Original Article

Efficacy of ^{99m}Tc-Ciprofloxacin and ^{67}Ga-Citrate scintigraphy to discriminate infection foci induced by Staphylococcus aureus from sterile inflammation induced by Carrageenan in rat

Alireza Doroudi[1], Mostafa Erfani[2], Fatemeh Kooshki[1], Seyyed Mostafa Saadati[3], Farzad Ahmadi[3], Ali Kiasat[3], Mohammad Javad Khodayar[1], Behrooz Etessami[3], Hossein Meghdadi[4]

[1]School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Nuclear Science Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran, Tehran Iran
[3]Nuclear Medicine Department, Golestan General Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[4]Department of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

(Received 12 August 2014, Revised 24 November 2014, Accepted 1 December 2014)

ABSTRACT

Introduction: This study was launched to evaluate the sensitivity and specificity of ^{99m}Tc-Ciprofloxacin to distinguish infection foci induced by staphylococcus aureus and inflammation lesions induced by carrageenan in the rat foot in comparison with ^{67}Ga-Citrate scintigraphy.

Methods: The labeling and quality control of ^{99m}Tc-Ciprofloxacin kits have been performed according to the manufacturer's instructions. A total number of 40 adult, male NMRI rats were randomly divided into two equal groups, one group for ^{99m}Tc-Ciprofloxacin and the other group for ^{67}Ga-Citrate scintigraphy. Every group was subdivided into two groups equally. Septic lesion was induced by Staphylococcus aureus. Aseptic inflammation lesion was induced by carrageenan in the rat foot in the other group. The ^{99m}Tc-Ciprofloxacin and ^{67}Ga-Citrate scintigraphy studies have been performed to evaluate the efficacy of radiotracers.

Results: The images showed ^{67}Ga uptake at the infection and inflammation sites. The infection foci could be visualized by ^{99m}Tc-Ciprofloxacin scintigraphy due to selective binding of ciprofloxacin to DNA gyrase of bacteria. The inflammation sites have been observed by non-specific uptake of ^{99m}Tc-Ciprofloxacin. None of both imaging studies have shown preferentially diagnosis of septic and aseptic inflammation lesions. The sensitivity, specificity and positive predictive value of both scintigraphic techniques were 100%, 50% and 50%, respectively.

Conclusion: The ^{99m}Tc-Ciprofloxacin scintigraphy is sensitive for visualization of the lesion, but it could to discriminate between septic and aseptic inflammation lesions. Other modalities must be considered for interpretation of images obtained by ^{99m}Tc-Ciprofloxacin scintigraphy.

Key words: Carrageenan; ^{67}Ga; ^{99m}Tc-Ciprofloxacin

Iran J Nucl Med 2015;23(2):96-102
Published: June, 2015
http://irjnm.tums.ac.ir

Corresponding author: Dr Alireza Doroudi, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. E-mail: Doroudi-a@ajums.ac.ir

INTRODUCTION

An early diagnosis of infection from sterile inflammation is one of the most common challenging problems in clinical practice. Several techniques have been suggested to solve this dilemma. Plain radiography is usually considered the first step to assess the lesions induced by infection or inflammation. The other available techniques, such as ultrasonography, computerized tomography (CT) and magnetic resonance imaging (MRI) have high sensitivity although these modalities lack of specificity for preferential diagnosis of infectious sites, particularly in early phase of disease when anatomic structures have not distorted considerably [1]. Radioisotope scintigraphy can be considered a part of the diagnostic procedures for discrimination between infectious and sterile inflammatory lesions. Therefore, several radiopharmaceuticals have been developed to tag the infection foci.

^{67}Ga-citrate is the most primitive radionuclide for this purpose, but unfortunately it has several disadvantages including long physical half-life, high and multiple energy gamma photons causing high radiation absorbed doses, non-availability as a generator and high sensitivity for both infection and non-infectious inflammation [2, 3]. Three phase bone scan with ^{99m}Tc-MDP radiopharmaceutical is commonly used in nuclear medicine. ^{99m}Tc-MDP scintigraphy is highly sensitive especially when uptake of ^{99m}Tc-MDP is positive in all three phases.

