SYNTHESIS, QUALITY CONTROL AND BIODISTRIBUTION OF TECHNEIUM-99M TRIAMCINOLONE ACETONIDE (^{99m}Tc-TA) COMPLEX: AN INFLAMMATION TRACER AGENT

FAHEEM ASKARI RIZVI[1], SYED ALI RAZA NAQVI[1], MUHAMMAD MEHDI[1], SAMINA ROOHI[2], AMEER FAWAD ZAHOOR[1], ZULFIQAR ALI KHAN[1], MUHAMMAD SOHAIB[4] AND RASHID RASHEED[1,3]*

[1]*Department of Chemistry, Government College University Faisalabad, Pakistan.*
[2]*Isotope Production Division, Pakistan Institute of Nuclear Science and Technology, P.O. Nilore, Islamabad, Pakistan.*
[3]*Institute of Nuclear Oncology and Radiology (INOR), Abbottabad, Pakistan.*
[4]*Department of Medical Sciences, Pakistan Institute of Engineering and Applied Sciences, (PIEAS) Islamabad Pakistan.*

ABSTRACT

In present study synthesis of ^{99m}Tc-triamcinolone acetonide (^{99m}Tc-TA) complex and its stability using set of quality control parameters such as ligand concentration, reducing agent concentration, pH, temperature and reaction time was assessed. ^{99m}Tc-TA complex was characterized in terms of percent (%) yield, stability in saline and serum using chromatographic procedures. Radiochemically the ^{99m}Tc-TA complex was found quite stable in saline and serum. After 30 min of reaction the complex showed maximum radiochemical yield of 96.32% which decreased to 96.25 % after 4 h incubation period. In serum, the % yield of radiochemical was remained same up to 2 h which decreased to 93.5% at 24 h time point. Normal biodistribution pattern in Sprague-Dawley rats revealed liver, stomach and kidneys as areas of high ^{99m}Tc-TA complex uptake (8.44 ± 1.32, 8.75 ± 1.03 and 12.67 ± 1.21%, respectively) at 1 h post injection time point. Scintigraphy of ^{99m}Tc-TA in rabbits showed similar eco as observed in biodistribution study. Based on the promising results obtained in context of in vitro and in vivo stability and biodistribution, ^{99m}Tc-TA complex could be further studied to identify the inflammation based diseases.

Key words: ^{99m}Tc-Triamcinolone, Electrophoresis, Biodistribution study, Scintigraphy of Rabbits, Serum Stability.

1. INTRODUCTION

With the invention of radiopharmaceuticals to study disease status, extensive debate was reported to manage worldwide infection threat. Within few decades thousands of repots were published to address early diagnosis strategy using radiolabeled compounds.[1-3] Although very sensitive and sophisticated diagnostic modalities such as computed tomography (CT) and magnetic resonance imaging (MRI), and number of biochemical testsare in use worldwide to assess infectious and malignant diseases, the radiopharmaceuticals were developed for early diagnosis and targeted therapy. The only bottlenecks associated with CT and MRI scan are their diagnostic ability after certain morphological and entomological changes in diseased body tissues, and absence of discrimination potential between infection and inflammation.[4] Various radiopharmaceuticals, such as radiolabeled white blood cells, [5,6] antibiotics,[7] synthetic organic moieties,[8] antimicrobial peptides,[3] antibodies,[9] and peptide hormone analogue[10,11] have been developed in pursuing early detection of infection and malignancies.

The radiolabeled leukocytes,[12] citrate,[13] human polyclonal immunoglobulin (HIG),[14] ubiquicidin-29-41 peptide[15]and antibiotic[1,7] were studied to diagnose and discriminate infections from inflammations.[16] One of the clinically most popular antibiotics, ciprofloxacin was labeled with Technetium-99m (Tc-99m/^{99m}Tc) and provided to oncological centers with trade name infecton® for specific imaging of infections.[17] However, previous and recently reported data showed contradiction while discriminating infection from inflammation.[18] Other antimicrobial agents belonging to different classes of antibiotics specifically from flouroquinolones and cephalosporin family were also investigated to overcome non-specificity issue but the required and potential results were not achieved[16,19]

Triamcinolone acetonide (TA), a synthetic glucocorticoid i.e. fluoroquinolones used as a potential anti-inflammatory agent under certain conditions such as eczema, psoriasis, arthritis, allergies also including ulcerative colitis, sympathetic ophthalmia, and ocular inflammations.[20]It also appeared as an anticancer agent. The anti-inflammatory properties of this compound have been demonstrated to inhibit the expression of the VEGF gene.[21,22]

In present study, we describe the radiolabeling of TA with Tc-99m (Figure 1), in vitro saline and serum stability, normal biodistribution in rats and scintigraphic imaging using rabbits for potential inflammationimaging agent.

