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Labeling, quality control and biological evaluation of ^{99m}Tc-vibramycin for infection sites imaging

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The use of radiolabeled antibiotics as diagnostic agents is an emerging area of medical research. Vibramycin is a semisynthetic antibiotic of the tetracycline family prescribed to treat a variety of infections. We present in this study a new technetium-99m labeled vibramycin radiopharmaceutical using $SnCl_2.2H_2O$ as a reducing agent. The stability of ^{99m}Tc-vibramycin was evaluated in human serum at 37 ^{0}C. Biodistribution studies of ^{99m}Tc-vibramycin were performed on a model of bacterially infected Sprague-Dawley rats. *In vitro* studies were performed to determine the binding interaction of the labeled antibiotic with bacteria and its stability. Scintigraphic study was done with a γ-camera 1 h, 4 h and 24 h after radiotracer injection in rats having infectious intramuscular lesions. It was confirmed through this study that ^{99m}Tc-vibramycin possesses high radiolabeling yield (95%) as determined by instant thin-layer chromatography. The binding assay shows good binding with *S. aureus*. Scintigraphy in rabbits showed uptake of ^{99m}Tc-vibramycin in the infectious lesions 1 h, 4 h and 24 h after injection. Biodistribution studies of ^{99m}Tc-vibramycin revealed that the radiopharmaceutical accumulated significantly at the infection sites and showed the renal route of excretion. Target-to-non target ratios for ^{99m}Tc-vibramycin for the infectious lesion and the control muscle were found to be significantly different. The study demonstrated that ^{99m}Tc-vibramycin shows preferential binding to living bacteria. The biological activity (*in vitro*) of ^{99m}Tc-vibramycin was studied using the optimized parameters and the ^{99m}Tc-vibramycin was found to be a good infection imaging agent.

Key Words: ^{99m}Tc-vibramycin; biodistribution; *Staphylococcus aureus*; infection imaging; ascorbic acid; scintigraphy.

INTRODUCTION

Infection is well-defined as an injury caused by bacteria, viruses, fungi and parasites leading to trauma, ischemia, and neoplasm. Signs and symptoms such as fever, swelling and pain may also appear and the diagnosis is regularly based on clinical, pathological and microbiological results. Due to lack of specificity, the diagnosis of infection is a major challenge in clinical practices. In molecular biology, immunology and medical biotechnology various approaches offer new insights for infection and inflammation imaging. Morphologic imaging and functional imaging tests are used for infection diagnosis. Morphologic imaging tests rely on structural abnormalities of tissues as a result of combination of microbial invasion and inflammatory reaction of the host such as ultrasonography, magnetic resonance imaging (MRI) and computed tomography (CT). Functional imaging studies use small quantities of radioactive material that is taken up by body cells, tissues, and organs directly, or attaches to other substances and migrates to the site of infection. Nuclear medicinal techniques can be used for *in vivo* characterization of cellular structure, function and biological changes at molecular level on infection associated tissues [1]. Radiopharmaceuticals are unique medicinal preparations comprising a radionuclide and a nonradioactive part and are designed to interact with a biological pathway, or target molecule in the body. Radiopharmaceuticals labeled with technetium-99m are used for medical imaging due to its superior properties of labeling and availability.Technetium-99m is normally eluted from a commercially available ^{99}Mo/^{99m}Tc generator system and is available at all radiopharmacy centers. Nuclear medical imaging techniques require radionuclides which decay with single photon emission and ^{99m}Tc is the radioisotope universally used in nuclear medical centers for gamma imaging [2]. Various technetium-99m biomolecules, such as cytokines, chemotactic peptides [3], monoclonal and polyclonal immunoglobulins, human defensin [4-6] and antibiotics [7] have been introduced for

