The need for rapid diagnosis of infections on one hand and avoid unnecessary surgery on the other hand, can be very effective in the treatment process. Currently available diagnostic methods, such as Computerized Tomography (CT-scan) and Magnetic Resonance Imaging (MRI) which had ability to provide precise images for imaging abnormalities needed to make morphological changes, the process is time consuming and cannot be useful in the early stages of infection (Kumar R et al. 2008, Stokkle M et al., 2002) Expanding the use of radionuclide’s, mainly due to the ease of use, determination and high sensitivity of methods that make use of specific radionuclide (Bylund DB, Toews ML 1993). So far, a wide variety of radiopharmaceuticals have been developed for the detection of inflammation and infection but already marked leukocyte as gold standard for diagnosis of infection are known. (Mirshojaei S et al., 2011) However, because of the in vitro preparation of leukocytes is complicated and expensive and not always available to work also, direct contact with the blood products increase the risk of secondary infections in patients, have not been warmly welcomed (Beker W et al., 1996, Palestro C et al., 2001). Now a lot of progress has been made in the field of imaging anti-infective agents. In 2002, the role of nuclear medicine techniques in the diagnosis of infection was studied and then progress on the use of antimicrobial agents labeled as selective markers for the diagnosis of bacterial infections, tuberculosis and fungal increased (Wareham D et al., 2005). These radiopharmaceuticals accumulate in infected sites and are metabolized by bacteria. So far, the largest pharmaceutical group that was studied in this context is the group of fluoroquinolones.

The use of radiopharmaceuticals is a good way to track and treat infections. In this study, Gemifloxacin a broad-spectrum antibiotic from the family of fluoroquinolones by using of Technetium 99m (Tc 99m) and direct method was labeled and used.Gemifloxacin by using of technetium 99m and direct method was labeled, then the stability of the drug, bind to Escherichia coli and bio-distribution in Mice was examined then possible changes in tissue by Hematoxylin and eosin (H&E) staining and immunohistochesmal (IHC) staining for Desmin marker was evaluated. By using of High performance liquid chromatography (HPLC), labeling efficiency, 96.94 % and binding to bacteria 95.43 % was determined. The results of radiopharmaceutical biodistributions indicate its tendency to the position of infection. Presence of lesions in the muscle tissues after infection was confirmed by histopathological methods and since excretion of drug, effects diminished. Desmin was shown in all samples. The combination can be used as a factor in taking pictures of places with Escherichia coli infections, without causing tissue damage.

**Keywords:** Gemifloxacin, Escherichia coli, Desmin, Infection, Technetium 99m.
(Mirshojaei S et al., 2011) the first preparation of radiopharmaceuticals from this branch is labeled ciprofloxacin with technetium. This group of drug has strong antimicrobial activity. For wide range of Gram-negative bacteria DNA gyrase is target and in many Gram-positive bacteria, topoisomerase IV is the site which drug effect on it. (Fournier B et al., 2000, Solanki K et al., 1993). In research by Syed Qaiser Shah • Muhammad Rafiullah Khan was released in December 2010 , the composition of, Gemifloxacin - technetium- 99 , introduced as a special agent for imaging of infections caused by Streptococcus pneumonia. (Syed Qaiser Shah & Muhammad Rafiullah Khan 2011)

In this study, the effect of Gemifloxacin on Escherichia coli, as one of the most common infectious agent, and its histopathological effects on skeletal muscle have been examined. To investigate damage to muscle tissue, Desmin as one of the effective factors in the contraction can be studied. This protein is one of the intermediate filaments; create scaffold around the lines (Z) and attach them into the cell skeleton under the plasma membrane as well as keeps muscle fiber’s sides together. So that is being an effective on contracting process. Desmin also have an impact on the proper functioning of mitochondria and is not detectable by conventional staining methods. To investigate it, the immunohistochemical techniques are used. (Desmin 2013)

MATERIALS AND METHODS

All materials used, were manufactured by Merck and Sigma. For immunohistochemical staining Nova link products were used.

All institutional and national guidelines for the care and use of laboratory animals were followed.

For the quality control radiopharmaceutical, model JASCO 880-PU HPLC apparatus with multi wave length detector and gamma-ray test-gabi detector with column cc250-4.6 nucleosil 120/5 c18 with the following gradient system was used.

Mobile phase A: solution containing 0.1% TFA (tri-chloro acetic acid) in distilled water.
Mobile phase B: acetonitrile 100 %
Gradient system as follows:
Zero minute A 95%, B5% - five minutes A95%, B5% - five minutes A0%, B100% - 30 minutes A0%, B100%.
Gamma counter EG & G / ORTEC / Model 4001M was used to determine activity.
For taking pictures Gamma camera, models Siemens smallarea mobile device was used.
Technetium $^{99m}$ from internal home generator Molybdenum$/^{99m}$/ Te$^{99m}$ was achieved.

