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Research Article

The efficiency of new developed ^{99m}Tc-ceftazidime radiotracer to distinguish infection induced by *Staphylococcus aureus* and sterile inflammation induced by carrageenan assay in the rat model

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ABSTRACT

Despite our understanding of microorganisms has significantly progressed and antibiotic therapy has become increasingly available, infections a major cause of patient morbidity and mortality. The discrimination of septic from aseptic inflammation is still critical step in nuclear medicine. Therefore, a variety of radiopharmaceutical investigated to solve this problem. Recently, new developed ^{99m}Tc-Ceftazidime has been introduced as an ideal infection-seeking agent in the preclinical study. The principle of this approach is to examine the efficiency of labeled antibiotic with technetium 99m to differentiate infection from sterile inflammation lesions which were created in rat's foot. The cold ceftazidime kits have been reconstituted by ^{99m}Tc radioisotope. Yield of radio complex and radiochemical impurities analyzed by ITLC and Radio-HPLC investigations. Twenty adult, male NMRI rats were randomly divide into two groups equally. Infection was induced by S aureus and sterile inflammation created by Carrageenan assay. All lesions were induced in the rat's foot and then radioisotope assessments have been undertaken. Radiolabeling yield was above 90%. All affected foot could be easily visualized by scintigraphy imaging. The sensitivity of ^{99m}Tc-Ceftazidime was 100% to visualize the injury areas. Semi qualitative and quantitative investigations indicated that the radiotracer uptake at the septic lesion were higher than the aseptic lesions. Imaging with ^{99m}Tc-Ceftazidime radiotracer is high sensitive to tag infection or inflammation foci. The increased radiotracer uptake at the infection versus inflammation foci may be helpful for intelligent interpretation of scintigraphy imaging.

Key words: *Carrageenan, Cephalosporin, Infection-seeking agent, Radioisotope imaging, Sterile Inflammation, ^{99m}Tc-Ceftazidime*

INTRODUCTION

Discrimination of infection from sterile inflammation foci is still challenging step in clinical practice. Because of infections are the results of invasion and localize of microorganism pathogens in the body. Sterile inflammation is the natural response of the immune system against any other type of disorder or injury to the host. Infection without inflammation or inflammation without infection may be happened. It depends upon the cause of injury or disease. Different medical modalities have been recommended for the distinct identification of septic from aseptic lesions. Plain radiography is widely used as a first step practically in order to assess the lesions caused by infection or sterile inflammation. The other available imaging techniques, like ultrasonography, computerized tomography (CT) and magnetic resonance imaging (MRI) are highly sensitive, but these modalities have lack of selectivity for distinction of septic from aseptic foci, especially in early phase of disease when anatomic structures have not been considerably distorted.[1,2] These techniques are dependent on morphologic abnormality

changes that may not be present in the early stage of patient's disease. Radioisotope imaging can be considered as a part of the diagnosis procedures in this regard. This imaging technique can be very helpful, because the entire body is assessed and providing information on physiologic changes of organs. It is provided the condition that the specific organic function can be visualized and investigated by tracing the dynamic bio-distribution of a radiotracer in a specific organ. The advantage of radioisotope imaging is that the amount of radiotracer required for images is too low and usually in physiological range. Therefore, different radiopharmaceutical agents have been evaluated for potential detecting infection [3-5] or sterile inflammation [6-8] in both preclinical and clinical investigations. None of the currently available radiotracer is ideal with regard to bio-distribution, pharmacokinetics or selective uptake at the infection or sterile inflammation foci. For this reason, different investigations are made in order to develop an ideal radiotracer that has suitable characteristic for this propose. Recently, ^{99m}Tc-Ceftazidime has been examined as an ideal radiotracer to identify the septic lesion in preclinical investigation[9]. It is highly desirable to establish a reliable experimental test in preclinical step in order to determine the selectivity of any radio tracer agents which are investigated as an infection-seeking agent. Carrageenan test is commonly used as an experimental assay for inducing sterile inflammation lesion in the animals. This in-vivo experimental model has been frequently used to assess the anti-inflammatory effect of natural or synthetic compounds [10-13]. This investigation was conducted to assess the efficiency radioisotope scintigraphy with new developed ^{99m}Tc-Ceftazidime radiotracer to detect and distinct infection induced by Staphylococcus aureus (S aureus) and sterile inflammation lesions induced by Carrageenan assay in rat's foot.

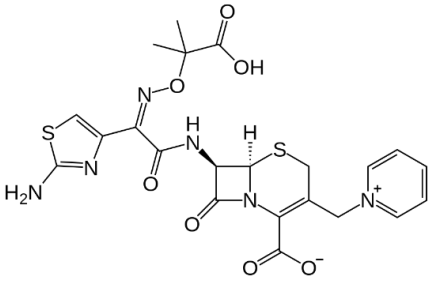

Figure 1: Structure of Ceftazidime

MATERIAL AND METHODS

All chemical materials have been purchased from Merck and Sigma. The chemicals and solvents were the highest purity and analytical grade and used without further purification. The new developed ceftazidimekits and ^{99}Mo/^{99m}Tc generators have been provided by Radioisotope Division of Atomic Energy Organization of Iran (AEOI). Technetium 99m as sodium pertechnetate was obtained from an in-house ^{99}Mo/^{99m}Tc generator using 0.9 % saline.