2. EXPERIMENTAL

2.1. Materials and methods

Triamcinolone acetonide was obtained from Sigma-Aldrich, ITLC from Agilent (Germany), Deluxe electrophoresis chamber (Gelman) system (Germany), Rats (Spragur-Dawley) from NationalInstitute of Health (NIH) Islamabad. All chemical used were on analytical grade (Sigma-Adrich). Technetium-99m was obtained from a locally situated fission based Pakistan generator (PAKGEN)^{99}Mo/^{99m}Tc generator (PINSTECH, Islamabad). The animal ethics committee of the institute gave an ethical approval for the animal experiments.

2.2. Synthesis of ^{99m}Tc-TA complex

In six sterilized nitrogen filled vials, reducing agent ($SnCl_2.2H_2O$) 2-5 µg with an increment of 0.5 µg were taken. Then, ~ 370 MBq^{99m} TcO_4^-saline solution (0.8 mL) was added in each vial. Thereafter, 100 to 600 mg TA with an increment of 100 mg was added in each vial. The pH of each vial (reaction mixture) was adjusted 3 to 8 with 1 unit rise followed by incubation at room temperature and starts to monitor radiolabeling from 5 min to 6 h with definite time intervals.

2.3. Quality control

Radiochemical yield of ^{99m}Tc-TA was assessed by ITLC-SG strips Whatman No. 3 paper. For this purpose 4 µL of reaction mixture was spotted at base line on Whatman No. 3 paper and developed with acetone to determine free $^{99m}TcO_4^-$using. While 2 µL reaction mixture was spotted at base line on ITLC-SG strip and developed with 0.5 M NaOH solution to determine reduced and hydrolyzed fractions After complete development the both radiochromatograms were dried and scanned with 2π-Scanner (Berthold, Germany). The radiochemical complex stability was monitored periodicallyfor 24 h at room temperature. Each experiment was repeated thrice.

2.4. In vitro stabilityin human serum

In vitro effect of blood serum on stability of the ^{99m}Tc-TA was studied by mixingserum (1.8 mL) with ^{99m}Tc-TA (0.2 mL) followed by incubation at 37 ºC. At pre-defined time points up to 24 h, 0.2 mL aliquots were withdrawn and spotted at ITLC to determine the percent of ^{99m}Tc-TA, reduced hydrolyzed ^{99m}Tc and free $^{99m}TcO_4^-$.

2.5. Electrophoresis of ^{99m}Tc-TA

The qualitative net charge on ^{99m}Tc-TA was determined with electrophoresis procedure. The process was carried out by spotting 0.2 mL ^{99m}Tc-TA at the center of the 30 cm long strip of Whatman No. 1 chromatographic paper. Electrophoresis was run for 60 to 90 min at a voltage of 300 V usingphosphate buffer of pH 6.8. After completion of electrophoresis, the strip was scanned by using 2π scanner to know the charge on ^{99m}Tc-TA complex.

2.6. Biodistribution study of ^{99m}Tc-TA in normal rats

Biodistribution in normal animal model was performed usingmale Sprague-Dawley rats weighing ~250 g. The distribution protocol was used as described previously.[2] Before administration of ^{99m}Tc-TA, the rats were properly monitored atleast for one day.On the day of biodistribution study, 0.2 mL of ^{99m}Tc-TA (~110 MBq) was injected into the tail vein. Three rats were used for one set of experiment. After a definite time, the rats were sacrificedby the decapitation under chloroform anesthesiaat 1, 4 and 24 h post-injection. One milliliter samples of blood were collected by cardiac puncture at the time of

e-mail: draliraza@gcuf.edu.pk

decapitation. Activity in total blood was calculated by assuming blood volume is 5% of the total body weight. Thereafter all organs (muscle, liver, spleen, lungs, kidney, stomach, femur, heart and brain) were dissected, weighed and their radioactivity was measured using a well-type NaI(Tl) detector connected witha single channel c-counter (SR-7). Results were expressed as percent of theinjected dose per organ. The experiment was performed in a group of three rats for one time point.