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infection imaging. Radiolabeled antibiotics are an accurate tool for detection of infectious diseases, because they precisely bind to the bacterial machineries making possible the discrimination of bacterial infection and sterile inflammation [8], ^{99m}Tc labeled antibiotics such as ^{99m}Tc-ceflizoxime[9], ^{99m}Tc-cefuroxime and ^{99m}Tc-sitafloxacin kit [10], ^{99m}Tc-ciprofloxacin [11,12], ^{99m}Tc-dextran [13],^{99m}Tc-enrofloxacine [14], ^{99m}Tc-ethumbutol [15], ^{99m}Tc-erythromycin [16], ^{99m}Tc-flucanazole [17], ^{99m}Tc-infecton [18], ^{99m}Tc-kanamycin [19], ^{99m}Tc-lomefloxacin,^{99m}Tc-ofloxacin complexes [20], ^{99m}Tc-pefloxacin [21], ^{99m}Tc-piroxicam [22], ^{99m} Tc-tetrofosmin [23], and ^{99m}Tc-vancomycin [24] have been developed for imaging purposes and some of them are routinely employed in diagnostic nuclear medical centers [25,26]. Vibramycin is a wide-spectrum antibiotic of the family of tetracycline synthesized from oxytetracycline that controls the ability of bacteria to produce proteins crucial to them. Vibramycin has pronounced activity against a broad range of gram-positive and gram-negative organisms. Vibramycin is used to treat infections of the urinary tract, genitals, lungs, or eyes caused by infecting bacteria [27].

The present work aims at labeling vibramycin with technetium-99m, characterization, quality control, biodistribution and scintigraphic studies of ^{99m}Tc-vibramycin for infection sites diagnosis.

EXPERIMENTAL

Vibramycin for intravenous injection was obtained from Ameer Medical and Superstores, Islamabad, Pakistan. $Na^{99m}TcO_4$ was eluted from a locally produced fission based PAKGEN ^{99}Mo/^{99m}Tc generator, with 0.9% saline. All other reagents used were of analytical grade and acquired from E. Merck, Germany. *Staphylococcus aureus* was obtained from the National Institute of Health, Islamabad. All animal experiments were performed following the principles of laboratory animal care and were approved by the Institutional Animal Ethics committee. Three animals (rabbits and rats) were used for each set of experiments for biodistribution and scintigraphy. The animals were kept under standard conditions with free access to food and water. Tissue and organ radioactivity was measured with a γ-counter (Ludlum model-261). Gamma scintillation camera (Capintec Caprac-R1) was used for imaging of experimental animals.

Labeling of vibramycin with technetium-99m

Vibramycin (0.2 mg) was directly labeled with ^{99m}Tc. Optional amount of 2-4 µg of $SnCl_2.2H_2O$ was used as a reducing agent (pH 3). Ascorbic acid (4 mg) was used as a stabilizer in the mixture. After addition of all reagents ~ 370 MBq $^{99m}TcO_4^-$ in saline was injected into the vial at room temperature.

Determining labeling efficiency by ITLC analysis

Stability and labeling yield of ^{99m}Tc-vibramycin was done by instant thin-layer chromatography. One µL sample of the preparation was spotted on ITLC strips (Gelman Laboratories) using 0.5M NaOH as the mobile phase. In this system, ^{99m}Tc-vibramycin migrated with the solvent front of the mobile phase (R_f = 1.0) and the colloid was found at the origin of the strip (R_f = 0). To determine the pertechnetate content of the preparations, a strip of Whatman Paper No. 3 was developed using acetone as the mobile phase. In this system, pertechnetate migrated with the solvent front of the mobile phase (R_f = 1.0).

Electrophoresis

Electrophoresis of the prepared ^{99m}Tc-vibramycin was done on Whatman No. 1 paper in phosphate buffer (pH 6.8) in a deluxe electrophoresis chamber (Gelman) system. Whatman No. 1 paper of 30 cm was marked as L at the left side of the strip and R at the right side of the strip. A drop of ^{99m}Tc-vibramycin was applied to the middle of the strip which was put in the midpoint of the electrophoresis chamber having buffer in such a way that the left side was dipped at anode and right side at cathode. The electrophoresis was run for 60 to 90 min at a voltage of 300V. After completion of electrophoresis, the strip was scanned by using 2π scanner to identify the charge on vibramycin.