Animal Study

This approach was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences and allocated the ethical code IR.AJUMS.REC:139.5.416. All the ethics issues were considered based on the Ahvaz University of Medical Sciences Protocols (AUMP) on animal experiments. The rats with average weight 160±15 g were obtained from research center and experimental animal house of Ahvaz Jundishapur University of Medical Sciences. Therefore, twenty adults, male NMRI were acclimated to the conditions for one week before the experiment. All subjects were participated in this study were kept in individually wire-bottom stainless steel cages in an air-conditioned room at 24±1ºC with a 12 h light-dark cycle. The animals were fed with standard pellet diet and had free access to water. The rats were randomly assigned into two groups equally. Infection lesions were created by S aureus in the rat's foot in one population group. Sterile inflammation foci were induced by Carrageenan solution in the rat's foot in the other group.

Septic induced by S aureus

The samples of S aureus bacteria were taken from patients admitted to the infection department of teaching center of Razi hospital, Ahvaz, Khuzestan, Iran. Therefore, the specimens were taken from the infected wounds, using a sterile swab when possible or by fine needle aspiration in case of closed infections. The inoculation was on blood agar and MacConkey agar culture media and was followed by incubation at 37°C aerobically for 24 hours. On the basis of Clinical and Laboratory Standards Institute (CLSI) recommendations, all gram positive cocci occurring in pairs, short chains or cluster that they were catalase-positive and coagulase-positive by test tube and DNase-positive on agar. They were recognized as S aureus and chosen. By using a sterile-tip applicator, touch the surface of one to four morphologically identical, isolated colonies. Immerse the applicator into a tube containing Mueller Hinton broth. Rub the applicator against the wall of the tube slightly to release a small amount of growth into the liquid. Cap the tube and mix the cells using a vortex to form a suspension, 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 of the tube above the fluid level wall. The Mueller-Hinton agar plates were streaked in three dimensions. The plates were inoculated at 35°C for 24 hours. The turbidity was adjusted to a number 0.5 McFarland (each milliliter of 0.5 McFarland contains 1.5×10^8 microorganisms).The rats were anesthetized by diethyl ether. The right muscle thigh was disinfected with povidone (Poly vinyl pyrrolidone iodine) antiseptic solution followed by aqueous alcohol. Half milliliter of inoculums has been injected to each right foot of subjects. The injury area was irrigated and disinfected with normal saline and povidone solution respectively. Then the animals were returned back to their cages and kept individually. Septic lesion was induced in the right thigh muscle of animals by intramuscular injection of bacteria suspension. The radioisotope analysis with ^{99m}Tc-Ceftazidime radio tracer was undertaken 48 hours post inoculation of bacteria. The animals were inspected regularly for clinical manifestation of local or systemic infection during the above mentioned time.

Sterile inflammation induced by Carrageenan assay

Carrageenan powder was dissolved in normal saline in order to create sterile inflammation in the rat's foot on the experiment

day. Under brief synesthesia with diethyl ether, half milliliter of 3% Carrageenan solution was administrated intramuscularly in the right thigh muscle of rats. Visible redness and pronounced swelling was induced by Carrageenan and persisted for more than twenty hours.

The reconstitution ceftazidime by ^{99m}Tc radioisotope and quality control

The new developed freeze-cold kits were place at room temperature for 30 min. The 555MBq (15 mCi) sterile and pyrogen-free freshly eluted Na $^{99m}TcO_4$ in 2 ml saline was aseptically added to the evacuated vial and then the kit was put in the lead shield container, and allowed the mixture incubated for15 min. The contents of vial were slowly shaked by shaker for 2 min. The shielded vial in upright position was heated on the water bath for 15 min and finally the vial was removed from the water bath device and waited for 15 min at room temperature. The radiopharmaceutical investigations have been performed by ascending Instant Thin Layer Chromatography (ITLC) and Radio- High Performance Liquid Chromatography (Radio-HPLC). ITLC analysis was undertaken in order to identify and quantify $^{99m}TcO_2$, $^{99m}TcO_4$ and ^{99m}Tc-Ceftazidime radio complex respectively. Therefore, ITLC developed by using silica gel 60 (Merck) filter paper chromatography solid or stationary phase and two different mobile systems. The strips with 10 cm in length and 2 cm in width were used for each chromatogram. The strips were marked with pencil 1 cm (origin) and 0.5 cm (solvent front) from the base. A single spot of sample was applied with a capillary pipette on the 1cm line. The strips were allowed to dry at the room temperature and placed in air-tight containers. The radiochemical analysis by ITLC evaluation using acetone as the mobile phase, $^{99m}TcO_2$ and ^{99m}Tc-Ceftazidime remained at the spotting, while the free $^{99m}TcO_4$traveled to the solvent front. Using citrate dextrose solution (CDS contains: Citric acid anhydrous 0.073 g, Sodium citrate dihydrate 0.22 g, Dextrose monohydrate 0.245 g and Water for injection qs, pH 4.5-5.5) as the another mobile phase ^{99m}Tc-Ceftazidime remained at the spotting while $^{99m}TcO_2$ and $^{99m}TcO_4$ moved to the solvent front. After migration of mobile phase 1 cm from the top, the strips were removed from the air-tight containers and dried at the room temperature and cut to⅓ lower and ⅔ upper pieces. Each piece was counted by using a single channel gamma counter with NaI (Tl) detector. The Radio-HPLC analysis was performed with analytical reverse-phase on a JASCO 880-PU intelligent pump HPLC system (Tokyo, Japan) equipped with a multi wavelength detector and a flow-through Raytest-Gabi g-detector CC 250/4.6 Nucleosil 120-5 C-18 column from Teknokroma was used for HPLC. For radionuclide analysis of ^{99m}Tc-Ceftazidime complex by HPLC, a volume of 10µl of the test solution was injected into the C-18 reverse-phase column. A mixed solvent containing 10% ethanol and 0.2M phosphate buffer, pH 7.2 and flow rate 0.5 ml/min was used for Radio-HPLC analysis Fig. 2.

