot of the reaction mixture was applied at this line, then the strip was developed in an ascending manner in a closed jar filled with N_2 gas to prevent oxidation of the labeled complex. The developing solvents were acetone and ethanol:water:ammonium hydroxide mixture (2:5:1) purged with N_2 gas. After complete development, the strip was dried and counted in a well-type γ-scintillation counter. The organic solvent acetone was used to calculate the percentage of free $^{99m}TcO_4^-$ which moved with the solvent front (R_f = 1) leaving the labeled complex and colloid at the origin. Ethanol:water:ammonium hydroxide mixture (2:5:1) was used to check the amount of reduced hydrolyzed technetium which 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 % of colloid and free pertechnetate from 100%. The radiochemical yield is the mean value of three experiments.

Bio-distribution studies: The biodistribution of the two ^{99m}Tc-fluoroquinolones 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 *Staphylococcus aureus* suspended in 0.2 ml of saline was 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-fluoroquinolones solution was injected into the tail vein of rats after 24 hours of bacterial induction. Then the rats were killed and blood was obtained by cardiac puncture. Samples of infected muscle, contralateral normal muscle, blood, liver, spleen, lungs, kidney, stomach, intestine, bone and heart were dissected and weighed, and their activity was measured in a shielded well-type γ-scintillation counter. To correct for physical decay and to calculate uptake of the radiolabel in each tissue sample as a fraction of the injected dose, aliquots of the injected dose were counted simultaneously. The results were expressed as percentage injected dose per gram of tissue or organ (% ID/g).[18]

Results and discussion

Effect of substrate concentration

As shown in Fig. 2, at low substrate concentration (0.5 mg) the yield was small and equal to 33.3 and 63.9% for ^{99m}Tc-lomefloxacin and ^{99m}Tc-ofloxacin, respectively. These low labeling yields were due to the substrate concentrations being insufficient to form the complex with all of the reduced technetium while the percentage of colloid was high (66.7 and 34.9%, respectively). Increasing the substrate concentration led to higher labeling yield and the maximum yield was achieved at 2.5 and 2 mg, respectively. By increasing the substrate concentration over the optimum values, the labeling yield was slightly decreased.

Effect of $SnCl_2$ concentration

At 10 μg $SnCl_2$, the labeling yield of ^{99m}Tc-lomefloxacin was small (69.2%) due to partial reduction of pertechnetate (13.9%). It is observed that the yield significantly increased by increasing the amount of $SnCl_2$ from 10 to 50 μg (optimum content), at which maximum labeling of 93.6% was obtained. By increasing the amount of $SnCl_2$ above 50 μg, the yield drastically decreased because excess $SnCl_2$ was converted to colloid (81.9% at 150 μg $SnCl_2$) as shown in Fig. 3. For ofloxacin, the yield was high at low $SnCl_2$ concentration and the maximum yield of 96.6% was achieved at 50 μg $SnCl_2$. This yield was decreased with increasing the $SnCl_2$ concentration above 70 μg.

Effect of pH of the reaction mixture

Figure 4 clearly show that the optimum pH range required to give high labeling yield for ^{99m}Tc-lomefloxacin and ^{99m}Tc-ofloxacin was pH 3.5–5. Above and below this pH range the yield was low (70.7 and 79.4%, respectively, at pH 2; 8.4 and 17.8%, respectively, at pH 6.5).