Stability of ^{99m}Tc-vibramycin in human serum

The stability of ^{99m}Tc-vibramycin was checked in human serum at 37 °C. Normal human serum (1.8 mL) was mixed with 0.2 mL of ^{99m}Tc-vibramycin and incubated for 30 min. Aliquots of 0.2 mL were withdrawn during the incubation at different time intervals up to 24 h and subjected to ITLC analysis for the determination of ^{99m}Tc-vibramycin, reduced/hydrolyzed ^{99m}Tc and free $^{99m}TcO_4^-$. The increase in the amount of free pertechnetate indicated the degree of degradation.

Bacterial strains

Bacterial strain of *Staphylococcus aureus* is frequently used in different microbiology labs. It was obtained from the American Type Culture Collection. Overnight cultures of the strain were

prepared in brain heart infusion broth (BHI, Oxoid) at 37 ºC in a shaking water bath. Aliquots of suspensions containing the viable stationary phase bacteria were rapidly frozen in liquid nitrogen and stored at -70 ºC. Before use, an aliquot of this suspension was immediately thawed in a water bath at 37 ºC and diluted with sodium phosphate buffer.

Bacterial binding assay

Bacterial binding of Tc-99m-vibramycin was assessed using *S. aureus*. Sodium phosphate buffer (0.1 mL) containing ~5MBq of ^{99m}Tc-vibramycin was transferred to a test tube. A known volume (0.8 mL) of acetic acid (0.01M) in Na-PB containing around 1× 10^8 viable bacteria was added to the above tube. The mixture was incubated at 4 ºC for 1 h and centrifuged for 10 min. The supernatant was removed and the bacterial pellet was gently resuspended in 1 mL of ice cooled Na-PB and recentrifuged. The supernatant was removed and the radioactivity in the bacterial pellet and the supernatant was measured by gamma-counter. The radioactivity related to bacteria was expressed in percent of the added ^{99m}Tc activity to viable bacteria with respect to total ^{99m}Tc activity. For comparison purposes binding of ^{99m}Tc-ascorbic acid to bacteria was also performed. Ascorbic acid (1 mg) was dissolved in 1 mL of pure water, the pH adjusted to 5 with 0.1 M NH_4OH solution, and then 0.2 mL of $SnCl_2 \cdot H_2O$ (1 mg/1 mL 0.1 M HCI) and 0.5 mL of $Na^{99m}TcO_4$ (100 MBq) in saline were added to the vial. The reaction vial was left at 25°C for 20 min. The radiochemical purity of the labeled compound was checked with TLC [28].

Induction of infection with live S.aureus

A single clinical isolation of *S.aureus* from biological samples was used to produce focal infection. Individual colonies were further diluted to obtain a turbid suspension containing 2×10^8 colony-forming units (cfu) of *S. aureus* in 0.2 mL of saline and were intramuscularly injected into the left thigh of rats [29, 30].Then, the rats were left for 24 h to get a visible swelling in the infected thigh. Three rats were used for one set of experiments.

Induction of non-infected inflammation with heat killed S.aureus

Staphylococcus aureus suspension was heated at 100°C for 2 h to obtain killed *S. aureus*. Sterile inflammation was induced by injecting 0.2 mL of heat killed *S.aureus*, intramuscularly in the left thigh muscle of the rats. Two days later, swelling appeared.

Induction of non-infected inflammation with irradiated S. aureus

Staphylococcus aureus (1 mL suspension) containing about 2×10^8 colony-forming units (cfu) was gamma irradiated with a 3 KGy dose to get irradiated *S. aureus*. The non-viability of bacteria was tested by cultivating them in different media and 0.2 mL suspension were injected intramuscularly in the left thigh of rats.

Induction of inflammation with turpentine oil

Sterile inflammation was induced intramuscularly by injecting 0.2 mL of turpentine oil [31] in the left thigh muscle of the rats. After two days the swelling appeared.