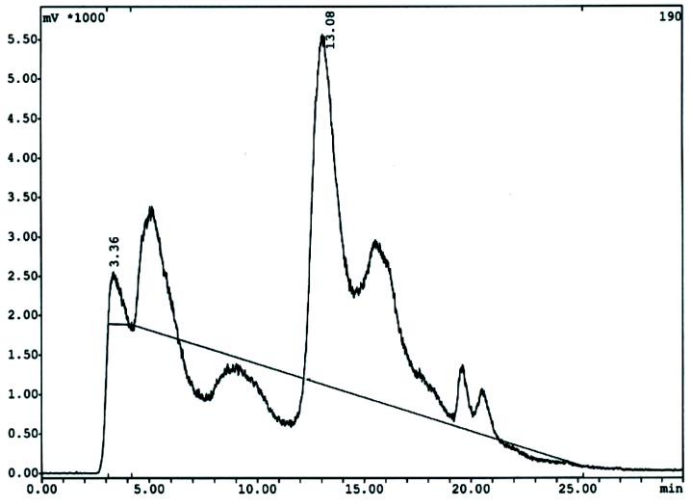

Figure 2: Radio-HPLC chromatogram of ^{99m}Tc-Ceftazidime

Radio-HPLC profile of ^{99m}Tc-Ceftazidime radio conjugate has been obtained 1 h post reconstitution. ^{99m}TcO4 and of ^{99m}Tc-Ceftazidime species were readily identified by Radio-HPLC. The retention times of ^{99m}TcO4 and main^{99m}Tc-Ceftazidimeradio complex were approximately 3.36 and 13.08 min respectively.

Radioisotope investigation

Each rat was placed in the restrainer device, and the 37 MBq (1 mCi) ^{99m}Tc-Ceftazidime radiotracer injected intravenously by contra lateral tail vein. The animals were returned back to their cages and kept them individually. The rats were anesthetized with diethyl ether approximately 1 h post injection. The anesthetized subjects were placed in a prone position with limbs spread out and fixed on the board with surgical tape for radioisotope imaging. A single-headed gamma camera (E-Cam, Siemens USA) was used in all studies. Imaging was undertaken 1 h post injection of radiotracer. Anterior and posterior static images were acquired by using a large field of view gamma camera peaked to 140 keV with a 15% window and low –energy all-purpose collimator for 500 kilo counts per image. Acquisition parameters were as follow: matrix size 256×256, zoom factor ×3, anterior and posterior views for 5 min and energy window 140 keV and reconstitution method: filter back projection. The gamma camera was positioned in order to image the affected foot and contra lateral healthy site in all subjects Fig 3. Three criteria haven been considered for radioisotope analysis. First, the visual inspection of accumulation of radio complex at the affected foot versus to the contra lateral healthy unaffected foot in all animals Fig. 3. Second, the specific radiotracer uptake factor (SUF) was measured at the affected foot in comparison to the contra lateral healthy site. Therefore, by using available commercial software, the activity at the affected foot versus contra lateral healthy foot was quantified in all subjects. For this reason, the region of interest (ROI) as target was generated on the affected foot, second ROI was created on the contra lateral healthy foot as non-target in interior view. The SUF was calculated by dividing count per pixel in target to non-target. The back ground subtraction was not used in all studies. All images were interpreted by three independent nuclear physicians and their final option was achieved by

consensus. The observers were completely unaware about the nature of induced injuries on the rat's foot. Third, the quantitative analysis has been undertaken after imaging study. The animals were sacrificed by diethyl ether and the organ of interest like affected foot, unaffected foot, liver, kidneys, stomach, intestine, bladder, lungs, heart and spleen were removed and weighed. The relative activity of each organ to the interest organs was measured for all subjects. The results have been shown in table 1.

