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Preparation and Biological Evaluation of ^{99m}tc-Sarafloxacin and ^{99m}tc-Danofloxacin Complexes as a Model for Infection Imaging

M. A. Motaleb, M. T. El-Kollaly, A. B. Ibrahim and A. Abd El-Bary
Hot Labs Center, Atomic Energy Authority, Cairo, Egypt.
*Faculty of pharmacy, Cairo University, Cairo, Egypt.

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Abstract: *Infection and inflammation remain a major cause of mortality and morbidity globally. This promotes research into better and more accurate diagnostic and therapeutic methods. This investigation focused on the labeling of sarafloxacin and danofloxacin for infection imaging. The radiolabeled antibiotic ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin were assessed as an infection imaging agent in a mouse model. ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin were obtained at pH 11 with a radiochemical yield of 96, 90%, respectively by adding ^{99m}Tc to 1 mg sarafloxacin or danofloxacin in the presence of 50 µg $SnCl_2.2H_2O$. Biodistribution studies in mice were carried out in experimentally induced infection in the left thigh using Staphylococcus aureus. Both thighs of the mice were dissected and counted and the ratio of bacterial infected thigh/contralateral thigh was then evaluated. ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin showed high uptake (T/NT =3.8±0.1 and 4.9±0.1, respectively) in the infectious lesion and abscess to normal muscle ratio indicating that ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin could be used for infection imaging.*

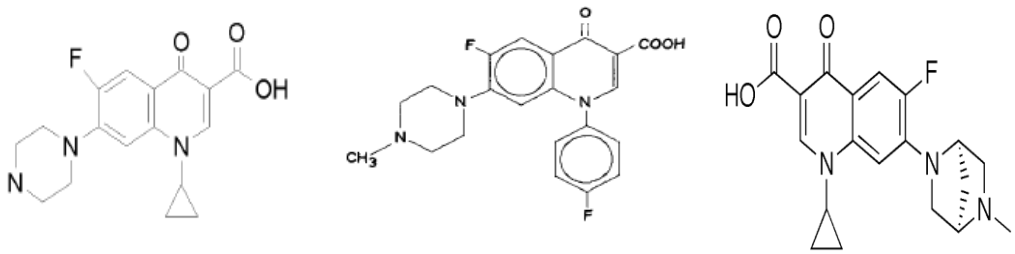
Key words: *sarafloxacin/ danofloxacin/ Technetium-99m/ Infection/ inflammation/ Diagnosis*

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)].

Quinolone drugs are large and widely used class of synthetic antibacterial compounds. [(3–5)] First generation quinolones include naldexic acid and oxolinic acid. Subsequent generations have been modified to increase spectrum and potency. The most significant modification has been the addition of a fluorine atom in drugs such as sarafloxacin and danofloxacine (Figure 1) is the 4th generation of quinolone antibiotics that has activity against a wide range of Gram-negative and Gram-positive microorganisms. Sarafloxacin and danofloxacin showed widespread distribution into body tissues and it was stereochemically stable in plasma and urine.

Sarafloxacin and danofloxacin undergoe limited metabolism in humans and excreted as unchanged drug in the urine that used safely in patients with impaired renal functions and hepatic insufficiency. Quinolones target bacterial type topoisomerase II 'DNA gyrase' in Gram-negative bacteria and DNA topoisomerase IV in Gram-positive bacteria.2 Nuclear medicine techniques are used in the context of infection localization. Inflammation imaging agents such as polyclonal and monoclonal antibodies, peptides, [6–8] cytokines, [9] and HMPAO-leukocytes [10, 11] cannot discriminate between septic and sterile inflammatory sites. A novel approach using a bacterially binding radiolabeled antimicrobial agent to detect infections was introduced since 1996 by Vinjamuri and Co-workers [12] when they labeled ciprofloxacin with technetium-99m, and clinically used under the trade name 'Infecton'. ^{99m}Tc-ciprofloxacin preparation has some disadvantages related to radiochemical purity (81%±4)[14] and stability which are discussed in details in the literature.[1,13,14–18] Later, many fluoroquinolones antimicrobial agents which not have the drawbacks of ciprofloxacin on patients suffering from kidney failure, congenital abnormalities of the urinary tract, urinary tract obstruction, pyelonephritis were labeled with technetium-99m and evaluated as infection imaging agents.[19] During this study, sarafloxacin and danofloxacin were labeled with technetium-99m and the parameters affecting this labeling reaction were studied. The labeled sarafloxacin and danofloxacin were evaluated biologically in normal and in inflamed mice.