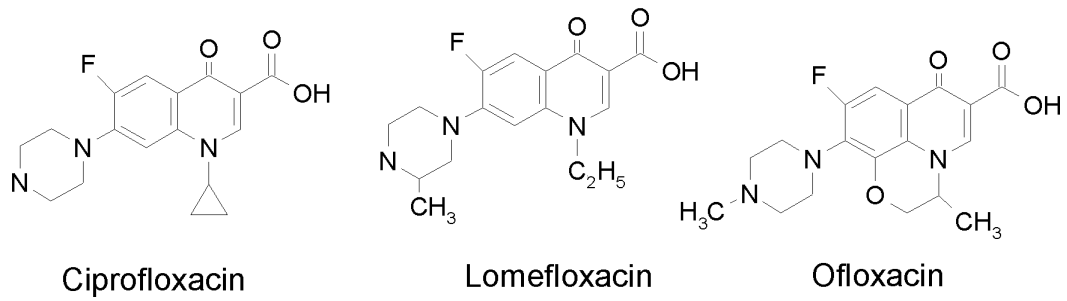

Fig. 1. Chemical structure of ciprofloxacin, lomefloxacin and ofloxacin

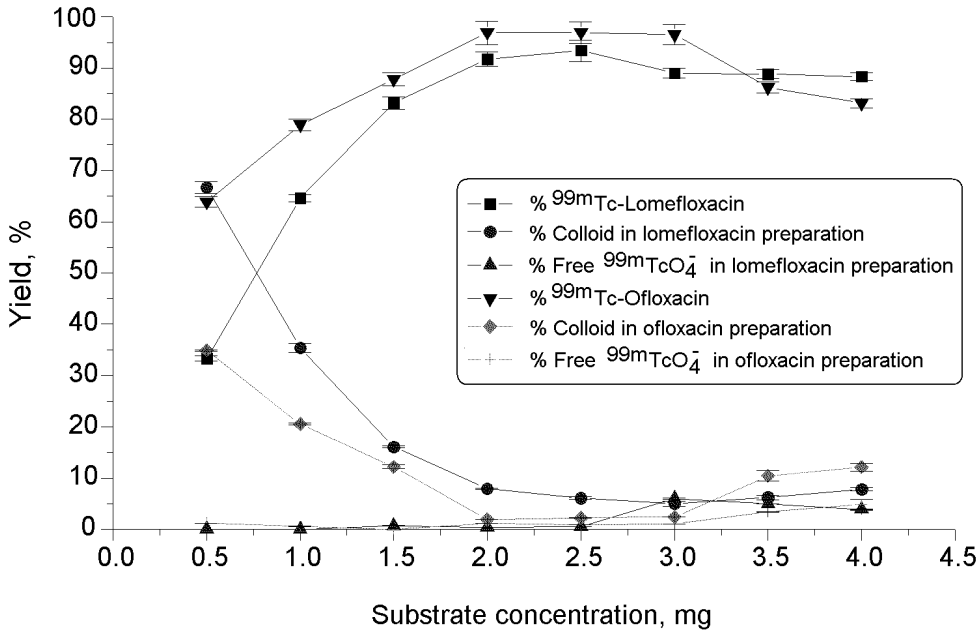

Fig. 2. Effect of the amount of lomefloxacin and ofloxacin on the radiochemical yield

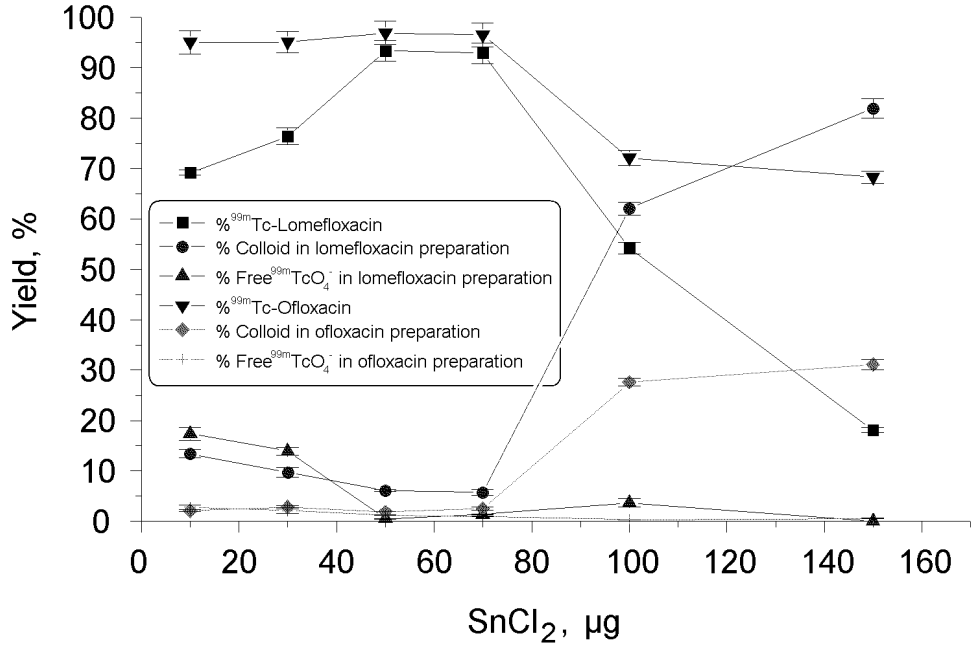

Fig. 3. Effect of $SnCl_2$ concentration on the labeling yield of ^{99m}Tc-lomefloxacin and ^{99m}Tc-ofloxacin