2.7. Scintigraphy of ^{99m}Tc-TA in normal rabbit

A single headed Siemens Integrated ORBITER Gamma Camera System interfaced with high-resolution parallel hole collimator was used for scintigraphic study. It was connected to an on-line dedicated computer (Macintosh® Operating System 7.5 Software used on the ICON™ Workstation). Previously reported protocol was adopted for scintigraphic study,[2] briefly 0.2 mL of ^{99m}Tc-TA (~110MBq) was injected into the ear vein of each rabbit which was then placed on a flat hard surface with both hind legs spread out, and all legs fixed with surgical tape. Diazepam (5 mg) was injected to left thigh muscle for anesthetic purpose. Biodistribution of ^{99m}Tc-TA in the body was assessed by whole body acquisition at1, 4 and 24 h of post-injection.

3. RESULTS AND DISCUSSION

3.1. Effect of different parameters on radiochemical yield

Radiochemical yield was assessed using a set of different parameters such as reducing agent, reaction pH, ligand concentration, reaction incubation period while keeping the radionuclide activity constant i.e. 370 MBq to achieve stable and maximum radiochemical yield and purity.

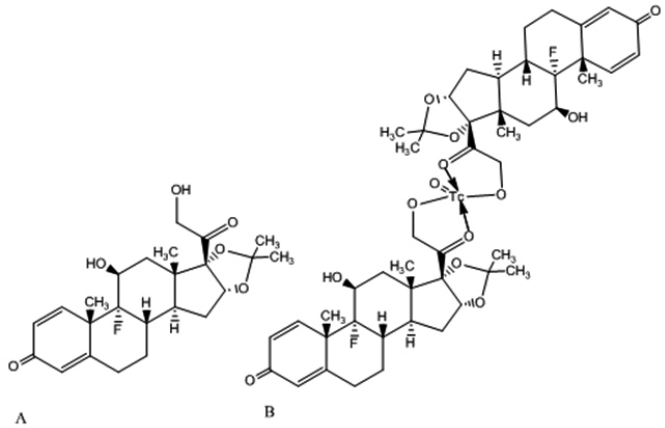

Figure 1.A) Structure of anti-inflamatory agent TA B) the proposed structure of ^{99m}Tc-TA complex; the oxygen from hydroxyl and carbonyl carbon from two molecule of TA make coordination bond and present at apex of the complex while oxygen from reduced TcO core present at pyramid of the complex.

3.2. Effect of reducing agent

Stannous chloride dihydrate in different concentrations i.e. 1.5 to 4 µL with 0.5 µL increment (µg/µL 0.01 N HCl) were used to reduce $^{99m}TcO^{-}_{4}$. In acidic medium, Sn(II) reduces ^{99m}Tc to oxidation states of +6 to +1; for Tc-99m, the +5 oxidation state is best suited for complexing with most antibacterial agents [23]. Stannous chloride dihydrate appeared well famed in Tc-99m complex chemistry due to its easy availability and solution formation for reduction process. It was observed in its lower oxidation state Tc-99m firmly tagged with TA and resulted a stable ^{99m}Tc-TA complex. The maximum radiochemical yield (97.18 %) was obtained with 2.5 µg $SnCl_2.2H_2O$ after 30 min of reaction mixture incubation at room temperature.

As shown in Figure 2 only optimal concentration (2.5 µg) of $SnCl_2.2H_2O$ results maximum yield; less than optimal concentration incomplete reduction of Tc-99m lead to weak complexation and finally low radiochemical yield. While in case of higher concentration it hydrolyzed and interfere in Tc-99m complexation with TA which results in lowering the overall percentage yield of ^{99m}Tc-TA complex.

3.3. Effect of pH

Figure 3 shows the results obtained at different pH of reaction mixture. Similar trend was observed in radiochemical yield as was observed in reducing agent concentration yield curve. The pH 5 was appeared suitable to maintain the stability of the ^{99m}Tc-TA complex up to 96.33 %. Below and above this pH the complex was found unstable and