Radiopharmaceutical preparation

10 ml of distilled water was mixed with 100 ml of hydrochloric acid so 0.1 normal hydrochloric acid was obtained. We solved 0.0031 grams of Sncl2 in 10 ml of hydrochloric acid 0.1 normal. 320 milligram (mg), Gemifloxacin in 10 ml of deionized water twice distilled solved, Then 62.5 micro liters (µl) of the above solution, which is equivalent to 2 mg of the drug, with 32.25 µl of Sncl2 solution equivalent to 100 mg Sncl2, mixed and 1 ml of radioisotope added to it, until 15 minutes, it was shaken every 2 minutes.

Evaluation of labeling Performance

Solution obtained with the features mentioned was analyzed by HPLC.

Evaluation of stability

100 µl of labeled antibiotic added to 1 ml of human serum and leave it for 60 minutes in a water bath at 37 ° Centigrade (C), Then 200 µl of serum added to 200 µl of alcohol, mixed and centrifuged at 15,000 revolutions per minute (rpm) for 5 minutes. After sinking a denatured protein, the supernatant analyzed by HPLC with features mentioned.

Determining the amount of labeled antibiotic binding to bacteria

100 µl labeled bacteria was added to 1 ml of antibiotic at a concentration of $1 \times 10^8$ colony forming unit (CFU), and for 60 minutes, put it in a water bath at 37 ° C then 200 µl of alcohol solution added into a 200 µl mixture of bacteria then centrifuged at 1500 rpm for 5 minutes. After sinking the supernatant denatured proteins took the activity from supernatant and lower fluid by following gamma counter: EG & G / ORTEC / Model 4001M.

Biodistribution and imaging studies in animal body

100 µl of a suspension containing 1 cfu bacteria were injected into the leg muscle of mice. 24 hours after injection of bacteria, 100 ml of a solution containing 0.5 millicurie (mCi) of radio
labeled antibiotics were injected via the tail vein. 1, 4 and 24 hours after an antibiotic injection, binary groups of mice were anesthetized under CO2 gas and follow Single Photon Emission Compute Tomography (ESPECT) Imaging was performed. Mice dissected and organs of interest, such as lung, stomach, spleen, intestine, liver, kidney, infectious and non- infectious muscle and ... was collected and the average percentage of injected dose per gram (%ID/g) of each organ was calculated.

Preparation of histological sections and histopathological evaluation

After remove the capsule and fascia, infectious muscle and non- infectious, fixed in 10% neutral formalin solution, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were stained with (H&E) and mounted on glass slides for light microscopic analyses. Desmin intermediate filament by Nova link polymer detection system was used, according to kit instructions.

At the end, slides were examined by light microscopy.

RESULTS

Gemifloxacin structure with C18H20FN5O4 formula and molecular weight of 389.381 g/mol, have shown in Fig. 1.

Labeling efficiency of Gemifloxacin by Technetium by using of 100 mg Sncl2, 2 mg antibiotic, 23.7 mCi Pertechnetate (TcO4^-) and PH between 4-5, 96.94 % was obtained. (Fig. 2) the first peak, Pertechnetate, released in time of 5:46 minutes and the second peak is related to labeled Gemifloxacin, released in 14.55 minutes.

One hour after labeling, stability of radiopharmaceuticals in serum was 32.96%.

Labeled antibiotic binding to bacteria an hour after labeling was equal to 95.43%

Biodistribution of labeled Gemifloxacin in animal models:

Considering the numbers on the graph in Figure 3, it is clear that the percentage of injected dose per gram (%ID/g) of infectious muscles in 1, 4 and 24 hours after injection in compare with healthy muscles respectively are , 1.49, 1.89 and 2.36 times more, which shows the greater tendency of the drug to the infected tissue. Its high levels in the liver and kidney also suggests excretion pathway of the drug.

Imaging results from animal models

Pictures 4, 5 and 6 have been taken respectively, 1, 4 and 24 hours after injection. Warmer colors (blue to yellow) indicate the presence and extent of radiation. Sites of infection are shown with green circle, as is known, the best time for imaging is between 1 to 4 hours after injection of the radiopharmaceuticals.

24 hours after injection, due to drug elimination and 6 hours half-life of radiiodotopse, no range of warm colors in the picture presents.

Figure 3 The graphs of pharmaceuticals biodistribution

Results from studies of tissue sections

Desmin intermediate filaments in all samples were found positive and brown.

Changes will be classified in accordance with Table 1.

