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^{99m}Tc-Labeled Erythrocin and Biological Evaluation in Mice for Detection of Bacterial Infection

Safaa B. Challan[1], A. A. Massoud[1*], M. El Tawoosy[2], M. A. Motaleb[2], I. H. Borei[3] and H. M. Ghanem[3]

[1]Chemistry Unit of Cyclotron, NRC, Egyptian Atomic Energy Authority, P.O.Box 13759, Cairo, Egypt.
[2]Department of Labeled Compounds, Hot Labs Center, Egyptian Atomic Energy Authority, Cairo, Egypt.
[3]Department of Chemistry, Faculty of Sciences, Ain Shams University, Cairo, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MAM, SBC and AAM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MET and IHB managed the analyses of the study. Author AAM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Nuclear medicine is a powerful diagnostic technique capable of detecting inflammatory points in human disease by using radiolabeled compounds. The ideal compound for imaging infection should be specific for an infected site with minimal side effects, low marrow, gut and renal uptake, be safe and easy to synthesize. The goal of this work was labelling of erythrocin with Technetiun-99m using stannous chloride as a reducing agent. Dependence of the yield of the ^{99m}Tc-erythrocin complex on the amount of Erythrocin, reducing agent, pH of the reaction mixture, reaction time and reaction temperature were studied. Under best conditions, the labelling yield of ^{99m}Tc-erythrocin complex was (85 ±1.1%) and was achieved using 5 mg of erythrocin, 5 µg of Sn (II), pH 10 and 30 minute reaction time at room temperature (25ºC). ^{99m}Tc- erythrocin complex was stable for 1 hour after labelling, then the yield decreased gradually until reaching 47.3 ±1.1% after 24 hours.

**Corresponding author: E-mail: ayman_mass@yahoo.com;*

Biodistribution studies were achieved in mice with left thigh infection using a bacterial effect such as *Staphylococcus aureus*. The biochemical parameters were done before and after inflammation process. The ratio of the bacterially infected thigh to normal thigh was evaluated. The time for the maximum accumulation of ^{99m}Tc- erythrocin at the site of infection was 1 hour after administration of the labelled compound. The ratio of abscess-to-muscle for ^{99m}Tc- erythrocin is (4.12 ± 0.15%) while in the commercially available ^{99m}Tc-Ciprofloxacin is (3.8 ± 0.5 %) under the same conditions. The results suggest that ^{99m}Tc- erythrocin could be used as infection imaging.

Keywords: Erythrocin; technetium-99m; labeling; inflammation; Staphylococcus aureus.

1. INTRODUCTION

Erythromycin is a macrolide antibiotic called also erythrocin that can be used to treat bacteria responsible for infections of the skin and upper respiratory tract including Streptococcus and Staphylococcus. Erythromycin was applied as an antibiotic drug from last mid-century [1]. Erythromycin shows great action for bacteriostatic activity or inhibits growth of bacteria. The mode of action of Erythromycin is by the reaction with the 50s subunit of rRNA, thus the protein synthesis, function processes for life or replication are inhibited. Also, erythromycin or eryhthrocin with aminoacyl translocation, stopping the relocate of the tRNA bound at the A site of the rRNA complex into the P site of the rRNA complex. Without this translocation, the A site remains busy and thus, the addition of an incoming tRNA and its attached amino acid to the chain is stopped. This interferes with the production of functionally useful protein so stop the rate of bacteria growth which is the basis of this antimicrobial action [2,3].

Inflammation is a biological phenomenon that takes place at the beginning of numerous pathological cases. Inflammation process takes place due to the liberation of histamine, kinins, serotonin, and prostaglandin. The anti-inflammatory compounds are those which can normally inhibit the liberation of these inflammatory mediators [4]. However, inflammatory processes can be imagined in early phases by various radionuclides, when anatomical changes are not yet apparent.

Radiopharmaceuticals play main role in providing the greatest possible solutions for inflammation diagnosis and treatment. Recently, a great number of radiopharmaceutical compounds are established for imaging of infection and inflammation. Radiopharmaceuticals were applied for scintigraphic imaging for infection and inflammation in their initial phases, while traditional methods of diagnosis depend on the culture of organism's examination from infected foci [2,5-8]. Radiopharmaceuticals are exclusive medicinal formulations covering radioisotopes which are applied in major clinical areas for diagnosis and/or therapy [9]. The development and wide application of diagnostic nuclear medicine have been mostly motivated by the easy availability (through the transportable ^{99}Mo/^{99m}Tc generator system). Today, over 70% of diagnostic investigations are still done with this single isotope such as ^{131}I or ^{99m}Tc. Recently a number of novel ^{99m}Tc labelled antibiotics compounds have been reported as promising radiotracers for the diagnosis of infection and its discrimination from inflammation [10–12]. The labelled compounds are able to determine the physiological and biochemical changes that occur during the early phases of inflammation than other methods. These radiopharmaceuticals such as chemotactic peptides, antibiotics, and antimicrobial peptides can be labelled with various radionuclides (^{67}Ga, ^{99m}Tc, ^{111}In, ^{18}F, ^{131}I, etc.) [13-15].