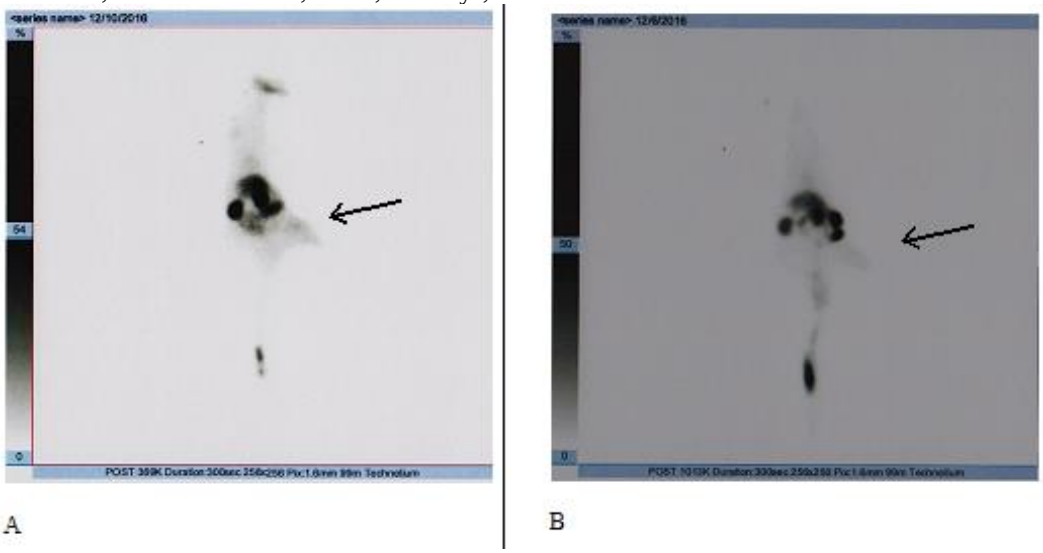

Figure 3: Scintigraphy imaging with ^{99m}Tc-Ceftazidime

Table 1: Relative bio-distribution of 99mTc-Ceftazidime in rat

Organ / Relative Uptake	Liver	Kidneys	Intestine	Affected foot	Unaffected foot	Stomach	Lungs	Spleen	Bladder	heart
Infection	32.88± 0.14	20.96± 2.07	32.02± 2.41	5.34± 0.17	2.65± 0.2	0.89± 0.01	1.19± 0.7	0.84± 0.26	2.00± 0.45	1.22± 0.36
Inflammation	22.43± 3.16	28.11± 2.16	31.86± 1.26	6.85± 0.19	4.97±0.1	2.03± 0.1	1.3± 0.13	0.19± 0.05	1.28± 0.6	0.98± 0.1

^{99m}Tc-Ceftazidime imaging has been undertaken 1h post injection of the 37MBq (1 mCi) radiotracer due to contra lateral tail vein, the posterior view images demonstrated lesions A: infection induced by Saureus B: sterile inflammation induced by Carrageenan test.

Quantitative analysis has been performed after radioisotope imaging. Therefore, the rats were killed by diethyl ether and organ of interest like liver, kidneys, intestine, affected foot, unaffected foot, stomach, lungs, spleen, bladder and heart removed and weighted. The relative activity of each organ to the interest organs was calculated. The data in top row belong to the radiotracer bio-distribution in the rats (n= 10) infected with S aureus. The data in bottom row belong to the radiotracer bio-distribution in the rats (n = 10) with sterile inflammation lesion, which were induced by Carrageenan assay

STATISTICAL ANALYSIS

All the results were expressed as mean± SD. The calculations were performed by SPSS software in this approach. Paired samples t test was performed to assess the bio-distribution of radiotracer between the two principle group animals which were participated in this experiment. Statistics analysis based on the cases with no missing or out of range data for any variable in the analysis. Differences at the 95% confidence level $p< 0.05$ were considered significant.

RESULTS

Each cold lyophilized Ceftazidime kits contained 2 mg of antibiotic ligand and 0.5 mg Stannous chloride dihydrate as a reducing agent. ^{99m}Tc as pertechnetate form has the highest oxidation state in elution solution. It must be reduced to the lower oxidation state in order to react with the ligand molecule. The various complex samples with ^{99m}Tc radioisotope may be formed by interactions between electron donor groups with empty orbitals of reduced ^{99m}Tc. Ligand structure has electron donors such as nitrogen, oxygen, phosphorus and sulfur in order to form bonds with ^{99m}Tc. The exact radio complex structure is not elucidated, results showed that ceftazidime coordinated with ^{99m}Tc because of its electron donor groups in its structure [9].Free $^{99m}TcO_4$ and reduced technetium $^{99m}TcO_2$ are two main radiochemical impurities formed during the labeling of new developed ceftazidime kits with ^{99m}Tc radionuclide.

The $^{99m}TcO_4$, $^{99m}TcO_2$ and ^{99m}Tc-Ceftazidime radio complex were readily detected and measured by ITLC analysis. The $^{99m}TcO_2$ cannot be identified and quantified by Radio-HPLC assay. The ratio of $^{99m}TcO_4$ and radio complex can only be identified and quantified by Radio-HPLC. According to the