This has provided to discriminate soft tissues from bony infections. In spite of high sensitivity of three phase bone scan, unfortunately it is not specific for infection. The uptake of radiotracer can be increased non-specifically due to presence of inflammation [4]. Leukocytes labeled with radioisotope have been recommended as a gold standard to differentiate infection from sterile inflammation [5]. In order to label the leukocytes, it is necessary to take the blood from the patient. Leukocytes must be separated, labeled and finally re-injected to the patient. This technique is time-consuming and has potential high risk of contamination or transmission of blood-borne pathogens to patient or technician. In addition to above mentioned factors, the process of labeling of leukocytes with radioisotope requires specialize facilities. The broad spectrum antibiotic agents have been suggested as a promising diagnostic test for detection of infectious lesions. The antibiotic molecules can accumulate at the site of infection, because they are taken up and metabolized by microorganisms [6-11]. The majority of the various antibiotics that have been studied in this regard are those of the quinolones family, second and third generation of cephalosporin. Ciprofloxacin is a synthetic fluoroquinolone derivative with bactericidal activity against a wide range of gram-negative and gram-positive bacteria with a wide distribution in the entire body. Recently, ^{99m}Tc-Ciprofloxacin known as Infecton, has been developed as a radiotracer for scintigraphy to distinct between infection and sterile inflammation [12-14]. The ^{99m}Tc-Ciprofloxacin radiopharmaceutical combines the advantages of technetium-99m labeling and the broad spectrum bacterial localizing capability due to attachment and inhibition of bacterial DNA gyrase. The experience in literature reported that the specificity of scintigraphy with ^{99m}Tc-Ciprofloxacin for the localization of bacterial infection exceeds 90 % [1, 15]. Carrageenan has been used to induce experimental inflammation in laboratory animals. Carrageenan-induced paw rats as an investigational, in-vivo model of inflammation has been frequently used to study the anti-inflammatory effect of natural products and drugs [16].

This study was conducted to evaluate the efficacy of ^{99m}Tc-Ciprofloxacin and ^{67}Ga-Citrate scintigraphy to differentiate infectious lesions induced by Staphylococcus aureus and inflammation lesions induced by carrageenan in foot's rats.

METHODS

All chemical materials have been purchased from Merck, Fluka and Sigma. The chemicals and solvents were of the highest purity and analytical grade and used without further purification. The freeze-dried kits of Ciprofloxacin and ^{99}Mo/^{99m}Tc generator have been provided by Radioisotope Division of Atomic Energy Organization of Iran (AEOI).

Production of ^{67}Ga was performed at the Agricultural Medical and Industrial Research School (AMIRS, Karaj, Iran) using a 30 Mev cyclotron (Cyclone-30, IBA, Belgium). Enriched Zinc 68 chloride (enrichment >95%) was obtained from the Ion Beam Separation Department at AMIRS.

Bacteria samples

The sample wound swabs were taken from patients admitted to the infection department of teaching center of Imam Khomeini hospital, Ahvaz, Khuzestan, Iran. The specimens were transported in sterile, leak-proof containers to the department of microbiology of Ahvaz Jundishapur University of medical Sciences. The isolates were inoculated on blood agar and incubated overnight at 35 ° C aerobically. Gram-positive cocci occurring in pairs, short chains or clusters, Catalase-positive, Coagulase-positive by test tube and DNase-positive on agar were identified as S.aureus and selected. The disc diffusion method was used to determine the susceptibility of S.aureus to ciprofloxacin antibiotic. By using a sterile-tip applicator, the surface of one to