The work aims to label of erythrocin drugs using Technesium-99m and to determine the optimum conditions for labelling reaction between ^{99m}Tc and erythrocin. Moreover, the stability of complex was studied after labelling process and also the biodistribution study of the labelled erythrocin in Albino Swiss mice was investigated.

2. MATERIALS AND METHODS

2.1 Materials

All the chemical reagents which used in this study were in analytical grade and bi-distilled water was used for solution preparation, with nitrogen purging in the case of labelling studies. Erythrocin was obtained from GlaxoSmithKline, Pharmaceutical Company, Cairo Egypt. Erythrocin is called a macrolide antibiotic. Its structure is shown in Fig. 1. The biodistribution study was evaluated using Albino Swiss mice (25-40 g) [16].

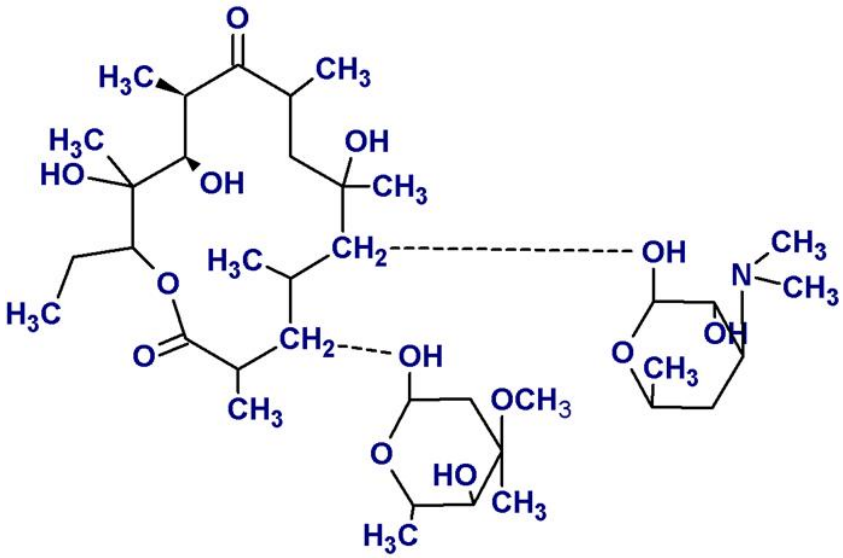

Fig. 1. Chemical structure of erythrocin

2.2 Methods

2.2.1 Labelling procedure

Erythrocin (0.5-7 mg) was taken separately in penicillin vial evacuated with nitrogen gas, then added (5-100µg) of $SnCl_2.2H_2O$ (with 5 µg in each vial). After gently swirling, the volume of the reaction mixture was completed to (1 ml) by N_2-purged distilled water, then the pH of the reaction mixture was in the range of 4 to 11 (with 0.5ml buffer pH 10 in each vial). Finally added 0.5 ml of freshly eluted $^{99m}TcO_4^-$ (~500MBq) through sterilized syringes to the above preparations followed by incubation at room temperature (25ºC) for 30 minutes. A similar procedure is in ref. [17].

2.2.2 Radiochemical purity

2.2.2.1 TLC analysis

For each labelling experiment, two drops of the reaction mixture were spotted on two thin layer chromatographic strips (each of 10 x 1.5 cm^2). One strip was developing with acetone and another strip was developing with the mixture, contain (ethanol: water: ammonium hydroxide) (2:5:1). After complete expansion, the two paper strips were dried, cut into 0.5 cm pieces and separately counted using the NaI (Tl) scintillation counter to determine the ratio of hydrolyzed ^{99m}Tc, free $^{99m}TcO_4^-$ and ^{99m}Tc-complex and each experiment was repeated three times to take the mean value. The results were further confirmed by electrophoresis analysis and the procedure was according to refs. [18-20].

2.2.2.2 Stability of ^{99m}Tc- Erythrocin

Stability of ^{99m}Tc-erythrocin was evaluated at different time intervals up to 24-hour post labelling by TLC for determination of the percent yield of ^{99m}Tc-complex, reduced hydrolyzed Technetium and free pertechnetate.

2.2.2.3 Induction of infectious foci and non-infected inflammation

The isolation of Staphylococcus aureus from biological samples was applied to obtain focal infection. Individual colonies containing 105-106 organisms were diluted with saline in order to get a turbid suspension. Groups of five mice weighing 25-40 g were intramuscularly injected with 200 µl of the suspension in the left lateral thigh muscle. After that, the mice were left for 48 hours to obtain the infection in the thigh [3]. Sterile inflammation was induced by injecting 200 µl of turpentine oil. The sterilization achieved by autoclaving at 120 ºC for 30 min, intramuscularly in the left lateral thigh muscle of the mice. Two days later, the swelling was appeared [21].