Ciprofloxacin **Sarafloxacin** **Danofloxacin**

Figure (1): Chemical structure of ciprofloxacin, sarafloxacin and danofloxacin

Experimental

Sarafloxacin and danofloxacin 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 1 mg sarafloxacin or danofloxacin was transferred to an evacuated penicillin vial. Exactly 50 µg $SnCl_2$ solution was added and the pH of the mixture was adjusted to 11 using 0.1N NaOH, 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.

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 cm2, 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, and 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 (*Rf* = 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 (*Rf* = 0) while other species migrate with the solvent front (*Rf* = 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

Induction of infectious foci

The inflammations were induced via intramuscular injection of the approximately 10^5–10^6 colony forming units of *Staphylococcus aureus* suspended in 0.2 ml of saline (model for septic inflammation). Groups of 3 mice, weighing approximately 25 g each, were injected with 200 µl of the above suspension in the right thigh muscle.(20) Twenty-four hours required to get gross swelling in theinfected thighs.

Induction of non-infected inflammation

Sterile inflammation was induced by injecting 200 µl of turpentine oil (21) which was sterilized by autoclaving at 121 °C for 20 minutes and heat killed *Staphylococcus aureus* (model for pyrogen induced inflammation), intramuscularly (i.m.) in the right lateral thigh muscle of the albino mice. Two days later, swelling appeared.

The animals were intravenously injected with 100 µl (100-150 MBq) ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin via the tail vein and kept alive in metabolic cage for different intervals of time under normal conditions.

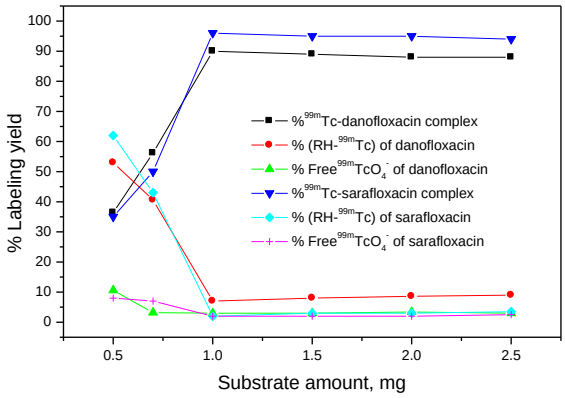
The mice were sacrificed at 2, 4 and 24-hour post-injection. Samples of fresh blood, bone and muscle were collected in pre-weighed vials and counted. The different organs were removed, counted and compared to a standard solution of the labeled sarafloxacin and danofloxacin. The average percent values 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. (22) Corrections were made for background radiation and physical decay during experiment. Both target and non-target thighs were dissected and counted.

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 35 and 36.4% for ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin, 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 (62 and 53%, respectively). Increasing the substrate concentration lead to higher labeling yield and the maximum yield was achieved at 1 mg for

sarafloxacin and danofloxacin. By increasing the substrate concentration over the optimum values, the labeling yield was slightly decreased.

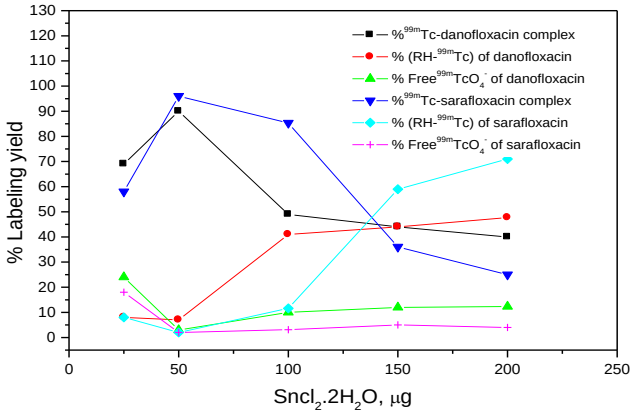


Reaction mixture: X mg substrate, 50 µg tin(II) at pH 10 and reaction time 30 min.