four morphologically identical, isolated colonies were touched. The applicator was immersed into a tube containing Mueller Hinton broth. The applicator was rubbed against the wall of the tube slightly to release a small amount of growth into the liquid. The tube was capped and the cells were mixed using a vortex to form a suspension, while being careful not to form froth or bubbles in the suspension when mixing the cells. The broth was incubated at 35 °C, and then the turbidity was adjusted to a number 0.5 McFarland turbidity standard. A sterile cotton swab on a wooden stick was dipped into the broth. Excess inoculum was removed by rotating the swab against the wall of the tube above the fluid level. The Mueller-Hinton agar plates were streaked in three dimensions. During 15 min after the surfaces of the agars were inoculated. Ciprofloxacin (5µg) disks were applied. The plates were inoculated at 35 °C for 24 hours. The diameter of each zone of inhibition was measured to the nearest millimeter through the underside of the plate by using a caliper. The isolates were considered to be resistant or susceptible to ciprofloxacin when the zone diameter of inhibition around a ciprofloxacin (5µg) disk was less than or equal to 15 mm. When zone diameters of inhibition were greater than or equal to 21 mm, the isolates were considered to be sensitive. The table of Clinical and Laboratory Standards Institute (CLSI) has been used in order to determine the resistance or susceptibility of S.aureus to ciprofloxacin. Then the isolates containing the susceptible bacteria were inoculated in normal saline. The turbidity was adjusted to a number 0.5 McFarland (each milliliter of 0.5 McFarland contains 1.5×10^8 microorganisms). Half milliliter of inoculums has been injected to each foot's rat. To make sure about the survival of S.aureus bacteria, 0.1 milliliter of the above mentioned inoculums was inoculated on blood agar. The antibiogram experiment has been repeated three times.

Labeling of ciprofloxacin by ^{99m}Tc

Technetium-99m as sodium pertechnetate ($Na^{99m}TcO_4$) was obtained from an in-house $^{99}Mo/^{99m}Tc$ generator using 0.9% saline. Commercial Ciprofloxacin kits (AEOI, Tehran, Iran) was used and the labeling and quality control were performed according to the manufacturer's instructions.

Animal studies

The rats with average weight 160±20 gr were obtained from research center and experimental animal house of Ahvaz Jundishapur University of Medical Sciences. This approach was approved by the ethics committee of Ahvaz Jundishapur University of medical Sciences. All the ethical issues were considered based on the Ahvaz Medical University Ethical Protocols (AMUP) on animal experiments. A total number of 40 adult, male NMRI rats were acclimated to conditions for one week before the experiment. These rats were kept in individually wire-bottom stainless steel in an air-conditioned room at 24±1°C with a 12 hours light-dark cycle and were fed with standard pellet diet and had free access to water. They were randomly assigned into two equal groups. One group has been allocated for ^{99m}Tc-Ciprofloxacin and the other group for ^{67}Ga-Citrate scintigraphy studies. Every group has randomly divided into two groups. Each group contained ten animals. S.aureus infection was induced in the right thigh muscle of animals by intramuscular injection of bacteria suspension. In the other group carrageenan was dissolved in normal saline in order to produce inflammation in animals. On the experiment day, one milliliter of 3 % carrageenan solution in saline was injected intramuscularly in the left thigh muscle of animals under brief diethyl ether anesthesia. Carrageenan caused visible redness and pronounced swelling that was developed two hours after injection, maximal between two to four hours after injection and persisted for more than twenty four hours.

Animal scintigraphy

Radioisotope scintigraphy studies have been performed 48 hours after inoculation of bacteria samples for visualizing of the infectious sites. These studies have been performed two hours after carrageenan induced-inflammation. In all studies, each rat was placed in the restrainer apparatus and the (37MBq) ^{99m}Tc-Ciprofloxacin or ^{67}Ga-Citrate was administered intravenously by contra lateral tail vein. Two hours after injection of ^{99m}Tc-Ciprofloxacin radiotracer and eight hours after ^{67}Ga-Citrate radioisotope, the anesthetized live rat was placed in a prone position with limbs spread out and fixed on the board with surgical tape for scintigraphy. For all studies a single-headed camera (E-Cam, Siemens USA) was used.