As is evident in Table 2 is not to harm healthy tissue and the extent of tissue damage in
The obtained images by light microscope:

**Figure 7:** Healthy muscles 1 hour after injection, Healthy Myocytes (arrowhead) with adjacent nucleus. Cross section (H & E 640X)

**Figure 8:** Infectious muscle 1 hour after injection, necrosis of muscle cells (arrowhead) and multi-core inflammatory cell infiltration (arrow) are seen with bacterial colonies (H & E 640X)

**Figure 9:** Infectious muscle 4 hours after injection, necrosis of muscle cells (arrowhead) and multi-core inflammatory cell infiltration (arrow) are seen with bacterial colonies (H & E 640X)

**Table 1.** Histopathological scoring in tissue samples

<table>
<thead>
<tr>
<th>Classification of changes</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis and inflammation</td>
<td>No necrosis / inflammation</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Local necrosis / inflammation, less than 25 %</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Local necrosis/inflammation, between 25 % - 50 %</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Necrosis/inflammation extended but local</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Complete tissue necrosis / inflammation, wide and across</td>
<td>4</td>
</tr>
</tbody>
</table>

The results of the sections review is given in Table 2

**Table 2.** The results of sections review

<table>
<thead>
<tr>
<th>Observations</th>
<th>Extent of necrosis</th>
<th>Extent of Inflammation</th>
<th>Sum</th>
<th>Desmin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mice No1-1h after injection, normal muscle</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Mice No1-4h after injection, normal muscle</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Mice No1-24h after injection, normal muscle</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Mice No2-1h after injection, normal muscle</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Mice No2-4h after injection, normal muscle</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Mice No2-24h after injection, normal muscle</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Infected tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mice No1-1h after injection, infected muscle</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>Mice No1-4h after injection, infected muscle</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>Mice No1-24h after injection, infected muscle</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>+</td>
</tr>
</tbody>
</table>

Infected muscle is less over time, after effect and dispose of drug.
multi-core inflammatory cell infiltration (arrow) are seen with bacterial colonies (H & E 640X)  
Figure 10: infectious muscle 24 hours after injection, necrosis of muscle cells (arrowhead) and multi-core inflammatory cell infiltration (arrow) are seen with bacterial colonies. Injuries sustained significantly decreased (H & E 640X)  
Fig. 11: healthy muscle, 4 hour after injection, Desmin intermediate filament (arrowhead) is seen in Myocytes (IHC 640X)  
Figure 12: infectious muscle 1 hour after injection, Desmin intermediate filament (arrowhead) within the Myocytes that surrounding by inflammatory cells (arrow) is seen. (IHC 640X)  
CONCLUSION  
At the first hour after injection highest uptake, respectively, in the liver, kidney, blood, spleen, lung and heart by amount of, 18.48, 14.26, 8.42, 5.63, 4.96 and 3.41 (%ID/g) were observed.  
Also uptakes in normal and infected muscle, respectively, were 1.1 and 1.64 (%ID/g). This biodistributions has many similarities with the survey by Syed Qaiser Shah and Muhammad Rafiullah Khan (2011) that following results were reported:
One hour after injection highest uptake, respectively, in the kidney, liver, blood, infected muscle by amount of, 18.50, 12.00, 11.35, 9.75, 7.85(%ID/g) were observed. They have introduced the combination of Gemifloxacin - 99mTc as an appropriate imaging agent for infections caused by Streptococcus pneumonia. Type text or a website address or translate a document.

In research by Erfani Mostafa et al., (2013), on combination of ofloxacin - 99mTc, highest uptake, respectively, in the liver, kidney, lung, spleen, and heart by amount of, 17.57, 16.47, 7.79, 4.43, 3.75, 3.55 and 0.84 and 1.96 (%ID/g) in normal and infected muscle were reported. He also suggest the best time for take a picture between 1 to 4 h after injection and he has proposed that combination is an appropriate Radiopharmaceutical to imaging of infections which have introduced by Staphylococcus aureus. These findings are also in good agreement with what we’ve achieved.

New antibiotics were evaluated in this study, the findings showed broad-spectrum antibiotics with high efficiency has the potential to be labeled by radioisotope Tc$^{99m}$. Also after labeling and intravenous injection, the internal infection points of gram-negative bacteria, Escherichia coli, which cause many infections, can be diagnosed. Results of histological studies also approved infection points and were accordance with imaging results. Over time and after injection of the radiopharmaceutical, histological changes reduced so these finds can be performer in future studies on effectiveness of antibiotics and its role.

The use of new antibiotics and its labeling has good performance in the detection of infectious points and histological studies verify production process and its effectiveness.

ACKNOWLEDGMENTS

I would like to express my appreciation to Dr Mostafa Erfani, Dr Pejman Mortazavi, Dr Iraj Pousty, Mr Mohammad Mazidi and Nuclear Science Research School, Nuclear Science and Technology Research Institute (NSTRI), Atomic Energy Organization of Iran (AEOI), Tehran, Iran, because of their Sincere help and guidance.

REFERENCES