Biodistribution studies in animal models

The animals were intravenously injected with 0.2 mL of ^{99m}Tc-vibramycin (~38 MBq) via the tail vein. After a definite time, the rats were sacrificed at 1, 4 and 24-h post-injection after ether anesthesia and biodistribution study was done. Blood (1 mL) was taken by cardiac puncture and activity in total blood was calculated by assuming blood volume equal to 6.34% of body weight. Samples of weighed infected muscle, normal muscle, liver, spleen, kidney, stomach, intestine, heart, brain, bladder and lungs were taken and activity was measured by the use of a gamma counter. The results were expressed as the percent uptake of injected dose per organ. The results of the bacterial uptake of ^{99m}Tc-vibramycin and other compounds were analyzed by analysis of variance setting the level of significance at 0.05.

Induction of experimental infection in rabbit

Saline (0.6 mL) containing viable *S. aureus* (4×10^8 cfu) was injected into the left thigh muscle of each rabbit. After 72 h when significant swelling appeared at the site of injection, scintigraphy was done.

^{99m}Tc-vibramycin scintigraphy

The model animal in triplicate was placed on a flat hard surface with both hind legs spread out and was fixed with the help of a surgical tape. Diazepam (5 mg) was injected into the right thigh muscle. Saline (0.2 mL) containing 15 MBq of ^{99m}Tc-vibramycin was then injected intravenously into the marginal ear vein. A single headed Siemens Integrated ORBITER Gamma Camera System interfaced with high-resolution parallel hole collimator and an on-line dedicated computer was used for imaging. Immediately after injection, dynamic acquisition with both thighs in focus was

done for 120 min. For the biodistribution study of the radiotracer, whole body acquisition was done at 1, 4 and 24 h after injection.

RESULTS AND DISCUSSION

Vibramycin (Fig. 1) was labeled with technetium-99m and labeling efficiency of 95 % was achieved (Fig. 2, 3). The effect of pH is shown in Fig. 4. At pH 2 the minimum labeling efficiency was achieved (80%), while at pH 3-4 the labeling efficiency of ^{99m}Tc-vibramycin increased to > 95%. In basic media (pH 8) the labeling efficiency decreased to 68%. A known quantity of 2-3 µg of reducing agent, $SnCl_2.2H_2O$ gave the highest labeling efficiency, and thus the value of 2.5 µg of $SnCl_2.2H_2O$ was chosen for further procedures (Fig. 5). The highest labeling efficiency was achieved at 200 µg of ligand (Fig. 6). The complexation of ^{99m}Tc with vibramycin was achieved after about 30 min and retained for up to 12 h (Fig. 7).

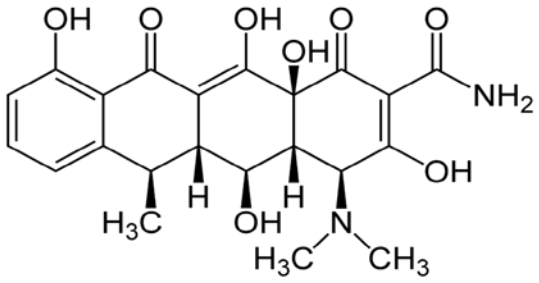


Fig. 1. Structure of vibramycin

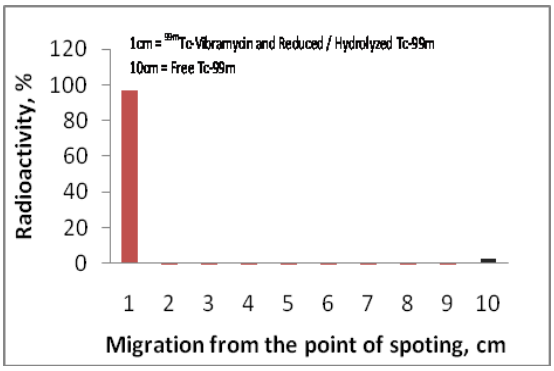


Fig.2. Paperchromatography pattern of the ^{99m}Tc-vibramycin, free, reduced/hydrolyzed ^{99m}Tc.