2.2.2.4 Induction of heat-killed Staphylococcus aureus non-infected inflammation

Sterile inflammation was induced by injecting 200 µl of heat-killed Staphylococcus aureus, sterilized by autoclaving at 121ºC for 20 min,

intramuscularly in the left lateral thigh muscle of the mice. Two days later, a swelling appeared [22].

2.2.2.5 Biochemical investigations

Some biochemical parameters were achieved before and after inflammation induction with living bacteria as markers for inflammation such as: Complement 3, Complement 4, LDH enzyme, CPK enzyme, alanine transaminase (ALT), Aspartate transaminase (AST), Albumin, Total protein, Urea, Creatinine, C-Reactive Protein (CRP) [23].

2.2.2.6 Biodistribution studies

Biodistribution of the sterile 99mTc-erythrocin complex was evaluated in male Albino Swiss mice weighing 25-40 g. Numbers of mice were applied for each experiment to the quantitative analysis of organ distribution using 0.1 ml of about 39 MBq of ^{99m}Tc-erythrocin solution and was introduced into the tail vein of mice after 24 hour of bacterial induction. The chloroform was used for anesthetize the mice and then applied for further experiments. Samples of infected muscle contralateral, normal muscle, blood, bone, liver, kidney, stomach, intestine, spleen, lung, heart, and urinary bladder were weighted, and the radioactivity was investigated using a gamma detector. For more details about the biodistribution studies is in ref. [24]. For statistical analysis: the results recorded were obtained as the mean values and statistical analysis was achieved using analysis of variance (ANOVA) with multiple comparison tests.

3. RESULTS AND DISCUSSION

Erythrocin was labelled with Technetiun-99m in previous work [25,26]. Erythrocin has more than functional groups which have donor atoms such as (OH, =O, and N) which made it able to form bonds with ^{99m}Tc. Therefore, the complexes of ^{99m}Tc-erythrocin may be formed by interaction between electron donor atoms or groups and technetium-99m in presence of stannous chloride as a reducing agent [27-29]. Radiochemical purity and stability of ^{99m}Tc-erythrocin complex were assessed by thin layer chromatographic method and electrophoresis. The electrophoresis radiochromatogram for ^{99m}Tc-erythrocin complex and free $^{99m}TcO_4^-$ is presented in Fig. 2 According to the results, there are two peaks, one of them is strong peak and was appeared at fraction number zero, which corresponds to ^{99m}Tc-erythrocin complex and the other is weak peak appeared at fraction number 8, which corresponds to free $^{99m}TcO_4^-$. On the other hand, the colloid removed by filtration using millipore filter [30-32].

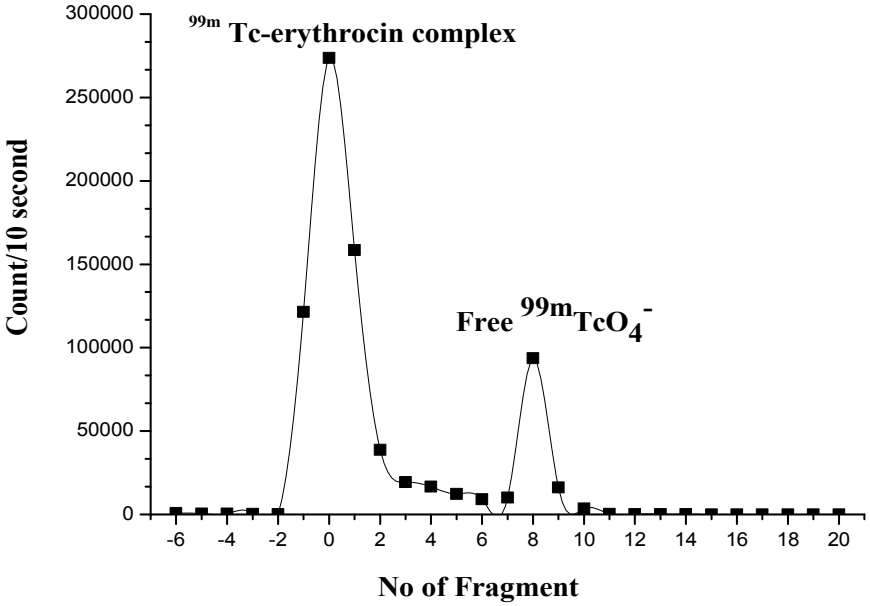

Fig. 2. Electrophoresis chromatogram of ^{99m}Tc-erythrocin, reaction conditions: 5mg of erythrocin, dissolved in1 ml pH 10, 5µg of $SnCl_2.H_2O$, 0.5 ml (~500MBq) of $^{99m}TcO_4^-$, the reaction mixture was kept at room temperature for 30 minute

3.1 Factors Affecting the Labelling Yield

3.1.1 Effect of substrate amount

The effect of erythrocin amount on the yield of the yield of ^{99m}Tc-erythrocin complex was investigated using (0.5 to 7) mg of Erythrocin with constant other factors and the results are as shown in Fig. 3. As can see in Fig. 3 the radiochemical yield of ^{99m}Tc-erythrocin complex increased with increasing the amount of erythrocin from 30.32 % at 0.5 mg of erythrocin to 85.2% at 5 mg erythrocin and then decreased again to reach 72.54% at 7mg. therefore, the optimum amount of erythrocin to obtain the highest ^{99m}Tc-erythrocin complex is 5mg. The optimum erythrocin amount was 1.5 mg according to ref. [25].