^{99m}Tc-Ciprofloxacin imaging

One hour after an intravenous injection of 37MBq ^{99m}Tc-Ciprofloxacin, imaging was performed. Acquisition parameters for ^{99m}Tc-Ciprofloxacin scintigraphy were as follows: matrix size 256×256, zoom factor ×3, anterior and posterior views for 5 min and energy window 140 keV. Anterior and posterior static images were acquired using a large field of view gamma camera peaked to 140 keV with a 15% window and a low-energy all-purpose collimator for 500 kilocounts per image. The gamma camera was positioned to image the affected part and contralateral healthy site.

^{67}Ga imaging

^{67}Ga-Citrate was injected intravenously by contralateral tail vein. Images were obtained eight hours later using a large field of view gamma camera with a medium-energy, general-purpose collimator. Acquisition parameters for ^{67}Ga-Citrate scintigraphy were as follows: matrix size 256×256, zoom factor ×3, anterior and posterior views for 5 min. Anterior and posterior images were acquired for 500 kilocounts, using three peaks of ^{67}Ga (93,185 and 300 KeV) with windows of 20% centered on each peak. The camera was positioned in the same way as for the above mention study.

Quantitative study

By using commercial available software the counts of affected foot (infectious lesion induced by S.aureus or inflammation lesion induced by carrageenan) and unaffected foot (contra lateral healthy side) were measured for each animal. Then the Relative Factor (RF) was calculated for each rat by dividing the counts of affected to unaffected foot.

RESULTS

Quality control of radiopharmaceutical kits has been performed by thin layer chromatography according to manufactures' instructions. The yield of ^{99m}Tc-Ciprofloxacin samples were approximately 78%. All images were interpreted by three nuclear physicians independently, and their final opinion was achieved by consensus (Figures 1 and 2). The observers were unaware of the nature of induced lesions in the rats. Infectious sites were induced in the right foot and carrageenan induced-inflammation in the left foot, in order to prevent any bias in interpretation of images. Preliminary investigation in our lab indicated that if the scintigraphies were performed 48 hours after inoculation of S.aureus and 3 hours after carrageenan inflammation, the quality of images were suitable for interpretation. The ^{99m}Tc-Ciprofloxacin scintigraphy studies showed both infectious and carrageenan induced-inflammation lesions. Images with good quality were obtained in each case and the quality did not change over the time. Radiotracer uptake was observed in all images. Therefore, ^{99m}Tc-Ciprofloxacin scintigraphy could not differentiate carrageenan induced-inflammation from infection foci induced by S.aureus. The ^{67}Ga-Citrate scintigraphy has been performed eight hours after radioisotope administration to obtain the images with good quality. The similar behavior has been observed by ^{67}Ga-Citrate images. The radiotracer uptake was observed in all images. The RF values in ^{99m}Tc-Ciprofloxacin scintigraphy to localize septic and aseptic lesions were 2.26 (n=10, range 1.16 to 3.1, mean 2.26) and 1.56 (n=10, range 1.16 to 2.26, mean 1.56), respectively. These values for ^{67}Ga-Citrate scintigraphy were 2.5 (n=10, range 1.9 to 3.2, mean 2.5) and 2.3 (n=10, range 1.53 to 3.1, mean 2.3), respectively.

Fig 1. Posterior view images have been obtained one hour after 37 MBq ^{99m}Tc-Ciprofloxacin administrated intravenously due to contra lateral tail vein. The images indicates lesions for carrageenan induced-inflammation (above), and infection site induced by Staphylococcus aureus (below)

Our achievement demonstrated that ^{99m}Tc-Ciprofloxacin scintigraphy has no advantage over ^{67}Ga-Citrate scanning to differentiate infectious lesions induced by S.aureus from carrageenan induced-inflammation lesions in the rats' foot. Both radioisotope scintigraphy images were highly sensitive but, not specific to distinct infectious from sterile inflammatory sites. The sensitivity, specificity and positive predictive value (PPV) of both

scintigraphic images were 100%, 50% and 50% to visualize the infectious sites.