Figure 2: Effect of substrate content on the percent labeling yield of ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin complex

Effect of $SnCl_2$ concentration

The results obtained in the figure 3 showed that, at pH 11 efficient labeling yield was achieved by using the optimum amount of Sn (II) µg at which a maximum labeling of 96 and which was equal to 50 90% for ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin, respectively was obtained. Below this value, stannous chloride is not sufficient for complete reduction of pertechnetate to form ^{99m}Tc-complex, this is an explanation of the presence of high percentage of free pertechnetate (18 µg tin (II)) and 24% at 25. By increasing the amount of $SnCl_2.2H_2O$ above 50µg, the yield drastically decreased while the amount of colloid increase and reach to 71 and 47.7% for sarafloxacin and danofloxacin at 200 µg $SnCl_2.2H_2O$, respectively. This may be due to excess $SnCl_2.2H_2O$ was converted to tin colloid which competes with saraflxacin and danofloxacin for reduced $^{99m}TcO_4^-$.

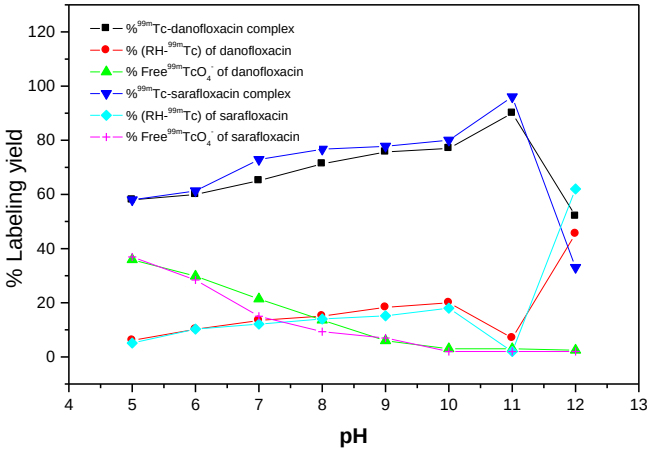


Reaction mixture: 1 mg danofloxacin, x µg tin(II) at pH 11 and reaction time 30 min

Figure 3: Effect of Tin (II) content on the percent labeling yield of 99mTc-sarafloxacin and 99mTc-danofloxacin complex

Effect of pH of the reaction mixture

Figure 4 clearly shows that the maximum radiochemical purity of the preparation was obtained at a pH 11 and was equal to 96 and 90% for sarafloxacin and danofloxacin, respectively. At pH below or above the optimum pH, the radiochemical purity is significantly decreased by forming RH-^{99m}Tc which is the main radiochemical impurities which was equal to 62 and 45.5%, respectively at pH 12.

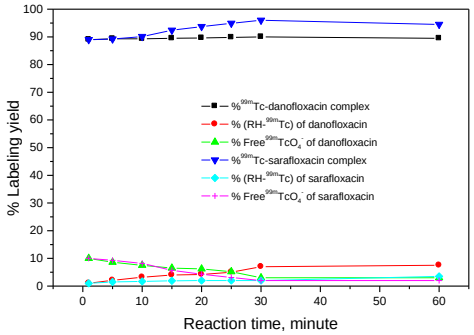


Reaction mixture: 1 mg danofloxacin and sarafloxacin, 50 µg tin(II) at pH = 5-11, and reaction time 30 min.

Figure 4: Effect of pH value of the reaction mixture on the percent labeling yield of ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin complex

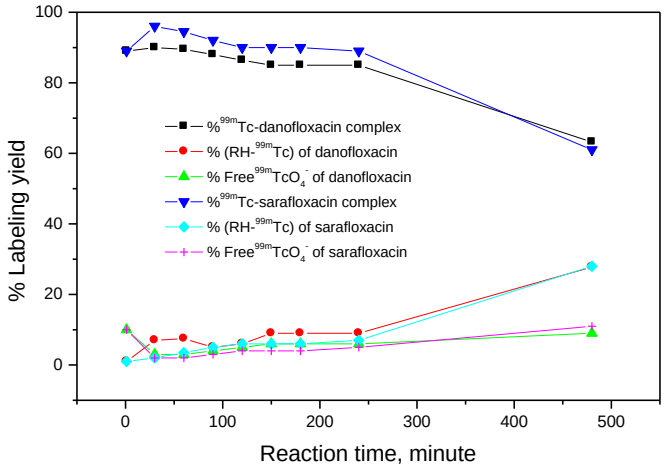
Effect of reaction time and stability test:

Figure 5 show the rate of formation of ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin started relatively slowly with a yield of ~ 89 and 85%, respectively at 1 minute. The highest yield of 96 and 90%%, respectively were achieved at 30 minutes reaction time. Figure 6 show stability test where the two ^{99m}Tc-complex were stable up to 4 hr, after that the yield decreased again. The colloid was the main impurity which was easily eliminated using a millipore filter (0.22 µm).



Reaction mixture: 1 mg danofloxacin and sarafloxacin, 50 µg tin(II) at pH = 11, at different reaction time

Figure 5: Effect of reaction time on ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin



Reaction mixture: 1 mg danofloxacin and sarafloxacin, 50 µg tin(II) at pH = 11, at different reaction time

Figure 6: Stability test for ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin

Biodistribution

Based on the data presented in Tables (1-3), which clearly show the biodistribution analysis of ^{99m}Tc-sarafloxacin, ^{99m}Tc-danofloxacin and ^{99m}Tc-ciprofloxacin complex in abscess and aseptic inflammation-bearing mice (heat killed bacteria and turpentine oil) at different time intervals post injection. These data depicted rapid distribution throughout the body and uptake in the inflamed areas was observed within 2 hours after intravenous injection of the tracer. As shown in figure (7, 8), mice with infectious lesions injected with ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin showed a mean abscess-to-muscle (target-to-non target, *T*/*NT*) ratio equal to 3.8±0.1 and 4.9±0.1, after 2 h post injection. These ^{99m}Tc-fluoroquinolones showed similar uptake in infected tissue to ^{99m}Tc-ciprofloxacin (*T*/*NT* = 3.6±0.4). [23] The above results showed that no significant difference in *T*/*NT* of ^{99m}Tc-sarafloxacin and ^{99m}Tc danofloxacin in septic and aseptic inflammation indicated that these ^{99m}Tc-fluoroquinolones can not differentiate between infection and sterile inflammation.

Table 1: Biodistribution of ^{99m}Tc-sarafloxacin 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	1.09±0.2	0.84±0.11	0.58±0.06	1.11±0.1	0.85±0.2	0.54±0.1	0.58±0.0
	0.38±0.2	0.23±0.1					
Control muscle	0.28±0.1	0.22±0.2	0.16±0.0	0.3±0.0	0.25±0.1	0.17±0.0	0.29±0.0
	0.23±0.0	0.14±0.0					
Liver	19.1±3.2	14.9±2.0	7.4±0.3	20.6±2.4	15.2±2.2	7.9±0.5	19.5±3.0
	15.2±2.2	8.1±0.6					
Urine	20.5±3.5	26.8±4.2	31.9±3.1	19.1±1.3	27.3±1.1	33.4±2.7	19.0±0.9
	27.4±1.8	31.8±2.4					
Kidneys	12.5±1.2	11.9±1.3	8.6±0.4	13.9±1.3	13.2±2.3	8.5±0.7	13.3±2.5
	13.2±1.2	7.9±0.3					
Blood	6.9±0.4	4.5±0.2	1.00±0.0	7810±0.2	6.0±0.2	1.3±0.1	6.0±0.2
	4.5±0.2	1.0±0.0					
Heart	0.8±0.1	0.2±0.0	0.1±0.0	0.3±0.09	0.2±0.0	0.1±0.0	0.4±0.08
	0.2±0.0	0.1±0.0					
Lung	1.4±0.2	0.4±0.0	0.1±0.0	1.2±0.09	0.3±0.0	0.1±0.0	1.4±0.09
	0.4±0.1	0.2±0.0					
Intestine and stomach	21.9±2.5	5.90±0.5	4.10±0.3	22.1±3.4	6.7±0.4	4.6±0.7	19.9±1.9
	6.7±0.8	4.4±0.4					
Spleen	2.10±0.1	1.10±0.2	0.3±0.1	2.5±0.3	1.6±0.0	0.5±0.1	2.3±0.1
	1.4±0.0	0.4±0.0					
Bone	1±0.1	0.60±0.1	0.30±0.0	1.2±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-danofloxacin 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	1.37±0.2	0.95±0.11	0.76±0.06	1.15±0.1	0.96±0.2	0.48±0.1	0.78±0.0		
	0.50±0.2	0.22±0.1							
Control muscle	0.28±0.1	0.21±0.2	0.19±0.0	0.29±0.0	0.26±0.1	0.15±0.0	0.31±0.0		
	0.25±0.0	0.13±0.0							
Liver	17.1±3.2	12.9±2.0	5.4±0.3	17.6±2.4	12.2±2.2	4.9±0.5	17.5±3.0		
	13.2±2.2	6.1±0.6							
Urine	18.5±3.5	24.9±4.2	29.9±3.1	17.1±1.3	24.3±1.1	29.4±2.7	18.0±0.9		
	25.4±1.8	28.8±2.4							