3.1.2 Effect of $SnCl_2$. $2H_2O$ amount

Effect of stannous chloride ($SnCl_2.2H_2O$) as a reducing agent is very important which reduce the charge of Technisium-99m which make it able to react with more than active site on the substrate (erythrocin) [33]. So, the effect of stannous chloride ($SnCl_2$. $2H_2O$) amount on the yield of ^{99m}Tc-erythrocin complex was studied using 0 to 100 µg under constant other parameters as shown in Fig. 4. The results showed that the maximum radiochemical yield of ^{99m}Tc-erythrocin complex was (85%) at 5 µg of $SnCl_2.2H_2O$, this behaviour may be due to its sufficient amount of $SnCl_2.2H_2O$ to reduce all pertechnetate which presents in the reaction mixture. On the other hand, below this amount of $SnCl_2.2H_2O$, the yield of ^{99m}Tc-erythrocin complex decreased due to insufficient of $SnCl_2.2H_2O$ to reduce all pertechnetate which exist in the solution mixture. Meanwhile, by increasing the amount of $SnCl_2.2H_2O$ more than 5 µg, the labeling yield decreased gradually until reached to (41.6%) at 100 µg of $SnCl_2.2H_2O$. This result may be related to the fact that most of the ligand molecules were consumed in the formation of complexes, so the pertechnetate is reduced to insoluble technetium (IV) $TcO_2.xH_2O$ in the absence of ligand [34] and/or due to the fact that the excess amount of stannous chloride leads to the formation of stannous hydroxide colloid Sn(OH) [34]. The optimum amount of stannous chloride was 15 µg according to ref. [25].

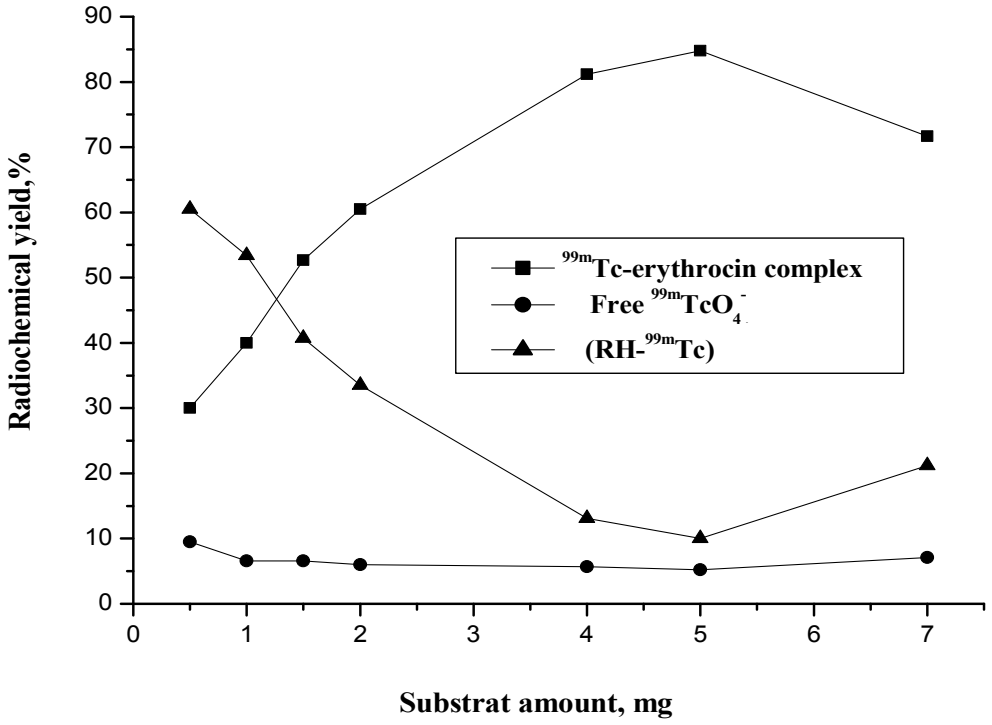

Fig. 3. Variation of the radiochemical yield of ^{99m}Tc-erythrocin as a function of erythrocin amount; [reaction conditions: xmg of erythrocin, dissolved in1 ml D.D H_2O, 5 µg of $SnCl_2.2H_2O$, 0.5 ml (~500MBq) of $^{99m}TcO_4^-$, the reaction mixture was kept at room temp.]