Stability studies

As shown in Fig. 5, the rate of formation of ^{99m}Tc-lomefloxacin started relatively slowly with a yield of 83.8% at 1 minute. The highest yield of 93.6% was achieved at 30 minutes. The complex was stable up to 120 minutes, after that the yield decreased again (>80%). In case of ofloxacin, ^{99m}Tc-ofloxacin was formed at once on the addition of ^{99m}Tc to the reaction mixture (96.6%), and the formed complex remained stable up to 120 minutes. At a reaction time greater than 2 hours, colloid was the main impurity which was easily eliminated using a millipore filter (0.22 µm). Accordingly, the labeling yields of ^{99m}Tc-lomefloxacin and ^{99m}Tc-ofloxacin were higher than that of ^{99m}Tc-ciprofloxacin up to 2 hours.

Biodistribution

As shown in Table 1, rats with infectious lesions injected with ^{99m}Tc-lomefloxacin showed a mean abscess-to-muscle (target-to-non target, *T*/*NT*) ratio equal to 6.5±0.5, while *T*/*NT* for ^{99m}Tc-ofloxacin was 4.3±0.6. Both ^{99m}Tc-fluoroquinolones showed greater uptake in infected tissue than ^{99m}Tc-ciprofloxacin (*T*/*NT* = 3.8±0.8).[13] The uptake of ^{99m}Tc-lomefloxacin in liver, spleen and lungs was lower than the uptake of ^{99m}Tc-ofloxacin in the same organs. Low activity of ^{99m}Tc-lomefloxacin and ^{99m}Tc-ofloxacin in the liver indicates low content of hydrolyzed forms of ^{99m}Tc.

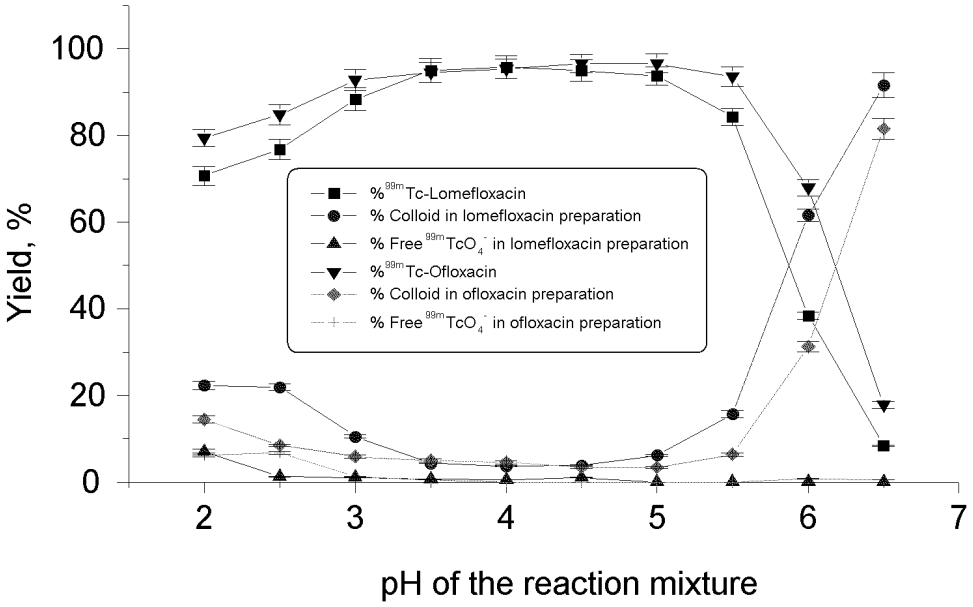

Fig. 4. Effect of pH on the labeling yield of lomefloxacin and ofloxacin with ^{99m}Tc