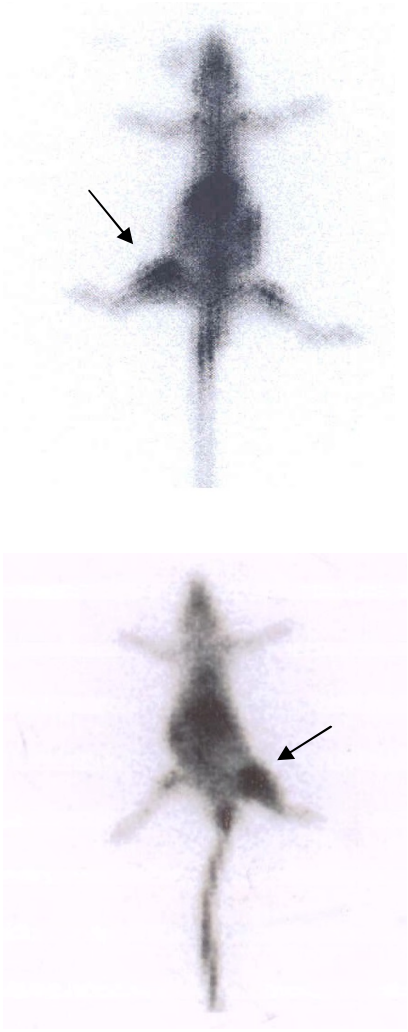

Fig 2. ^{67}Ga-Citrate scintigraphy imaging study has been performed eight hours after 37 MBq radiotracer injected by contra lateral tail vein. The posterior view images demonstrated lesions for sterile inflammation induced by carrageenan (above) and infection induced by Staphylococcus aureus (below).

DISCUSSION

Ciprofloxacin is synthetic derivative of fluoroquinolone antibiotic with bactericidal activity against a wide range of Gram-positive and Gram-negative microorganisms. This molecule has a wide distribution in the entire body including the bone, joints and effective penetration into the inflamed tissues. Ciprofloxacin is incorporated by bacteria and binds to the DNA gyrase selectively. The mechanism of therapeutic characteristic of the drug has been used for diagnostic purposes. Recently ciprofloxacin has been developed as a freeze-dried radiopharmaceutical kit by Radioisotope Division of Atomic Energy Organization of Iran (AEOI) for radioisotope imaging to visualize infection foci. The formulation contains 2 mg of ciprofloxacin. Therefore, the dose of ciprofloxacin for scintigraphy compared to the therapeutic dose for eradication and treatment of infection (500mg to 2gr per day) is very low. ^{99m}Tc-Ciprofloxacin scintigraphy has been offered several advantages over the other techniques.

The ^{99m}Tc-Ciprofloxacin complex does not bind to dead bacteria, so sterile abscesses do not show radiotracer uptake .The labeling of ciprofloxacin by ^{99m}Tc can provide images with good quality and a shorter investigation time in comparison to ^{67}Ga radioisotope imaging. The preparation of ^{99m}Tc-Ciprofloxacin complex is simple and cheap, and it does not involve blood manipulation with associated risk of blood-borne infections. ^{99m}Tc-Ciprofloxacin scintigraphy is not dependent on the absolute white blood cell count and for this reason can be used in patients with neutropenia. There is an absence of bone marrow uptake when ^{99m}Tc-Ciprofloxacin scintigraphy is used. According to literature, several studies have been conducted to assess the efficacy, sensitivity and specificity of ^{99m}Tc-Ciprofloxacin scintigraphy study to detect infectious lesions preferentially. Yaper et al, reported that Infecton has a sensitivity of 85%, specificity of 92% and accuracy of 88% in detecting orthopaedic infections compared with 78%, 100% and 90%, respectively for combined bone and gallium imaging (13). They concluded that although the two techniques show a similar clinical outcome, availability and short investigation time of ^{99m}Tc-Ciprofloxacin scintigraphy study make it the better option for detection of orthopaedic infections. Britton et al, reported overall sensitivity of 85% and a specificity of 82% for detecting infective foci in orthopaedic infection [17].