3.1.3 Effect of pH of the reaction mixture

The charge of the substrate (erythrocin) and the speciation of metal ions are highly affected by pH values [35,36]. So, the effect of pH value on the formation of ^{99m}Tc-erythrocin complex was studied from 4 to 11 under constant other factors as shown in Fig. 5. In general, the labelling yield (^{99m}Tc-erythrocin complex) increases with increasing pH value from 4 to 10 and then decreased again at pH 11. The maximum labeling yield of ^{99m}Tc-erythrocin complex is 85% at pH 10. Below or above this value (pH 10), the interaction between ^{99m}Tc and erythrocin decreased, this may be related to the formation of reduced hydrolyzed technetium colloid. The optimum pH value for ^{99m}Tc-erythrocin complex formation was 4 [25].

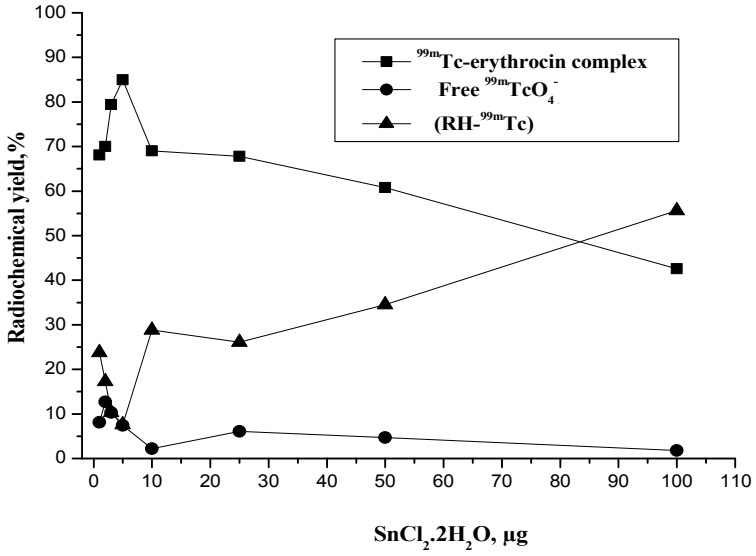

Fig. 4. Variation of the radiochemical yield of ^{99m}Tc-erythrocin as a function of $SnCl_2.2H_2O$ amount; [reaction conditions: 5mg of erythrocin, dissolved in1 ml D.D H_2O, x µg of $SnCl_2.2H_2O$, 0.5 ml (~500MBq) of $^{99m}TcO_4^-$, the reaction mixture was kept at room temperature for 30 min]

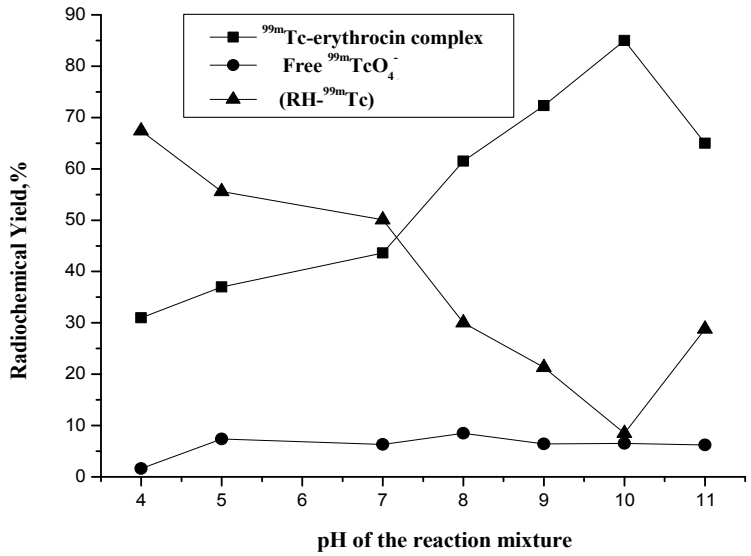

Fig. 5. Effect of pH of the reaction mixture on the radiochemical yield of ^{99m}Tc- erythrocin; [reaction conditions: 5 mg of erythrocin, dissolved in (1) ml pH x, 5 µg of $SnCl_2.2H_2O$, 0.5 ml (~500MBq) of $^{99m}TcO_4$, the reaction mixture was kept at room temperature for 30 min]

3.1.4 Effect of reaction time and stability

The contact time between the substrate and radionuclides is a very important parameter to determine the time which used to achieve the labelling process. The effect of contact time on the labelling reaction was studied from 0 to 24 hour with keeping the other parameters constant as shown in Fig. 6. The results revealed that the labelling reaction was completed after 30 min with a radiochemical yield of 85%. The formed complex (^{99m}Tc-erythrocin complex) was stable for a time up to 1 hour, after that, the yield decreased to reached 47.3% after 1440 min 24hour from the post-labeling. The optimum contact time to achieve the labelled reaction was 15 min according to previous work [25].

3.1.5 Effect of reaction temperature

Effect of temperature on the radiolabeling of erythrocin was evaluated from 20 to 95^{o}C with constant other parameters of labelling reaction as shown in Fig. 7. In general, the formation of ^{99m}Tc-erythrocin complex was significantly affected by the reaction temperature. The results showed that the radiochemical yield of ^{99m}Tc-erythrocin complex was maximum (85%) at room temperature 25ºC, then decreased gradually to reached (65.5%) at 95ºC. this behavior may be related to the increase of temperature lead to crack the bond between the substrate and the radionuclides.