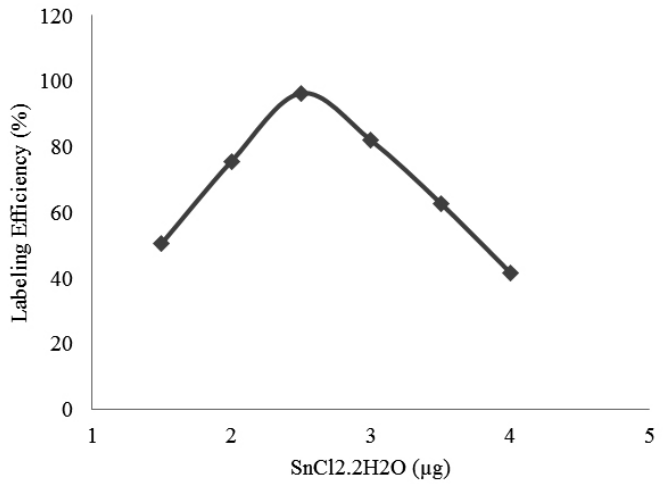

Figure 2. Effect of concentration of $SnCl_2.2H_2O$ (reducing agent) on the labeling efficiency of ^{99m}Tc-TA complex.

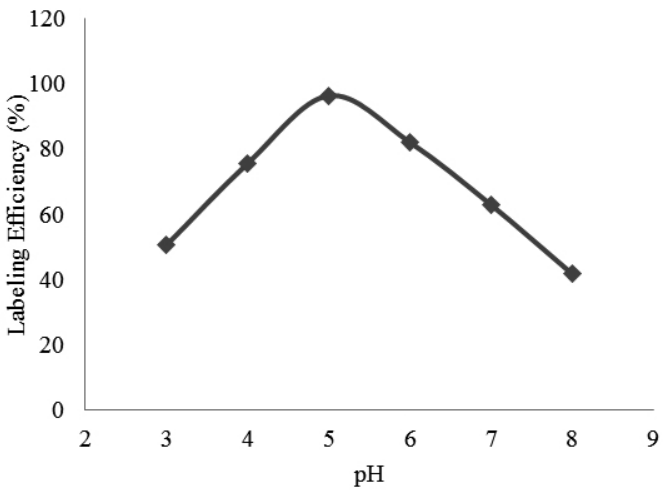

Figure 3. Effect of pH on the labeling efficiency and stability of ^{99m}Tc-TA complex.

consequently resulted in lower radiochemical yield while released (non-bounded) Tc-99m was converted into hydrolyzed form which is the main impurity of Tc-99m based radiopharmaceuticals.

3.4. Effect of TA concentration

As shown in Figure 4, the maximum radiochemical yield (96.89 %) was obtained at 300 µg substrate amount in reaction mixture. However, other than optimum ligand concentration the radiochemical yield was not greater than 96.89 %. The selection of ligand concentration while labeling with radionuclide based on experimental trails because stochiometric relation between Tc-99m and ligand concentration was not known; that's way particular ligand concentration could not be chosen. Low concentration of ligand resultedin the formation of hydrolyzed technetium colloid from reduced Tc-99m. A slight decrease in complex yield was observed at higher increments of ligand concentrations might be due to the exchanged ligand phenomenon.

3.5. Effect of incubation on ^{99m}Tc-TA complex

The stability of the complex was assessed up to 4 h. The results showed ^{99m}Tc-TA complex remained stable up to monitored time span and no decrease in complex yield was noted as shown in Figure 5. It apparently shows that^{99m}Tc-TA complex could be administered up to 4 h after reconstitution of radiolabeled compounds.

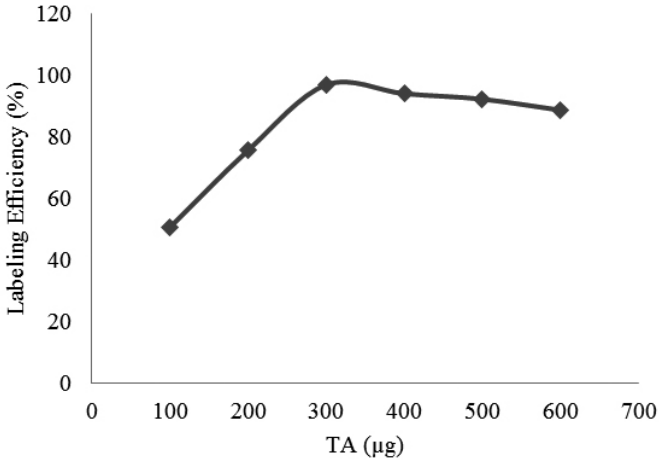

Figure 4. Effect of concentration of TA (complexing ligand)on the labeling yield of ^{99m}Tc-TA complex.