Sarda et al. reported a high sensitivity of 100%, low specificity 37% and high negative predictive value for the detection of bone and joint infection by using Infecton scintigraphy [18]. They concluded that the new developed modality could not discriminate between infected and aseptic osteoarticular diseases. Carrageenan is a natural polysaccharide obtained from edible red seaweeds. Carrageenan induced-inflammation test is widely used to investigate anti-inflammatory activity of any compounds and constitutes a simple and routine animal model for evaluation of pain at the site of inflammation, without any injury or damage to the inflamed tissue [19-21]. The development of inflammation following the injection of carrageenan has been described as biphasic in which various inflammatory mediators operate in sequence to produce the inflammatory response. There are several mediators involved in inflammation. Histamine, serotonin and bradykinin are the first detectable mediators in the early phase of

carrageenan induced-inflammation. Prostaglandins are involved in the increased vascular permeability and are detectable in the late phase of inflammation. Local and systemic inflammation is associated with enhanced levels of the pro-inflammatory mediators such as tumor necrozing factor (TNF), Interleukin 1(IL-1) and Il-6 [22]. Local neutrophil infiltration and activation also contribute to this inflammatory response. Oxygen-derived free radicals like superoxide anion and hydroxyl radicals have been suggested to involve in the inflammatory response induced by carrageenan [23]. Therefore, carrageenan induced-inflammation test is able to assess the capability of any radioisotope imaging to detect septic lesions. ^{67}Ga-Citrate has been used for visualizing infectious lesions in nuclear medicine.^{67}Ga is produced by cyclotron and emitted four principal gamma rays (93, 184, 296 and 388 KeV) suitable for imaging. When ^{67}Ga-Citrate is injected for scintigraphy approximately 90% of circulating ^{67}Ga is in the plasma, and nearly all of it is bound to transferrin. Increased blood flow and increased vascular membrane permeability result in enhanced delivery and accumulation of ^{67}Ga-transferrin at inflammation sites. ^{67}Ga can also bind to lactoferrin, which is present in high concentration at the inflammation sites. It has been suggested that ^{67}Ga is transported by binding to leukocytes. Direct uptake by certain bacteria has been observed in vitro, and this may account for ^{67}Ga uptake in infection foci. Siderophores, low-molecular weight chelates produced by bacteria, have a high affinity for ^{67}Ga. ^{67}Ga-siderophore complex is presumably transported into the bacterium, where it remains until phagocytosed by macrophages [24]. For the above mentioned factors, ^{67}Ga-Citrate scintigraphy could not differentiate the infection foci induced by S.aureus and the inflammatory lesions induced by carrageenan in the rats' feet. According to literature, the uptake of labeled ciprofloxacin is specific for the DNA gyrase of bacteria and therefore allows infected sites to be visualized. For this reason all the infectious lesions induced by S.aureus have been visualized by ^{99m}Tc-Ciprofloxin scintigraphy in our approach. The exact mechanism of localizing ^{99m}Tc-Ciprofloxacin at the inflammation foci is not elucidated completely. The following assumptions are suggested for non-specific uptake of ^{99m}Tc-Ciprofloxacin at the inflammation foci. The method of the reconstitution of this labeled compound is critical. A significant amount of colloid is formed, which, in turn, accumulates at the inflammation sites yielding false-positive results. A new labeling technique for ciprofloxacin tartaric acid and tin (II) chloride, instead of tin (II) chloride has minimized colloid formation [25]. It has been reported in the literature that neutrophil and activated macrophage can take up ciprofloxacin antibiotic [26]. In addition to the above factors, the local congestion and specific receptors may have a role. Therefore, ^{99m}Tc-Ciprofloxin scintigraphy has not shown any advantages to ^{67}Ga-Citrate radioisotope imaging to discriminate between infection and sterile inflammation in our assessment. This investigation was one of the first studies performed to evaluate the efficacy and efficiency of ^{99m}Tc-Ciprofloxin scintigraphy to differentiate septic and sterile inflammation lesions by using an experimental animal model.