3.2 Biodistribution in Animals

Biodistribution studies are an important parameter to consider when designing and testing novel materials and can provide precious information early in the development [37-39]. Thus, the biological distribution of ^{99m}Tc-erythrocin complex was achieved in Swiss Albino mice. The results revealed that increase in the biochemical parameters results after inflammation induction in animals than before inflammation induction as shown in Table 1. Liver enzymes (aspartate aminotransferase AST, alanine aminotransferase ALT), kidney function test (serum creatinine, blood urea nitrogen, total protein) were increased in case of inflammation than in normal mice, while serum albumin was decreased in state of inflammation [40]. Moreover, there are great increases in the muscle markers enzymes (lactate dehydrogenase LDH, creatine phosphokinase CPK) in case of inflammation than in normal mice. Also, there is increase in the concentrations of complement 3, complement 4, c-reactive protein CRP which play an important role in host defence mechanisms against infection in case of inflammation than in normal state [41,42].

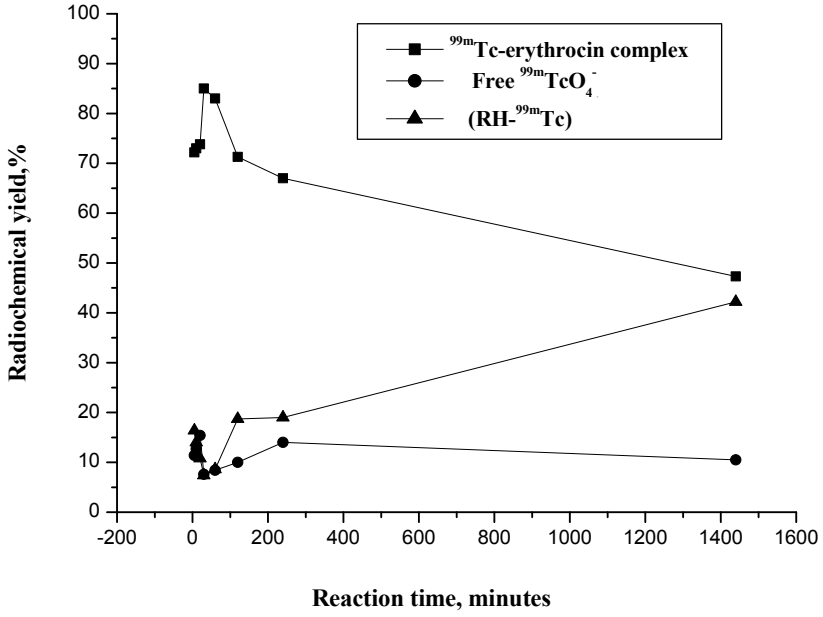

Fig. 6. Effect of reaction time on the radiochemical yield of ^{99m}Tc-erythrocin; [reaction conditions:5 mg of erythrocin, dissolved in1 ml pH 10, 5 µg of $SnCl_2.2H_2O$, 0.5 ml (~500MBq) of $^{99m}TcO_4$, the reaction mixture was kept at room temperature at different time intervals]

Table 1. Results of some biochemical parameters in serum of normal and inflamed mice

Biochemical parameters	Normal mice	Inflamed mice by living bacteria
Inflammation markers		
C3 (mg/dl)	48.9±4.0	156±3.8
C4 (mg/dl)	5.6±1.3	54±1.2
CRP (mg/l)	Negative (<6±0.0)	Positive (24±6.0)
(ESR (1st- 2nd h)	4-10±1.3 mm	15-25±2.2 mm
Muscles markers		
LDH (u/l)	560±151	6126±586
CPK (u/l)	320±46	7893±120
Liver enzymes		
ALT (u/l)	35±5.8	73±5.1
AST (u/l)	57±4.1	156±15.2
Kidney tests		
S. Creatinine (mg/dl)	0.8±0.2	1.8±0.2
Blood Urea (mg/dl)	34±5.5	86±7.6
T. protein (g/dl)	7.1±0.2	8.1±0.2
S. albumin (g/dl)	3.6±0.35	2.5±0.18

Table 2. Biodistribution of ^{99m}Tc-erythromycin in organs and body fluids for living *S. aureus* at 15, 30, 60 and 120 min