results have been obtained from ITLC assay, the yields of $^{99m}TcO_2$, $^{99m}TcO_4$ and ^{99m}Tc-Ceftazidime radiotracer (n= 15) were 4.07± 1.6, 4.18± 1.15 and 91.75± 2.25 respectively. As it has been stated in Fig 1, different electron donor atoms are present in the ceftazidime molecule. Therefore, the different radio complex could be formed during the direct radio labeling of ceftazidime. The retention time for $^{99m}TcO_4$ and main^{99m}Tc-Ceftazidime radio conjugate were approximately 4 and 13 min respectively and the yield of radiotracer complex samples were more than 96%. The Radio-HPLC analysis indicated that the reaction was leaded to multiple radio complex samples Fig 2. The above mentioned findings demonstrated that the reconstitution of the new developed cold kits with ^{99m}Tc could be successful in this approach. Radioisotope investigations have been performed 48 h post inoculation of bacteria mean while, these studies have been undertaken 2 h after the sterile inflammation lesions induced by carrageenan test on the experiment day. All injuries were induced in the right foot of subjects in order to exclude any misinterpretation of images. All affected areas were identified by visual inspection of images. The quality of images was appropriate in each case and did not change over time. Qualitative analysis indicates imaging with ^{99m}Tc-Ceftazidime was sensitive to tag and identify the affected region. Semi qualitative assessment has been performed in order to quantify the radiotracer uptake at the affected foot to the contra lateral healthy foot. The target to non-target ratios at the infection (n=10) and sterile inflammation (n=10) foci were 15.02± 3.12 and 6.27± 1.76respectively. Therefore, the proportional accumulation of radiotracer at the infection to sterile inflammation loci was approximately 2.40. This matter demonstrated that the radiotracer uptake significantly enhanced at the septic versus aseptic lesions. In spite of visual inspection of images with ^{99m}Tc-Ceftazidime radio complex could not differentiate the induced lesions, but semi qualitative analysis could show the relative selectivity of radiotracer to the septic in comparison to aseptic foci. The quantitative investigation has been done in order to provide the further information about the bio-distribution of ^{99m}Tc-Ceftazidime at the affected foot and the other organs of animals. As it has stated in table 1, the target to non-target ratios were 2.015 and 1.37 at the septic and aseptic foci. It revealed this matter that there is significant difference between the radiotracer uptake at the infection versus sterile inflammation lesions. This finding was completely consistent to the result has been obtained in semi qualitative analysis. The pathologic condition due to infection or sterile inflammation could not lead to a significant difference of organ distribution of radiotracer, except the accumulation of radio complex has been increased at the infection versus sterile inflammation lesions. The highest radioactivity has been observed in liver, kidney and intestine. Similar pattern of organ distribution of radiotracer has been seen between two groups of animals that they have been participated in this study. The data have been obtained by radioisotope investigations indicated that the imaging with ^{99m}Tc-Ceftazidime is high sensitive to detect the septic and aseptic lesions. In addition to the above characteristic, the relative selectivity could be observed to identify the infection foci. These promising characteristics reveal this matter that the imaging with new developed radiotracer is very suitable candidate for diagnostic of infection lesions in nuclear medicine. It can be recommended as an alternative radiopharmaceutical for infection-seeking agent.

DISCUSSION

The identification of infection lesions by radioisotope imaging depends on the physiological and biochemical changes that would be happened at the site of foci, which appear much earlier than anatomical changes. This imaging technique is high sensitive to distinct infection lesions, but the selectivity of this modality is low. Therefore, it cannot always discriminate between septic and aseptic lesions in clinical practice. Different radiopharmaceutical agents have been examined to localize infection and sterile inflammation for scintigraphy imaging. An ideal radiotracer agent has the following characteristics to differentiate infection or inflammation lesions. It has high sensitive toward infection or inflammation lesions. It should discriminate infection from inflammation. The toxicity and immunogenic reactions have not been observed after administration of radiotracer agent to the patient. It has a rapid clearance from the blood and no gastrointestinal uptake. In addition to the aforementioned factors, the reconstitution of cold kit is easy and low cost at the nuclear medicine departments. Since no currently available radiopharmaceutical agents is not ideal in this regard. For this reason, wide range radiotracers have been investigated in order to find the solution of this dilemma. Imaging with ^{67}Ga-Citrate radiopharmaceutical is the most primitive effort to detect infection lesions in nuclear medicine. But it has the following disadvantage characteristics. ^{67}Ga has long physical half-live decay, multiple gamma radiations causing high ionization radiation absorbed doses and it is not available as generator. It is the product of cyclotron and relative high expensive. In addition to the above mention factors, this radiotracer is high sensitive for both infection and sterile inflammation lesions.[14] Different radio-labeled ligands with ^{99m}Tc radionuclide such as monoclonal antibodies,[15] cytokines,[16] chemo tactic peptides[17] and human defensins[18] have been assessed to distinct infection foci and sterile inflammation processes. Each suggested radio-pharmaceutical agent has especial advantage and disadvantage characteristics to discriminate between septic and aseptic foci. Radio-labeled leukocyte can be considered as a gold standard to detect infection lesions. The blood must be taken from the patient in order to perform the imaging with radio-labeled leukocyte. Then, leukocyte must be separated and labeled with radioisotope. The radio-labeled leukocyte should be re-injected to the patient. The process of labeling is time-consuming and has the potential risk of contamination or transmission of blood-borne microorganisms to patient or technician. The imaging with radio-labeled leukocyte cannot used in neutropenic patients.[19] The antibiotic molecules are readily taken and metabolized by microorganisms. Theoretically, radio-labeled antibiotic agents have potentially to incorporate and metabolize by the pathogens that are present at the infection site.