CONCLUSION

In spite of high sensitivity of ^{99m}Tc-Ciprofloxin scintigraphy, it exhibited low specificity for differentiation between infection and sterile inflammation. It is necessary to consider the other modalities for interpretation of ^{99m}Tc-Ciprofloxin scintigraphic images.

Acknowledgment

This study is part of pharm-D thesis of Fatemeh Koshki and special thanks to Ahvaz Jundishapur University of Medical Sciences for the financial support and also to Radioisotope Division of Atomic Energy Organization of Iran for providing the freeze-dried Ciprofloxacin kits, ^{99}Mo/^{99m}Tc generator and ^{67}Ga-Citrate radioisotope.

REFERENCES

1. Britton KE, Vinjamuri S, Hall AV, Solanki K, Siraj QH, Bomanji J, Das S. Clinical evaluation of technetium-99m infecton for the localisation of bacterial infection. Eur J Nucl Med. 1997 May;24(5):553-6.
2. Palestro CJ. The current role of gallium imaging in infection. Semin Nucl Med. 1994 Apr;24(2):128-41.
3. Crerand S, Dolan M, Laing P, Bird M, Smith ML, Klenerman L. Diagnosis of osteomyelitis in neuropathic foot ulcers. J Bone Joint Surg Br. 1996 Jan;78(1):51-5.
4. Bohchelian HA, Klisarova AD, Koeva LA. Radioimmune imaging of diabetic foot infection - Tc-99m-labelled antigranulocyte antibody in combination with Tc-99m-methylene diphosphonate bone scintigraphy. Turk J Med Sci. 2002;32:255-59.
5. Weiner RE, Thakur ML. Radiolabeled peptides in diagnosis and therapy. Semin Nucl Med. 2001 Oct;31(4):296-311.
6. Mirshojaei SF, Gandomkar M, Najafi R, Sadat Ebrahimi SE, Babaei MH, Shafiei A, Talebi MH. Radio labeling, quality control and biodistribution of 99m Tc-cefotaxime as an infection imaging agent. J Radioanal Nucl Chem . 2011;287:21-25.
7. Shah SQ, Rafiullah Khan M. Radiocharacterization of the 99m Tc–rufloxacin complex and biological evaluation in Staphylococcus aureus infected rat model. J Radioanal Nucl Chem. 2011;288:373-78.

8. Motaleb MA. Preparation and biodistribution of 99m Tc-lomefloxacin and 99m Tc-ofloxacin complexes. J Radioanal Nucl Chem. 2007;272:95-99.

9. Benitez A, Roca M, Martin-Comin J. Labeling of antibiotics for infection diagnosis. Q J Nucl Med Mol Imaging. 2006 Jun;50(2):147-52.

10. Erfani M, Doroudi A, Hadisi L, Andishmand A, Mirshojaei SF, Shafiei M. (99m) Tc-tricabonyl labeling of ofloxacin and its biological evaluation in Staphylococcus aureus as an infection imaging agent. J Labelled Comp Radiopharm. 2013 Oct;56(12):627-31.

11. Erfani M , Doroudi A, Hadisi L, Andishmand A, Mazidi SM. Optimization condition in labeling of Ofloxacin with 99mTc and its biological evaluation in Staphylococcus aureus and Escherichia coli for infection imaging. Iran J Nucl Med. 2013;21(1):1-6.