Living *S. aureus* (n=5, value represent mean ± S.D)				
Organs & body fluids	**Percent injected dose/gram tissue**			
	Time post injection, min			
	15	**30**	**60**	**120**
Blood	7.77±0.2	3.0±0.1	1.55±0.1	1.28±0.08
Muscle (Inf.)	2.74±0.1	2.98±0.1	3.63±0.15	3.4±0.1
Muscle (C)	1.85±0.1	1.06±0.09	0.88±0.02	0.91±0.02
Bone	2.2±0.1	1.73±0.07	1.63±0.1	1.58±0.06
Stomach	2.1±0.09	0.47±0.03	0.27±0.02	0.56±0.03
Intestine	3.1±0.1	2.76±0.09	2.1±0.1	2.9±0.1
Kidneys	12.6±1.1	13.5±1.1	15.8±1.2	14.8±1.1
Liver	3.26±0.1	3.3±0.1	1.73±0.07	2.83±0.1
Spleen	0.8±0.03	1.86±0.07	0.41±0.03	0.53±0.01
Lung	4.5±0.5	2.15±0.1	1.1±0.06	1.2±0.05
Heart	4.1±0.2	2.2±0.17	0.77±0.06	0.74±0.05
Urine	6.1±1.4	33±2.8	65±4.5	70±4.7
T/NT	1.48±0.02	2.81±0.04	4.12±0.15	3.4±0.1

Tables 2- 4 show the biodistribution of ^{99m}Tc-erythrocin in the important body organs and fluids. The results showed that the accumulated activity in the left thigh (inflamed tissue) was 4.12±0.15% fold higher than that in the right thigh (control tissue) at (1 hour) post injection. In addition, renal excretion was observed for ^{99m}Tc-erythrocin, as indicated by the great increase of kidneys activity with time post-injection.

Fig. 8 shows T/NT for live *S. aureus*, killed *S. aureus* and turpentine oil at 15, 30, 60 and 120 min post-injection ^{99m}Tc- erythrocin, respectively. The retention was specific since the abscess uptake of ^{99m}Tc- erythrocin remained high (T/NT= 4.12) when compared with the uptake of septic foci at 1 hour post-injection, the high T/NT ratio for live *S. aureus* model in comparison to the turpentine oil and killed *S. aureus* models supports the hypothesis that ^{99m}Tc- erythrocin was retained at infectious site because of its binding to bacterial cells [43].

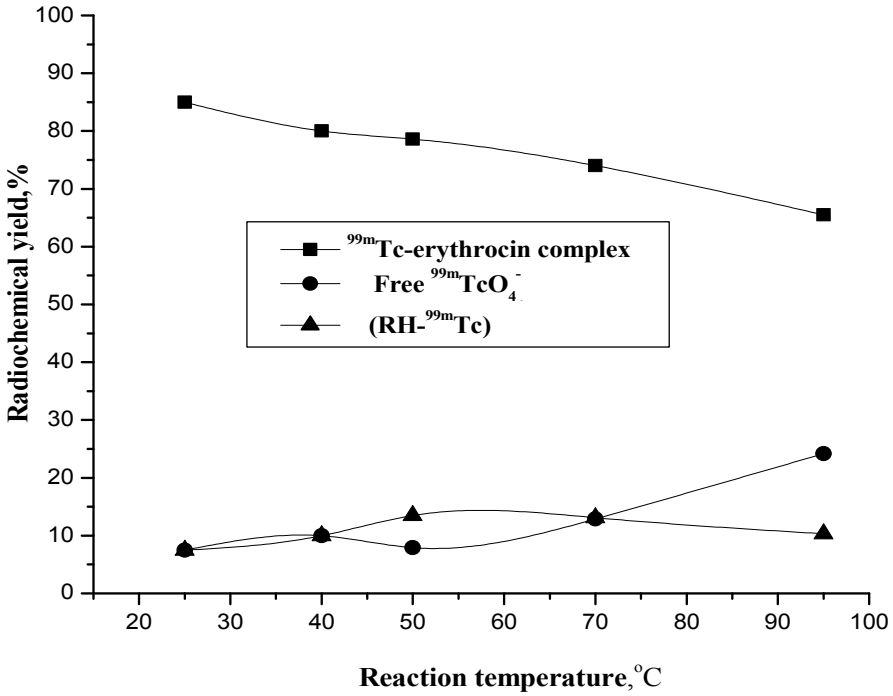

Fig. 1. Effect of temperature on the radiochemical yield of ^{99m}Tc-erythrocin; [reaction conditions: 5 mg of erythrocin, dissolved in1 ml pH 10, 5µg of $SnCl_2.2H_2O$, 0.5 ml (~500MBq) of $^{99m}TcO_4$ at different temperature for 30 minute]