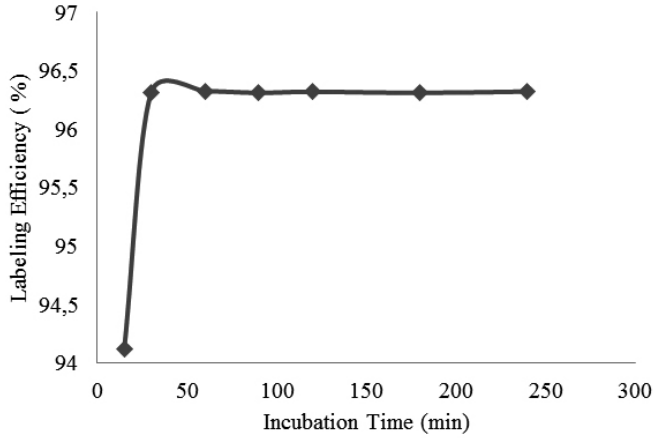

Figure 5. Incubation period effect on ^{99m}Tc-TA complex stability at room temperature.

3.6. Chromatographic characterization

Radiochemical purity and yield were assessed with ITLC and paper chromatography. In paper chromatography using acetone as mobile solvent, free $^{99m}TcO^-_4$ moved towards the solvent front (Rf = 1), while ^{99m}Tc-TA and reduced/hydrolyzed Tc-99m remained at the point of spotting (Figure 6a). While in ITLC-SG analysis using 0.5 M NaOH as mobile solvent, reduced/hydrolyzed Tc-99m remained at the point of spotting (Rf = 0), whereas ^{99m}Tc-TA and free $^{99m}TcO^-_4$ moved toward the solvent front (Rf = 1) (Figure6b). Radio-species such as hydrolyzed $^{99m}TcO^-_4$, free $^{99m}TcO^-_4$, and ^{99m}Tc-TA complex were calculated fromtwo chromatographic patterns (Figure 6).

After studying all parameters it was observed that using 2.5 µL solution of $SnCl_2.2H_2O$, 370 MBq $^{99m}TcO^-_4$ activity, 300 µg of TA, 2 mL reaction volume, 30 min reaction incubation at room temperature with 5.5 pH, yield highest ^{99m}Tc-TA complex yield. Other than optimized set of conditions the radiochemical complex reaction showed less than maximum yield of radiochemical.