The measured radioactivity is proportional to the number of pathogens present at the septic foci in order to visualize the affected area by radioisotope imaging. It is important to consider this point; the tiny amount of antibiotic is always used in diagnostic modalities. Therefore, the new developed

antibiotic radiopharmaceutical does not have any therapeutic effect. Majority of various antibiotics examined as infection-seeking agents are those of the quinolones, second and third generation cephalosporines.[20] The radio-labeled antibiotic agents could demonstrate promising sensitivity in diagnosing a wide variety of infection lesions.^{99m}Tc-Ciprofloxacin, also known as Infecton, was the first radiolabeled antibiotic examined in human to identify infection lesions.[21] According to the literature, controversial results have been reported in sensitivity and selectivity of radioisotope imaging with Infecton to discriminate infection and sterile inflammation foci. These controversial and variable data may be depended on the type and site of infections, strain of microorganisms, concomitant of antibiotic therapy and lack of interpretation criteria. Some researchers have reported that Infecton is an infection-seeking agent to distinct pulmonary or extra pulmonary tuberculosis, fever unknown origin (FUO), osteomyelitis, hip or knee prosthesis and orthopedic infection.[22-25] But the other investigations, Sarda et al,[26] Appelboom et al,[27] De Winteret al,[28] Pucar et al[29] and Doroudi et al[30] have reported high sensitivity but low selectivity for imaging with Infecton. These investigations have been performed in different kind of infection models and images interpreted with different methods. But they concluded that imaging with Infecton is not able to differentiate infection from sterile inflammation foci. Cephalosporin agents have been labeled with radioisotope in order to detect infection lesions. El-Tawoosy investigated the optimum radio-labeling condition of cefazolin with ^{99m}Tc and its bio-distribution in infected mice and turpentine oil as control. The researcher reported that the radiolabeled cefazolin could be able to discriminate the early stage of infection from sterile inflammation lesions.[31] Ceftriaxone is third generation cephalosporin and has been evaluated infection-seeking agent by various researchers. Mostafa et al studied the radio-labeling of ceftriaxone and its bio-distribution in a mice model, infected with a live Escherichia coli (E. coli), heat killed bacteria and turpentine oil as control. They reported the potential ability of radio-labeled antibiotic to discriminate infection from sterile inflammation lesions.[32] Fazli et al conducted another study to evaluate the efficiency of imaging with ^{99m}Tc-Ceftriaxone to identify infection induced by S aureus and sterile inflammation induced by turpentine oil in mice. They reported that the radio-labeled antibiotic could not discriminate septic from aseptic lesions.[33] Sohaib et al confirmed the ability of this radiotracer to differentiate the infection from sterile inflammation. ^{99m}Tc-Cefriaxone has been examined in rats, infected with E coli or S aureus, while turpentine oil used in control subjects. The outcome of this approach indicated that accumulation of radiotracer in the infected region in animals injected with E coli rather than aureus or turpentine oil.[34] They concluded that imaging with ^{99m}Tc-Ceftriaxone may be recommended as an infection-seeking agent for E coli. Ceftazidime is the third generation of cephalosporin with the biological half-life of 1.6-2 h. Like the other third generation cephalosporines, it has broad spectrum activity against gram positive and gram negative bacteria and resistant to β lactamase. Ceftazidime interfere synthesis of the peptidoglycan layer of bacterial cell walls by binding to penicillin binding proteins (PBP), causing the cell walls to break down and eventually the bacteria die. Since cephalosporin agents like ceftazidime interrupt on the processes that are unique to bacteria, it has been examined that radio-labeled ceftazidime can be able to discriminate septic from aseptic lesions by radioisotope imaging. Therefore, therapeutic characteristics of ceftazidime were used for diagnostic imaging to localize the infection loci. Mirshojaei et al assessed the radio-labeling of ceftazidime with ^{99m}Tc and examined its bio-distribution in normal and S aureus infected mice and turpentine oil as control. They reported that ^{99m}Tc-Ceftazidime may be considered as an ideal infection-seeking agent.[9] Its' completely justified to evaluate the sensitivity and specificity of ^{99m}Tc-Ceftazidime radiotracer to detect infection or inflammation by the valid and reliable experimental method in order to introduce this radio-pharmaceutical to nuclear medicine departments. Carrageenan is a natural polysaccharide obtained from edible red seaweeds. Carrageenan test is widely used as an investigational assay to examine anti-inflammatory any compounds in animals without any injury or damage to the inflamed tissue. Reliable and reproducible sterile inflammation lesions can be created by carrageenan test like the real inflammation processes. According to the literature, different mechanisms have been recommended for Carrageenan in order to induce aseptic lesions in the animals. Several inflammatory mediators like histamine, bradykinin and serotonin could be released in early phase of sterile inflammation process. Prostaglandins are accompanied and caused the enhanced vascular permeability at the inflammation foci. The levels of other inflammatory mediators such as Tumor Necrozing Factor (TNF), Interleukin I (IL-1) and IL-6 are increased. These mediators are responsible for local and systemic inflammation. Local neutrophil infiltration and activation are also involved in producing inflammation process.[35-37] Exact mechanism for non-specific uptake of radio-labeled antibiotic is not determined at the sterile inflammation lesions induced by Carrageenan assay. The following mechanisms may be considered for the radio complex uptake at the inflamed region. The local congestion and increased vascular permeability induced by carrageenan test, the radio-labeled antibiotic could be transferred at the inflamed tissue. The non-specific attachment of radiotracer to the other receptors may be considered. The diagnostic dose of ceftazidime in comparison to pharmacologic dose for bactericidal effect is low. The crucial role of antibiotic molecule is a carrier for ^{99m}Tc to transfer to the infection site specifically. Therefore, the radio conjugate could be traveled to the affected area in the appropriate concentration to detect inflamed region by imaging.