The radiochemical purity was assessed by a combination of ascending paper chromatography and instant thin-layer chromatography on silica gel. In paper chromatography acetone was used as solvent for free $^{99m}TcO_4^-$ while in ITLC-SG 0.5M NaOH was the solvent used for ^{99m}Tc-vibramycin and reduced/hydrolyzed ^{99m}Tc. By following the above mentioned procedures the results were in excellent agreement. In this study, vibramycin was labeled with ^{99m}Tc with high radiochemical yields. During the labeling of vibramycin <2% colloid and <2% free pertechnetate were observed.

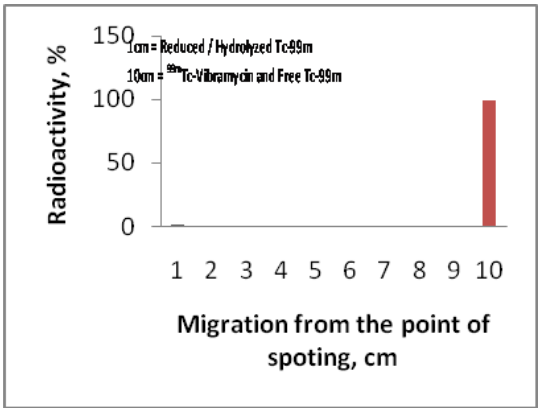


Fig.3. ITLC-SG pattern of the ^{99m}Tc-vibramycin, free, reduced/hydrolyzed ^{99m}Tc.

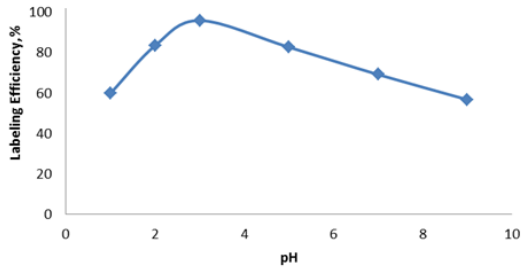


Fig.4. Effect of pH on the labeling efficiency of ^{99m}Tc-vibramycin

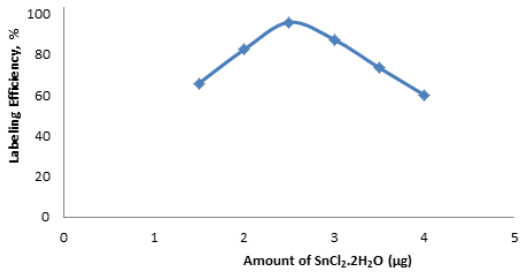


Fig.5. Effect of amount of stannous chloride dihydrate on the labeling efficiency of ^{99m}Tc-vibramycin

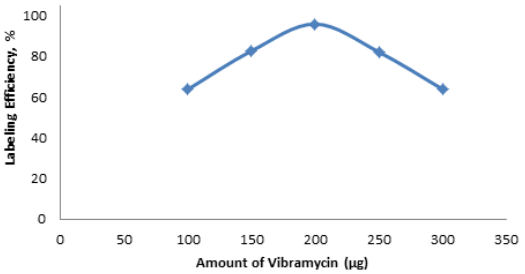


Fig.6. Effect of amount of ligand on the labeling efficiency of ^{99m}Tc-vibramycin

The stability of the radiopharmaceutical was checked by its incubation in human serum. ^{99m}Tc-vibramycin was found to be fully stable as determined by ITLC. Up to 98% labeling was found at 24 h of incubation at 37ºC and there was almost no increase in reduced/hydrolyzed ^{99m}Tc,

while very little increase in free pertechnetate was observed. The total impurities were <2% (Table 1). The results of electrophoresis illustrate the neutral behavior of the ligand (Fig. 8).