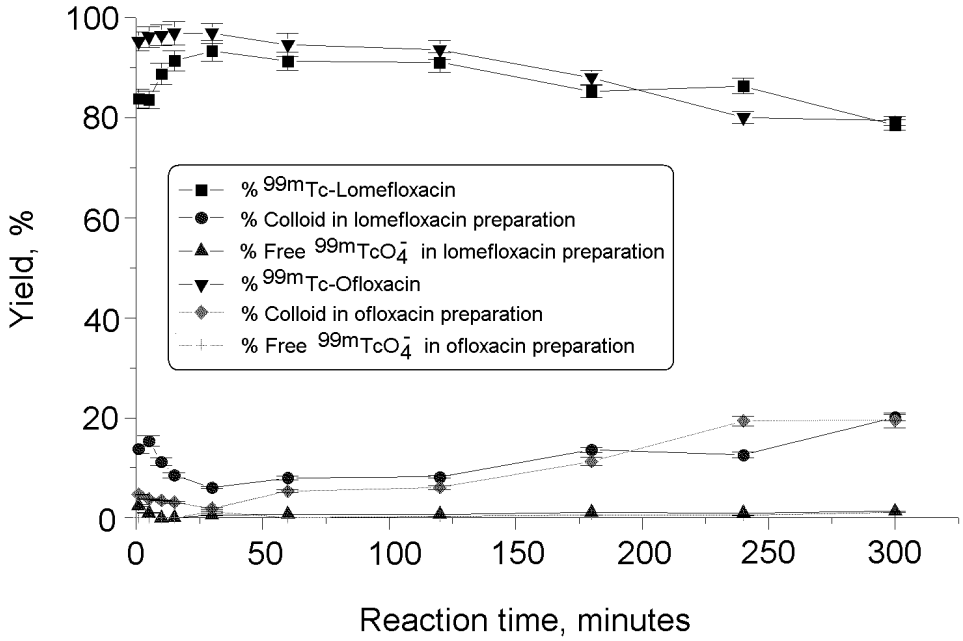

Fig. 5. ^{99m}Tc-lomefloxacin and ^{99m}Tc-ofloxacin yields vs. reaction time

Table 1. Biodistribution of ^{99m}Tc-lomefloxacin and ^{99m}Tc-ofloxacin complexes

Organs or body fluids	^{99m}Tc-lomefloxacin, %ID/g	^{99m}Tc-ofloxacin, %ID/g
Inflamed muscle	0.750 ± 0.171	0.570 ± 0.140
Control muscle	0.115 ± 0.040	0.132 ± 0.015
Blood	0.299 ± 0.052	0.371 ± 0.061
Liver	0.312 ± 0.041	0.385 ± 0.041
Spleen	0.147 ± 0.024	0.152 ± 0.040
Lung	0.319 ± 0.041	0.392 ± 0.050
Kidney	3.204 ± 0.432	3.312 ± 0.620
Stomach	0.091 ± 0.050	0.157 ± 0.051
Intestine	0.379 ± 0.042	0.421 ± 0.052
Bone	0.119 ± 0.054	0.234 ± 0.039
Heart	0.099 ± 0.062	0.079 ± 0.011

Reaction conditions: 2.5 mg lomefloxacin, 50 μg $SnCl_2$, pH 3.5–5 and 30-minute reaction time; 2 mg ofloxacin, 50 μg $SnCl_2$, pH 3.5–5 and 30-minute reaction time.

Conclusions

^{99m}Tc-lomefloxacin and ^{99m}Tc-ofloxacin were labeled easily at pH 3.5–5 using 50 μg $SnCl_2$ as a reducing agent with a high labeling yield of 93.6 and 96.6%, respectively. The formation of ^{99m}Tc-lomefloxacin starts slowly and reaches its maximum at 30 minutes. The formed complex was stable up to 2 hours, then the yield decreased (>80%). ^{99m}Tc-ofloxacin was formed upon addition of ^{99m}Tc to the reaction mixture and the formed complex was stable up to 2 hours, then the yield decreased (>80%). At reaction times greater than 2 hours, ^{99m}Tc-lomefloxacin and ^{99m}Tc-ofloxacin behave like ^{99m}Tc-ciprofloxacin, with a yield of about 80%. Colloid was the main impurity which was easily eliminated using a 0.22 μm millipore filter. As a result, ^{99m}Tc-lomefloxacin and ^{99m}Tc-ofloxacin could be used instead of ^{99m}Tc-ciprofloxacin for detecting infection sites without any purification within 2 hours after preparation. ^{99m}Tc-lomefloxacin was accumulated at the site of infection with *T*/*NT* higher than that of ^{99m}Tc-ofloxacin and ^{99m}Tc-ciprofloxacin. ^{99m}Tc-lomefloxacin showed higher yield and better biodistribution properties than ^{99m}Tc-ciprofloxacin.

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