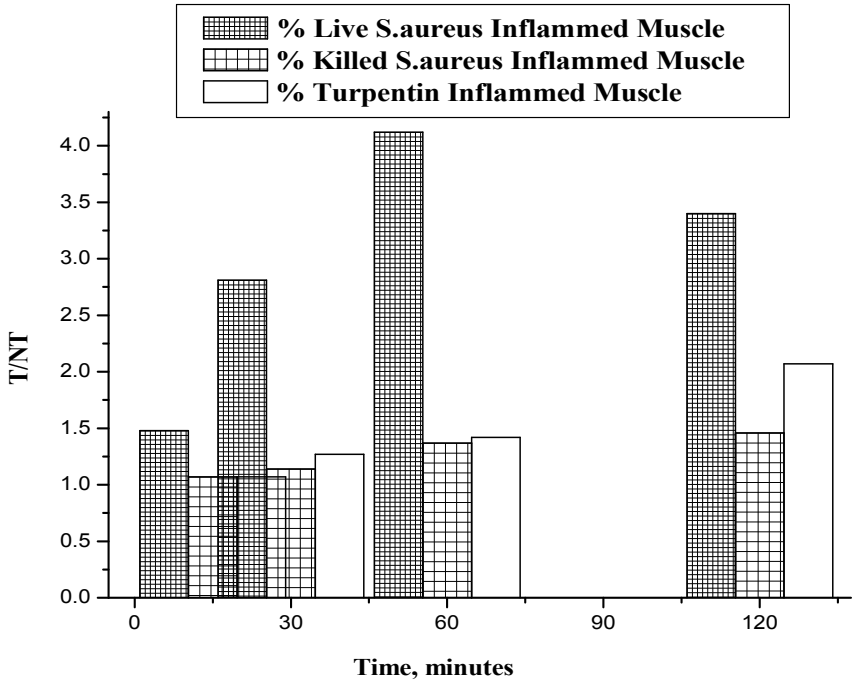

Fig. 8. Target (T) –to– Non-target (NT) ratio of live *S. aureus*, killed *S. aureus* and turpentine oil at 15, 30, 60 and 120 min. post injection of ^{99m}Tc-erythrocin

Table 3. Biodistribution of ^{99m}Tc-erythromycin in organs and body fluids for with killed *S. aureus* at 15, 30, 60, 120 min

Killed *S. aureus* (n=5, value represent mean ± S.D)				
Organs & body fluids	**Percent injected dose/gram tissue**			
	Time post injection, min			
	15	**30**	**60**	**120**
Blood	6.89±0.12	4.12±0.1	2.56±0.1	1.15±0.08
Muscle (Inf.)	2.73±0.1	1.83±0.04	2.17±0.08	1.59±0.03
Muscle (C)	2.55±0.1	1.61±0.05	1.58±0.05	1.09±0.04
Bone	2.82±0.1	2.32±0.08	2.84±0.1	2.2±0.1
Stomach	0.65±0.02	0.8±0.03	1.6±0.08	0.3±0.02
Intestine	1.65±0.07	5.06±1.2	5.2±1.5	2.77±0.14
Kidneys	13.2±1.1	25.4±1.6	22.5±1.2	8.5±0.6
Liver	4.42±0.2	4.0±0.18	3.0±0.1	1.5±0.07
Spleen	1.52±0.04	1.95±0.03	0.85±0.03	0.31±0.01
Lung	4.9±0.7	4.65±0.5	2.54±0.1	1.27±0.07
Heart	3.45±0.2	3.55±0.17	1.68±0.1	1.16±0.5
Urine	3.5±0.1	65±3.8	24±2.7	49±4.1
T/NT	1.07±0.05	1.14±0.02	1.37±0.03	1.46±0.03

Table 4. Biodistribution of ^{99m}Tc-erythromycin in organs and body fluids for turpentine oil at 15, 30, 60, 120 min

Turpentine oil (n=5, value represent mean ± S.D)				
Organs & body fluids	**Percent injected dose/gram tissue**			
	Time post injection, min			
	15	**30**	**60**	**120**
Blood	3.6±0.18	6.53±0.36	3.25±0.17	1.95±0.1
Muscle (Inf.)	3.35±0.12	3.67±0.1	3.35±0.1	2.13±0.06
Muscle (C)	3.13±0.15	2.89±0.11	2.35±0.1	1.03±0.03
Bone	3.85±0.12	3.53±0.1	3.1±0.1	2.35±0.08
Stomach	1.16±0.05	2.39±0.1	3.1±0.11	0.73±0.03
Intestine	2.33±0.09	2.68±0.1	5.8±0.2	4.44±0.17
Kidneys	11.7±1.5	22.1±1.7	12±1.3	10±1.1
Liver	2.98±0.1	5.35±0.2	3.75±0.12	3.77±0.1
Spleen	1.16±0.08	2.43±0.09	3.73±0.1	1.61±0.04
Lung	3.78±0.1	2.75±0.1	2.15±0.1	1.93±0.03
Heart	2.71±0.1	4.03±0.2	2.87±0.1	1.44±0.02
Urine	8±1.2	41±2.8	55±4.6	68±5.7
T/NT	1.07±0.01	1.27±0.01	1.42±0.01	2.07±0.02

4. CONCLUSION

In this work, erythrocin was successfully labelled with technetium-99m with a labelling yield of 85 ±1.1% under optimum conditions, using the simple and instantaneous method. The stability of ^{99m}Tc- erythrocin complex up to (1) hour post labelling and T/NT ratio= 4.12 which consider better than the commercially existing ^{99m}Tc-ciprofloxacin (labelling yield ~ 80%, stable up to 2 hour post labelling and T/NT=3.8). Therefore, Erythrocin is a good radiotracer for imaging of infection at early stages and distinguishing infection from sterile inflammation.

ETHICAL APPROVAL

The authors state that all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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