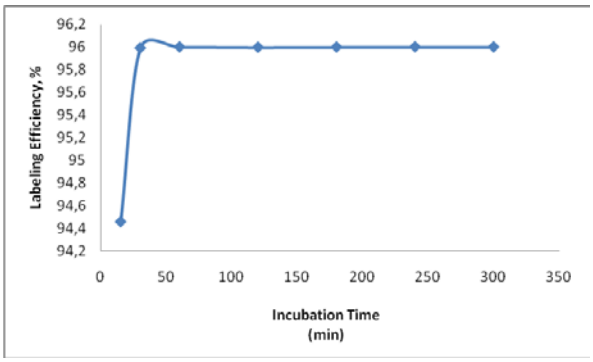


Fig.7. Rate of complexation and stability of ^{99m}Tc-vibramycin at room temperature.

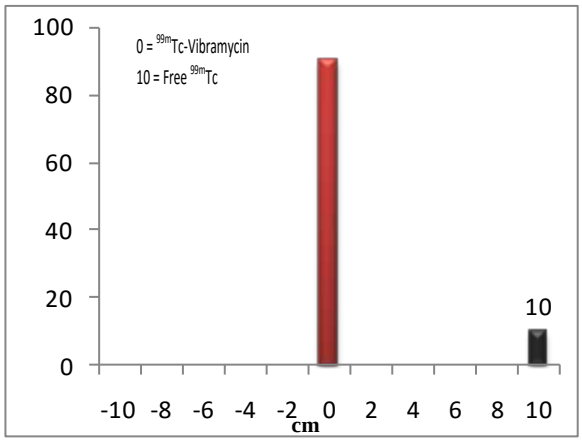


Fig.8. Electrophoresis of radiolabeled compound (^{99m}Tc-vibramycin at 0 cm; $^{99m}TcO_4^-$ at 10 cm).

Table 1. *In vitro* stability of ^{99m}Tc-vibramycin in normal human serum

Incubation time (h)	^{99m}Tc-vibramycin	Free pertechnetate	Colloid
0.5	99.9 ± 0.1	0.1 ± 0.0	0.0
1	99.8 ± 0.2	0.2 ± 0.01	0.0
2	99.7 ± 0.3	0.3 ± 0.03	0.0
4	99.6 ± 0.4	0.4 ± 0.04	0.0
24	99.5 ± 0.5	0.5 ± 0.06	0.0

In vitro binding of ^{99m}Tc-vibramycin to bacteria was comparable to that of ^{99m}Tc-ascorbic acid. Binding of varying amounts of ^{99m}Tc-vibramycin with *S. aureus* was in the range of 97-99% (Fig. 9), while binding of ^{99m}Tc-ascorbic acid was <5% (Table 2).

Table 2. *In vitro* binding of ^{99m}Tc-ascorbic acid to viable *Staphylococcus aureus*

^{99m}Tc-ascorbic acid	*Staphylococcus aureus*		
	1 h	4 h	24 h
10 µg	0.25	0.18	0.10
50 µg	0.39	0.29	0.16
100 µg	4.7	3.6	1.2