Kidneys	11.2±1.2	10.7±1.3	6.6±0.4	10.9±1.3	10.2±2.3	5.5±0.7	11.3±2.5	10.2±1.2	6.9±0.3
Blood	5.3±0.4	3.1±0.2	1.00±0.0	6.10±0.2	4.0±0.2	1.0±0.1	5.5±0.2	4.3±0.2	1.0±0.0
Heart	0.3±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	1.2±0.2	0.2±0.0	0.1±0.0	1.1±0.09	0.2±0.0	0.1±0.0	1.3±0.09	0.4±0.1	0.2±0.0
Intestine and stomach	19.9±2.5	3.90±0.5	2.10±0.3	19.1±3.4	4.7±0.4	2.2±0.7	18.9±1.9	4.7±0.8	2.4±0.4
Spleen	2.10±0.1	1.00±0.2	0.3±0.1	2.1±0.3	1.1±0.0	0.5±0.1	2.0±0.1	1.3±0.0	0.3±0.0
Bone	0.90±0.1	0.40±0.1	0.10±0.0	1.0±0.0	0.49±0.1	0.1±0.0	1.1±0.2	0.5±0.1	0.1±0.0

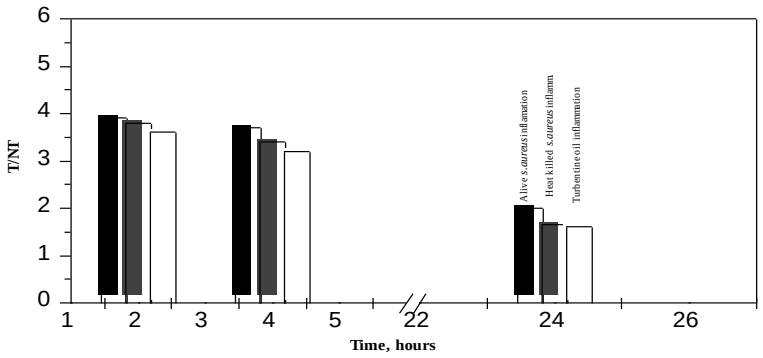


Figure (7) target (T) to non-target (NT) ratio of ^{99m}Tc-sarafloxacin when injected intravenous in mice at different post injection time

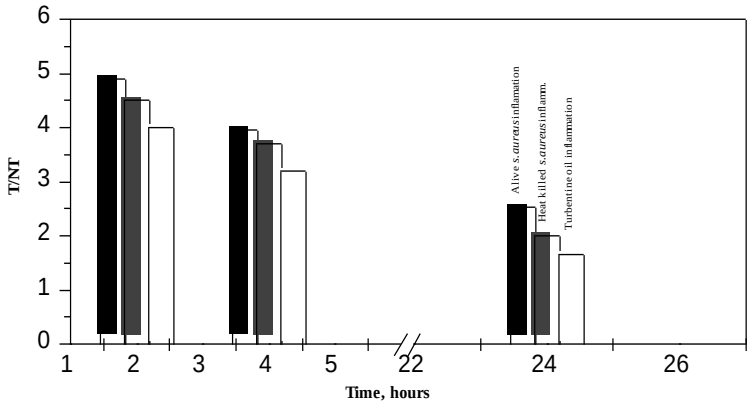


Figure (8) target (T) to non-target (NT) ratio of ^{99m}Tc-danofloxacin when injected intravenous in mice at different post injection time

Conclusions

^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin were labeled easily at pH 11 using 50 µg $SnCl_2.2H_2O$ as a reducing agent with a high labeling yield of 96 and 90%, respectively which higher than that of the commercially available ^{99m}Tc-ciprofloxacin. The formation of ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin start slowly and reaches its maximum at 30 minutes. The ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin complexes were stable up to 4 hours, then the yield decreased (>80%). Biodistribution studies showed that, ^{99m}Tc-sarafloxacin and ^{99m}Tc-danofloxacin can not distinguish infection from sterile inflammation.