The inflamed area was created at the septic and aseptic foci. Bacteria were present at the infection site and radio-labeled antibiotic could interact with bacteria in addition to the above mentioned factors that radiotracer was accumulated at the inflamed region. Therefore, the uptake of radiotracer at septic sites was higher than the sterile inflammation foci. The radiotracer was readily accumulated in inflammatory regions in the sufficient amount to yield the images with appropriate quality. Therefore, the discrimination of infection from sterile inflammation could not be observed by visual inspection of imaging. The relative selectivity could be observed by semi-qualitative and quantitative analysis in this investigation. This study was one of the first studies performed to investigate the sensitivity and specificity of ^{99m}Tc-Ceftazidime radioisotope imaging to distinct infection and sterile inflammation foci by

using a reliable experimental animal model. Carrageenan test is a new developed experimental tool to evaluate the potentially of any radiopharmaceutical kit has been examined as an infection-seeking or inflammation-seeking agent.

CONCLUSION

The results have been obtained from this investigation demonstrated that scintigraphy imaging with ^{99m}Tc-Ceftazidime radiotracer is high sensitive to subtle changes in physiological processes. Qualitative analysis could not differentiate septic or aseptic lesions. The other medical attempts must be used for intelligent interpretation of ^{99m}Tc-Ceftazidime imaging. The radiotracer uptake at the affected foot as target in comparison to unaffected foot as non –target is one of these modalities. This factor may be helpful to distinguish infection and sterile inflammation lesions. The promising characteristic of new developed radio-labeled antibiotic is very suitable for diagnostic of infection foci in nuclear medicine. It may be recommended as an alternative radiopharmaceutical for infection-seeking imaging agent.

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CONFLICT OF INTEREST

This approach is part of Pharm-D thesis of Yasin Ahmadi. Authors have no relevant financial interests related to the material in this manuscript. They also have no conflict of interests to declare.

ABBREVIATIONS

Bq: Becquerel, Ga: Gallium, H: Hour, Ci: Curie, Min: Minute, Mo: Molybdenum, TcO_4^-: Pertechnetate, Tc: Technetium

REFERENCES

1. Yuh WT, Corson JD, Baraniewsli HM et al. Osteomyelitis of the foot in Diabetic Patients: Evaluation with plain film, ^{99m}Tc-MDP bone scintigraphy and MR Imaging. AJR Am J Roentgenol 1989; 152(4): 795-800
2. Cavanagh PR, Young MJ, Adams JE, Vickers KL, Boulton AJ. Radiographic abnormalities in the feet of patients with diabetic neuropathy. Diabetes Care 1994; 17(3): 201-209
3. Erfani M, Doroudi A, Hadisi L, Andishmand A, Mazidi SM. Optimization condition in labeling of ofloxacin with ^{99m}Tc and its biological evaluation in Staphylococcus aureus and Escherichia coli for infection imaging. Iran J Nucl Med 2013; 21(1): 1-6
4. Erfani M, Doroudi A, Hadisi L, et al. ^{99m}Tc-tricarbonyl labeling of ofloxacin and its biological evaluation in Staphylococcus aureus as an infection agent. J Label Comp Radiopharm 2013; 56(12): 627-631
5. Doroudi A, Samaee H, Saadati SM, et al. Clinical evaluation of ^{99m}Tc-MDP three phase bone scan and ^{99m}Tc-UBI29-41 scintigraphy imaging in preferentially detection infection from sterile inflammation among diabetic patients with suspected foot infection. IOSR J Pharm 2015; 5(2): 20-26
6. El-Ghany EA, Amine AM, El-Sayed AS, El-Kolaly MT, Abdel-Gelil F. Radiochemical and biological characteristics of ^{99m}Tc-Piroxicam for scintigraphy of inflammatory lesions. J Radioanal Nucl Chem. 2005; 266(1): 125-130
7. Sanad MH, Amin AM. Optimization of labeling conditions and bio evaluation of ^{99m}Tc-Meloxicam imaging. Radiochem 2013; 55(5): 521-526
8. Erfani M, Sharifzadeh S, Doroudi A, Shafiei M. Labeling and evaluation of ^{99m}Tc-tricarbonyl-meloxicam as a preferential COX-2 inhibitor for inflammation imaging. J Label Comp Radiopharm 2016; 59(7): 284-290
9. Mirshojaei SF, Erfani M, Shafiei M. Evaluation of ^{99m}Tc-ceftazidime as bacterial infection imaging agent. J Radioanal Nucl Chem 2013; 298(1): 19-24
10. Thomson AW, Fowler EF. Carrageenan: a review of its effects on the immune system. Agents Actions 1981; 11(3): 265-273
11. Henriques MG, Silva PM, Martins MA, et al. A new model for inflammation? Braz J Med Biol Res 1987; 20(2): 243-249
12. Neelakandan Y, Venkatesan A. Antinociceptive and anti-inflammatory effect of sulphated polysaccharide fractions from Sargassum wightii and Halophila ovalis in male wistar rats. Indian J Pharmacol 2016; 48: 562-570
13. Vigilde Mello SV, da Rosa JS, Facchin BM, et al. Beneficial effect of Ageratum conyzoides linn (Astraceae) upon inflammatory response induced by carrageenan into the mice pleural cavity. J Ethnopharmacol 2016; 194: 337-347
14. Palestro CJ. The current rule of gallium imaging in infection. Sem Nucl Med 1994; 14: 128-141
15. Pak KY, Nedelman MA, Tam SH, Wilson E, Daddona PE. Labeling and stability of radiolabeled antibody fragment by a direct 99mTc-labeling method. Int J Rad Appl Instrum B 1992; 10(6): 669-677
16. Signore A, D'Alessandria C, Annovazzi A, Scopinaro F. Radiolabeled cytokines for imaging chronic inflammation. Braz Arch Biol Tech 2002; 45: 15-23
17. Weiner RE, Thakur ML. Radiolabelled peptides in diagnosis and therapy. Semin Nucl Med 2001; 31(4): 296-311
18. Gandomkar M, Najafi R, Shafiei M, et al. Clinical evaluation of antimicrobial peptide ^{9m}Tc/Tricine/ HYNIC]ubiquicidin$_{29-41}$ as a human specific infection imaging agent. Nucl Med Biol 2009; 36(2): 199-205
19. Crerand S, Dolan M, Laing P et al. Diagnosis of osteomyelitis in neuropathic foot ulcers. J Bone and Joint Surg 1996; 78: 51-55
20. Auletta S, Galli F, Lauri C et al. Imaging bacteria with radio-labelled quinolones, cephalosporins and siderophores for imaging infection: a systemic review. Clin Transl Imaging 2016; 4: 229-252
21. Vinjamuri S, Hall AV, Solanki KK et al. Comparison of ^{99m}Tc-Infecton imaging with radio-labelled white-cell imaging in the evaluation of bacteria infection. Lancet 1996; 347(8996): 233-235
22. Hall AV, Soluanki KK, Vinjamuri S, Britton KE, Das SS. Evaluation of the efficacy of ^{99m}Tc-Infecton, a novel agent