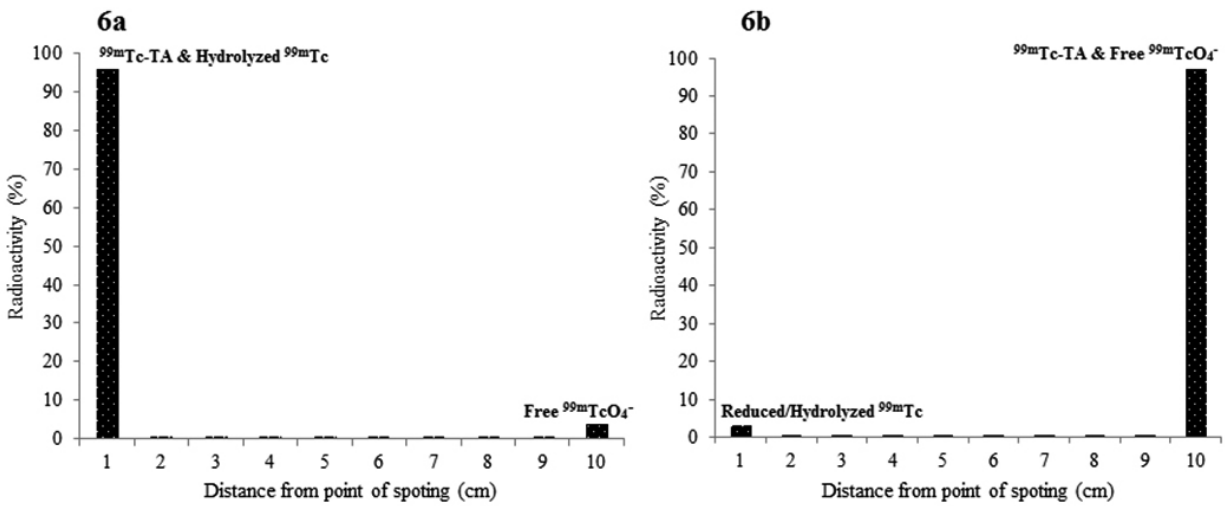

Figure 6. Chromatogram patterns obtained by 2π-scanning to assess ^{99m}Tc-TA complex, free TcO^-_4 and reduced/hydrolyzed ^{99m}Tc. 6a) Paper chromatogram showing ^{99m}Tc-TA complex and reduced/hydrolyzed ^{99m}Tc remained at base line (Rf=0) while free TcO^-_4at R_f= 1; 6b) ITLC chromatogram pattern showing reduced/hydrolyzed ^{99m}Tc at base line (R_f = 0) while ^{99m}Tc-TA complex and free TcO^-_4 traveled with solvent front (R_f = 1). The % formation of ^{99m}Tc-TA complex, free TcO^-_4 and reduced/hydrolyzed ^{99m}Tc was calculate using both chromatograms.

3.7. Stability in blood serum

As shown in Table, incubation of ^{99m}Tc-TA complex in normal human blood serum for 24 h at physiological temperature (37 ºC) resulted in slight decrease in ^{99m}Tc-TA complex % yield (2%). The complex appeared quite stable against action of serum on ^{99m}Tc-TA complex; indicating safe transportation of radio-complex to target site.

Table. In vitro stability of ^{99m}Tc-TA in normal human serum.

Incubation time (h)	^{99m}Tc-TA (%)	Free TcO^-_4 (%)	Colloid (%)
0.5	97.9 ± 0.1	2.1 ± 0.01	0.0
1	97.8 ± 0.2	2.2 ± 0.01	0.0
2	97.7 ± 0.3	2.3 ± 0.09	0.0
4	96.6 ± 0.4	3.4 ± 0.14	0.0
24	93.5 ± 0.5	6.5 ± 0.26	0.0

Electrophoresis analysis

Electrophoresis of ^{99m}Tc-TA complex showed it is a neutral complex moiety. The whole activity remained at place of sample introduction (center of the electrophoretic paper) while a small fraction of free $^{99m}TcO^-_4$ was traveled toward anode (Figure 7).

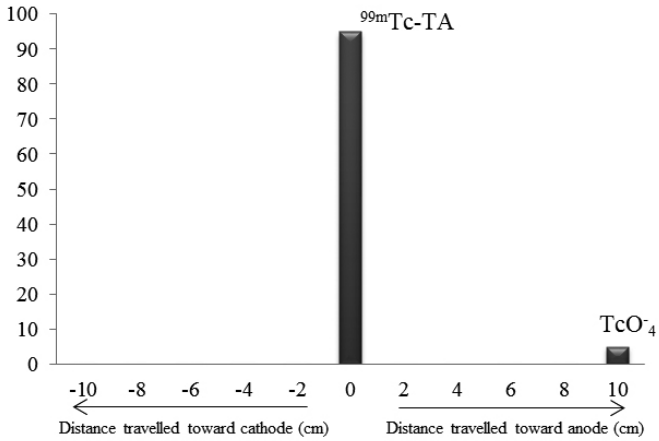

Figure7. Paper electrophoresis patern showing neutral behaviour of ^{99m}Tc-TA complexof as it didn't travelled to any electrode.

Cell permeability shows quite sensitivity for net charge on molecule. Polar and charged molecules do not diffuse easily through the lipid core of the plasmaand consequently unable to interact with cell machinery to play diagnostic or therapeutic role. Neutral structure of ^{99m}Tc-TA complex can approach into cell plasma through cell membrane.

3.8. Biodistribution and scintigraphy

The tissue distribution of ^{99m}Tc-TA in different organs of normal rat model (represented as percent of injected dose per organ (%ID/organ) at 1, 4 and 24 h after intravenous administration is shown in Figure 8. The only organs which showed high initial uptake were liver and stomach at 1 h post injection of ^{99m}Tc-TA (12.67 ± 1.67% & 8.44 ± 1.18%, respectively) due to initial high flux of blood stream toward these organs in addition to other body tissues. However after 24 h post injection period the initial activity was reduced to 5.45 ± 1.01% & 4.11 ± 0.87%, respectively in both organs.