References

1. K. E. Britton, S. Vinjamuri, A. V. Hall Et Al., Eur. J. Nucl. Med., 24 (1997) 553.
2. M. M. Welling, A. Paulusma-Annema, H. S. Balter, E. K.
3. J. Pauwels, P. H. Nibbering, Eur. J. Nucl. Med., 27 (2000) 292.
4. Drlica K. Curr Opin Microbiol 1999; 2: 504–508.
5. Drlica K, Zhao X. Microbiol Mol Biol Rev 1997; 61: 377–392.
6. Kuhlmann J, Dalhof A, Zeiler Hj. Quinolone Antibacterials,Vol.127.Springer: Berlin, Germany,1998.
7. Becker W, Palestro Cj, Winship J, Feld T, Pinsky Cm, Wolf F, Goldenberg Dm. Clin Orthop 1996; 329: 263–272.
8. Welling Mm, Feitsma Rij, Calame W, Pauwels Ekj. Nucl Med Biol 1997; 24: 649–655.
9. Welling Mm, Lupetti A, Calame W, Balter Hs, Lanzzeri S, Souto B, Rey Am, Savio Eo.
10. Paulusma- Annema A, Pauwels Ek, Nibbering Ph. J Nucl Med 2001; 42(5): 788–794.

Van Der Laken Cj, Boerman Oc, Oyen Wjg, Vande Ven Mtp, Van Der Meer Jwm, Cortens Fhm. J Nucl Med 2000; 41: 463–469.

11. Welling Mm, Nibbering Ph, Paulusma-Annema A, Hiemstra Ps, Pauwels Ekj, Calame W. J Nucl Med 1999; 40: 2073–2080.
12. Garvin Kl, Hanssen Ad. J Bone Joint Surg Am 1995; 77: 1576–1588.
13. Vinjamuri S, Hall Av, Solanki Kk, Bomanji Jb, Sirag Q, Shaughnessy Em, Das Ss, Britton Ke. Lancet 1996; 347: 233–235.
14. S. Vinjamuri, A. V. Hall, K. K. Solanki, Lancet, 347 (1996) 233.
15. H. S. Rien, J. R. Huub, C. B. Otto, D. Rudi, S. Guido, J. Nucl. Med., 45 (2004) 2088.
16. Pirmettis, G. S. Limouris, M. Papadopoulos Et Al., Eur. J. Nucl. Med., 26 (1999) 1108.
17. J. O. Seung, S. R. Jin, W. S. Joong, J. Y. Eun, J. H. Hyun Et Al., Appl. Radiation Isotopes, 57 (2002) 193.
18. J. E. T. Lima, C. Maliska, M. R. B. Goncalves, R. C. Lima, R. Corbo, Worled J. Nucl. Med., 3 (2004) 284.
19. L. Sarda, A. C. Cremieux, Y. Lebellec, A. Meulemans, R. Lebtahi Et Al., J. Nucl. Med., 44 (2003) 920.
20. El-Ghany Ea, El-Kolaly Mt, Amine Am, El-Sayed As, Abdel-Gelil Fj. Radioanal Nucl Chem 2005; 266(1): 131–139.
21. W. J. G. Oyen, O. C. Boerman, F. H. M. Corstens, J. Microbiol. Meth., 47 (2001) 151.
22. M. Asikoglu, F. Yurt, O. Cagliyan, P. Unak, H. Ozkilic, Appl. Radiation Isotopes, 53 (2000) 411.
23. B. A. Rhodes, Sem. Nucl. Med., 4 (1974) 281.
24. H. S. Rien, J. R. Huub, C. B. Otto, D. Rudi, S. Guido, J.Nucl. Med., 45 (2004) 2088.