Significant uptake of ^{99m}Tc-vibramycin was observed in liver, stomach, lungs and heart during biodistribution studies. *In vivo* stability of ^{99m}Tc-vibramycin was noticed in the body since stomach, lungs and intestine showed significant activity. The biodistribution results (% injected activity/g) of ^{99m}Tc-vibramycin in different organs of the animals infected with living, heat killed *S.aureus* and turpentine oil induced, 1, 4 and 24 h after intravenous administration are shown in Table 3. The results show that ^{99m}Tc-vibramycin accumulates significantly at the infected thigh muscle as compared to heat killed *S.aureus* and turpentine oil infected group of animals. Our studies in rats with intramuscular infection indicated that the uptake in the infected tissue is attributed to specific binding to living bacteria. Whole body images of infected rabbits at 1, 4, and 24 h post ^{99m}Tc-vibramycin administration are presented in Fig. 10 a, b and c, respectively. *S. aureus* infection in rabbit left thigh was visualized as the area of increased tracer accumulation just after injecting labeled vibramycin as shown in Fig 11. The infection is clearly visible 3 h post administration. The rats with infectious lesions injected with ^{99m}Tc-vibramycin showed a mean target-to-non target (T/NT) ratio of 2.6 ± 0.3, 1 h post injection (Table 3). ^{99m}Tc-vibramycin shows a higher T/NT ratio in the infected muscle (live *S.aureus*) at all time intervals than that of sterile inflamed muscle (heat killed *S.aureus* and turpentine oil). This ^{99m}Tc-vibramycin showed higher uptake in infected tissue than ^{99m}Tc-streptomycin (T/NT = 2.4 ± 0.1) [32]. The high T/NT ratio for the live S. *aureus* model as compared to turpentine, irradiated and heat killed *S. aureus* models provides evidence that ^{99m}Tc-vibramycin accumulated at the infectious site due to its specific binding to bacterial cells. Thus, in this study we can establish the basis for the potential of ^{99m}Tc-vibramycin to distinguish bacterial from non-bacterial infection.

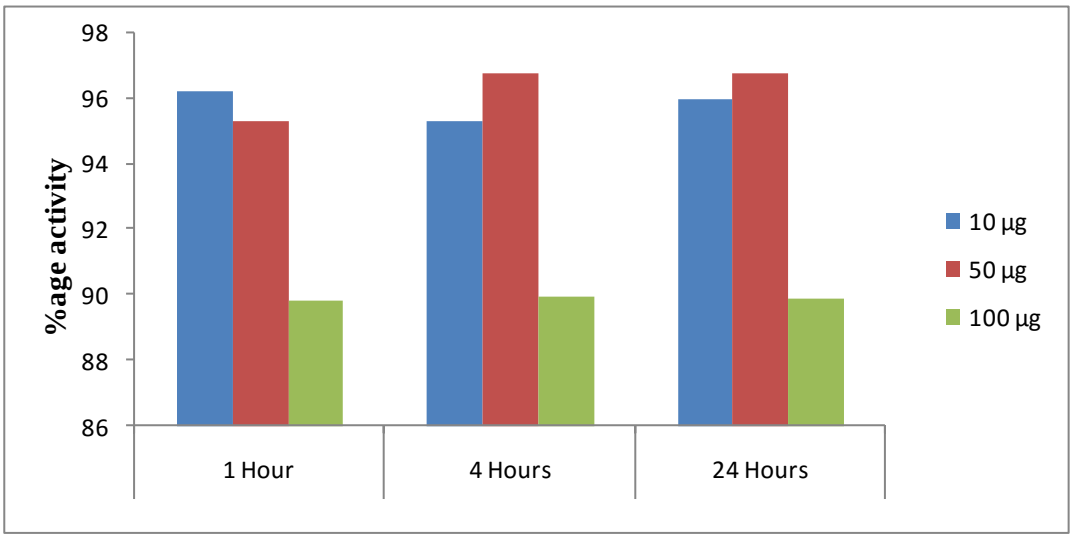
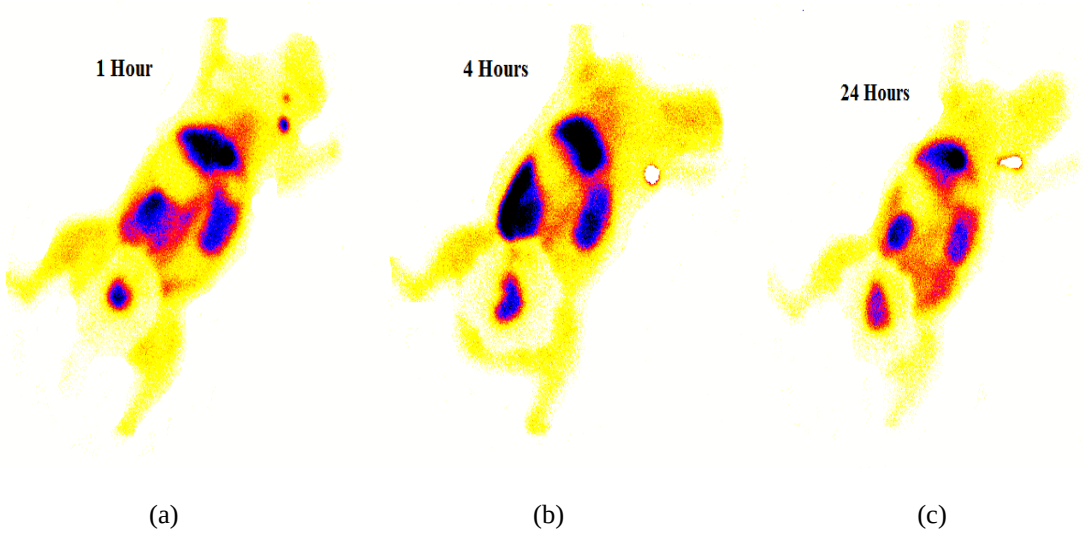