for detecting sites of infection. J Clin Pathol 1998; 51(3): 215-219
23. Falagas ME, Valotassiou VJ, Papadouli D, Papadopoulos A, Malamitsi J. ^{99m}Technetium-ciprofloxacin scintigraphy for the evaluation of spinal infections. Clin Orthop Rel Res 2006; 444: 34-37
24. Lee M, Yoon M, Hwang KH, Choe W. Tc-99m ciprofloxacin SPECT of pulmonary tuberculosis. Nucl Med Mol Imaging 2010; 44(2): 116-122
25. Fuster D, Soriano A, Garcia S, et al. Usefulness of 99mTc-ciprofloxacin scintigraphy in the diagnosis of prosthesis infections. Nucl Med Commun 2011; 32(1): 44-51
26. Sarda L, Cremieux AC, Lebellec Y, et al. Inability of ^{99m}Tc-ciprofloxacin scintigraphy to discriminate between septic and sterile osteoarticular diseases. J Nucl Med 2003; 44(6): 920-926
27. Appelboom T, Emery P, Tant L, Dumary N, Schoutens A. Evaluation of technetium-99m-ciprofloxacin (Infecton) for detecting sites of inflammation in arthritis. Rheumatol 2003;42(10):1179-1182
28. De winter F, Gemmel F, Van Laere K et al. ^{99m}Tc-ciprofloxacin planar and tomographic imaging for the diagnosis of infection in the postoperative spine: experience in 48 patients. Eur J Nucl Med Mol Imaging 2004; 31(2): 233-239
29. Pucar D, Jancovic Z, Dugonjic S, Popovic Z. Estimation of ^{99m}Tc-ciprofloxacin accumulation indexes in bone and joint bacterial infections. Vojnosanit Pregl 2009; 66(5): 395-398
30. Doroudi A, Erfani M, Kooshki F et al. 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. Iran J Nucl Med 2015; 23(2): 96-102
31. El-Tawoosy M. Preparation and biological distribution of ^{99m}Tc-cefazolin complex, a novel agent for detecting sites of infection. J Radioanal Nucl Chem 2013; 298(2): 1215-1220
32. Mostafa M, Motaleb MA, Sakr TM. Labeling of ceftriaxone for infective inflammation imaging using ^{99m}Tc eluted from ^{99}Mo/^{99m}Tc generator based on zirconium molybdate. Appl Radiat Isot 2010; 68(10): 1959-1963
33. Fazli A, Salouti M, Mazidi M. ^{99m}Tc-ceftriaxone as a targeting radiopharmaceutical for scintigraphic imaging of infection foci due to Staphylococcus aureus in mouse model. J Radioanal Nucl Chem 2013; 298(2): 1227-1233
34. Sohaib M, Khurshid Z, Roohi S. Labeling of ceftriaxone with^{99m}Tc and its bio-evaluation as an infection imaging agent. J Label Compd Radiopharm 20143; 57(11): 652-657
35. Sugishita E, Amagaya S, Ogihara Y. Anti-inflammatory testing methods: comparative evaluation of mice and rats. J Pharmacobiodyn 1981; 8: 565-575
36. Petersson M, Wiberg U, Lunderberg T, Uvans-Moberg K. Oxytocin decreases Carrageenan induced inflammation in rats. Peptides 2001; 22(9): 1479-1484
37. Cuzzocrea E, Sautebin L, De Sarro G, et al. Role of IL-6 in pleurisy and lung injury caused by Carrageenan. J Immunol 1999; 163(9): 5094-5104