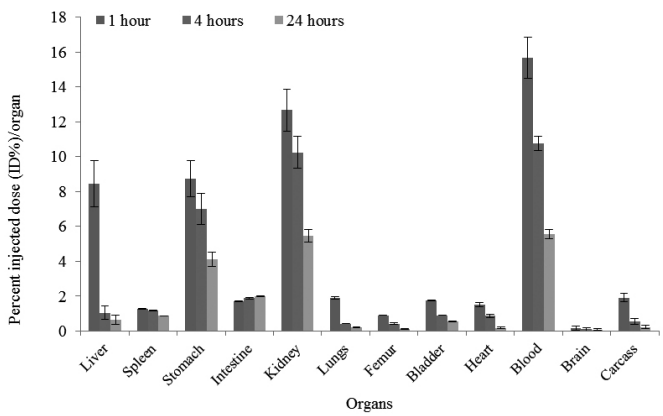

Figure8. The biodistribution of the ^{99m}Tc-TA complex in per gram of liver, spleen, stomach, intestine, kidney, lungs, femur, bladder, heart, blood, brain and carcase.

The high uptake in kidney after 1 h ^{99m}Tc-TA administration is due to the excretory passage which reduced to a trace quantity (0.65 ± 0.11%) after 24 h. Trace amount of activity at 24 h post injection might be due to the slow release of activity from liver, stomach and blood for excretion. Intestine showed slight increase in activity at each higher time point, possibly due to slow penetration into and difficult removal from intestine. Due to the uptake of ^{99m}Tc-TA in appreciable quantity by liver the tracer could not be suggested for liver disorder imaging; however rapid elimination from kidneys indicates the tracer is quite safe to use for different inflammatory diseases imaging. The normal scintigraphic distribution imaging study at 1 and 4 h time point well correlated with normal biodistribution study (Figure 9). The tracer is clearly visible at 1 and 4h post administration in liver, kidneys and bladder. However at 24 h time point activity was only seen in kidney and bladder and no activity counts was noted in liver that show the rapid clearance from non targeted organ accept kidney and bladder which are excretory pathway of metabolic products.

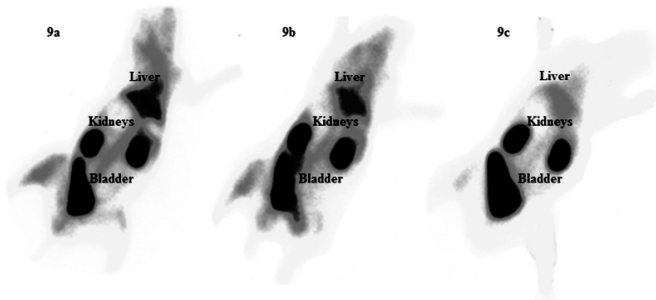

Figure 9. Gamma camera scintigraphic imaging of 1, 4 and 24 h post injection of ^{99m}Tc-TA into rabbit body. 9a) showing liver, kidney and bladder hot areas and similar pattern is appearing in image 9b) showing absence of activity in liver.

CONCLUSIONS

Different radio-tracers were developed previously to discriminate infections from inflammation either tagging ^{99m}Tc (in its lower oxidation state) with leukocytes, antibiotics or antimicrobial peptides but non-recognizable effort was done to develop inflammation imaging agent[3,16]. TA is an anti-inflammatory agent that specifically binds to inflammation associated cells to stop the inflammation process.The labeling of TA with Tc-99m could be advantageous in imaging inflammatory diseases. This study represents a comprehensive strategy to develop Tc-99m labeled TA using different reaction parameters which resulted > 96% labeling yield. Based on the results obtained the probability of ^{99m}Tc-TA complex for inflammation imaging could be further investigated.

ACKNOWLEDGEMENT

The authors are thankful to Mr. Ibrar Haider (PINSTECH) and Dr. Shabana Saeed (Department of Nuclear Medicine, Pakistan Institute of Engineering and Applied Sciences, Islamabad) for providing the gamma camera facility and to the Department of Chemistry, GC University Faisalabad for providing chemicals of AR grade.