12. Fuster D, Soriano A, Garcia S, Piera C, Suades J, Rodríguez D, Martinez JC, Mensa J, Campos F, Pons F. Usefulness of 99mTc-ciprofloxacin scintigraphy in the diagnosis of prosthetic joint infections. Nucl Med Commun. 2011 Jan;32(1):44-51.

13. Yapar Z, Kibar M, Yapar AF, Toğrul E, Kayaselçuk U, Sarpel Y. The efficacy of technetium-99m ciprofloxacin (Infecton) imaging in suspected orthopaedic infection: a comparison with sequential bone/gallium imaging. Eur J Nucl Med. 2001 Jul;28(7):822-30.

14. Singh B, Mittal BR, Bhattacharya A, Aggarwal A, Nagi ON, Singh AK. Technetium-99m ciprofloxacin imaging in the diagnosis of postsurgical bony infection and evaluation of the response to antibiotic therapy: A case report. J Orthop Surg (Hong Kong). 2005 Aug;13(2):190-4.

15. Hall AV, Solanki KK, Vinjamuri S, Britton KE, Das SS. Evaluation of the efficacy of 99mTc-Infecton, a novel agent for detecting sites of infection. J Clin Pathol. 1998 Mar;51(3):215-9.

16. Thomson AW, Fowler EF. Carrageenan: a review of its effects on the immune system. Agents Actions. 1981 May;11(3):265-73.

17. Britton KE, Wareham DW, Das SS, Solanki KK, Amaral H, Bhatnagar A, Katamihardja AH, Malamitsi J, Moustafa HM, Soroa VE, Sundram FX, Padhy AK. Imaging bacterial infection with (99m)Tc-ciprofloxacin (Infecton). J Clin Pathol. 2002 Nov;55(11):817-23.

18. Sarda L, Crémieux AC, Lebellec Y, Meulemans A, Lebtahi R, Hayem G, Génin R, Delahaye N, Huten D, Le Guludec D. Inability of 99mTc-ciprofloxacin scintigraphy to discriminate between septic and sterile osteoarticular diseases. J Nucl Med. 2003 Jun;44(6):920-6.

19. Sugishita E, Amagaya S, Ogihara Y. Anti-inflammatory testing methods: comparative evaluation of mice and rats. J Pharmacobiodyn. 1981 Aug;4(8):565-75.

20. Henriques MG, Silva PM, Martins MA, Flores CA, Cunha FQ, Assreuy-Filho J, Cordeiro RS. Mouse paw edema. A new model for inflammation? Braz J Med Biol Res. 1987;20(2):243-9.

21. Petersson M, Wiberg U, Lundeberg T, Uvnäs-Moberg K. Oxytocin decreases carrageenan induced inflammation in rats. Peptides. 2001 Sep;22(9):1479-84.

22. Cuzzocrea S, Sautebin L, De Sarro G, Costantino G, Rombolà L, Mazzon E, Ialenti A, De Sarro A, Ciliberto G, Di Rosa M, Caputi AP, Thiemermann C. Role of IL-6 in the pleurisy and lung injury caused by carrageenan. J Immunol. 1999 Nov 1;163(9):5094-104.

23. Salvemini D, Wang ZQ, Wyatt PS, Bourdon DM, Marino MH, Manning PT, Currie MG. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Br J Pharmacol. 1996 Jun;118(4):829-38.

24. Love C, Palestro CJ. Radionuclide imaging of infection. J Nucl Med Technol. 2004 Jun;32(2):47-57.

25. Rodriguez-puig D, Piera C, Fuster D, Soriano A, Sierra JM, Rubi S, Saudes J. A new method of [99mTc]-ciprofloxacin preparation and quality control. J Labelled Comp Radiopharm. 2006;49(13):1171-1176.

26. Easmon CS, Crane JP, Blowers A. Effect of ciprofloxacin on intracellular organisms: in-vitro and in-vivo studies. J Antimicrob Chemother. 1986 Nov;18 Suppl D:43-8.