REFERENCES

1. K. E. Britton, S. Vinjamuri, A. V. Hall, K. Solanki, Q. H. Siraj, J. Bomanji, S. Das. Eur. J. Nucl. Med. 24, 553, (1997)
2. S. A. R. Naqvi, M. M. Ishfaq, Z. A. Khan, S. A. Nagra, I. H. Bukhari, A. I. Hussain, N. Mahmood, S. A. Shahzad, A. Haque, T. H. Bokhari. Turk. J. Chem. 36, 267, (2012)
3. M. S. Akhtar, M. B. Imran, M. A. Nadeem, A. Shahid. Intern. J. Pept., 2012, 19, (2012)
4. M. M. Welling, G. Ferro-Flores, I. Pirmettis, C. P. J. Brouwer, M. Anti-Infect. Agent. Med. Chem. 8, 272, (2009)
5. S. Vinjamuri, K. K. Solanki, J. Bomanji, Q. Siraj, K. E. Britton, A. V. Hall, E. O'Shaughnessy, S. S. Das, Lancet. 347, 233 (1996)
6. K. Sonmezoglu, M. Sonmezoglu, M. Halac, I. Akgün, C. Türkmen, C. Önsel, B. Kanmaz, K. Solanki, K. E. Britton, I. Uslu, J. Nucl. Med. 42, 567, (2001)
7. A.; Doroudi, M.; Erfani, F.; Kooshki, S. M.; Saadati, F.; Ahmadi, A.; Kiasat, M. J.; Khodayar, B.; Etessami, H. Meghdadi, Iran. J. Nucl. Med., 23, 96, (2015)
8. X. Wang, D. Li, W. Deuther-Conrad, J. Lu, Y. Xie, B. Jia, M. Cui, J. Steinbach, P. Brust, B. Liu, et al., J. Med. Chem. 57, 7113 (2014)
9. J. Barbet, M. Bardies, M. Bourgeois, J. F. Chatal, M. Cherel, F. Davodeau, A. Faivre-Chauvet, J. F. Gestin, F. Kraeber-Bodere, Meth. Mol. Biol., 907, 681, (2012)
10. A. Frilling, F. Weber, V. Cicinnati, C. Broelsch, Exp. Rev. Endocr.Metab., 2, 517, (2007)
11. S. A. R. Naqvi, T. Matzow, C. Finucane, S. A. Nagra, M. M. Ishfaq, S. J. Mather, J. Sosabowski, Cancer. Biother. Radiopharm., 25, 89, (2010)
12. A. M. Peters, Semin. Nucl. Med., 24, 110, (1994)

13. D. K. Hughes, J. Nucl. Med. Technol., 31, 196, (2003)
14. G. Gerasimou, E. Moralidis, E. Papanastasiou, G. Liaros, T. Aggelopoulou, E. Triantafyllidou, N. Lytras, L. Settas, A.Gotzamani-Psarrakou, Hippokratia., 15, 37, (2011)
15. M. S. Akhtar, J. Iqbal, M. A. Khan, J. Irfanullah, M. Jehangir, B. Khan, I. Ul-Haq, G. Muhammad, M. A. Nadeem, M. S Afzal, et al., J. Nucl. Med., 45, 849, (2004)
16. C. Love, C. J. Palestro, J. Nucl. Med. Technol., 32, 47, (2004)
17. K. E. Britton, D. W. Wareham, S. S. Das, K. K. Solanki, H. Amaral, A. Bhatnagar, A. H. Katamihardja, J. Malamitsi, H. M. Moustafa, V. E Soroa,.et al., J. Clin. Pathol., 55, 817, (2002)
18. L. Sarda, A. C. Cremieux, Y. Lebellec, A. Meulemans, R. Lebtahi, G. Hayem, R. Genin, N. Delahaye, D. Huten, D. Le Guludec, J. Nucl. Med., 44, 920, (2003)
19. A. Kaul, P. P. Hazari, H. Rawat, B. Singh, T. C. Kalawat, S. Sharma, A. K. Babbar, A. K. Mishra, Int. J. Infect. Diseas. 17, e263, (2013)
20. A. T. Kay, D. M. Bolt, A. Ishihara, P. J. Rajala-Schultz, A. L. Bertone, Am. J. Vet. Res., 69, 1646, (2008)
21. O. Uckermann, F. Kutzera, A. Wolf, T. Pannicke, A. Reichenbach, P. Wiedemann, S. Wolf, Bringmann, A. J. Pharmac. Exper. Therap., 315, 1036, (2005)
22. X. Zhang, S. Bao, D. Lai, R. W. Rapkins, M. C. Gillies, Diabetes., 57, 1026, (2008)
23. D. K. Nayak, R. Baishya, K. K. Halder, T. Sen, B. R. Sarkar, S. Ganguly, M. K. Das, M. C. Debnath, Metallomics., 4, 1197, (2012)