Fig.9. *In vitro* binding of ^{99m}Tc-vibramycin to viable *Staphylococcus aureus*



(a) (b) (c)

Fig.10. Whole body gamma camera image of rabbit injection with ^{99m}Tc-vibramycin 1 h post administration (a), 4 h post administration (b), and 24 h post administration (c).

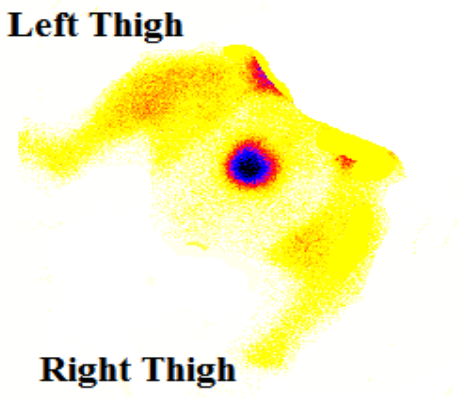


Fig.11. *S. aureus* infection in rabbit left thigh and right thigh visualized as area of increased tracer accumulation 3 h post injection of ^{99m}Tc-vibramycin

Table 3. Biodistribution of ^{99m}Tc-vibramycin in live *S.aureus*, heat killed *S.aureus*, irradiated *S.aureus* and turpentine oil inflamed rats at different time intervals (mean ± SD), (% ID/g).

Organs	Percentage of injected dose per gram of tissue weight (n = 3/time interval, ir)[1]											
	Live *S.aureus*			Heat Killed *S.aureus*			Irradiated *S.aureus*			Turpentine oil		
	1 h	4 h	24 h	1 h	4 h	24 h	1 h	4 h	24 h	1 h	4 h	24
Liver	4.60 ± 0.8	3.52 ± 1.2	1.48 ± 0.6	4.32±0.4	3.55±0.8	0.80±0.1	3.06±0.6	2.5±0.6	1.18±0.5	3.88 ± 0.8	2.25 ± 0.2	0.50 ± 0.9
Spleen	0.20 ± 0.6	0.19 ± 0.05	0.12 ± 0.03	0.10±0.2	0.15±0.2	0.05±0.5	0.12±0.5	0.10±0.06	0.10±0.2	0.05± 0.4	0.09 ± 0.8	0.03± 0.1
Stomach	0.19 ± 0.2	0.18 ± 0.03	0.11 ± 0.01	0.90±0.3	1.09±0.9	0.9±0.1	0.25±0.9	0.15±0.1	0.09±0.02	0.50± 0.5	0.77 ± 0.8	0.32 ± 0.2
Intestine	4.28 ± 1.1	3.81 ± 0.9	1.98 ± 0.4	3.59±1.0	3.1±1.5	1.50±0.3	1.05±0.3	1.30±0.4	1.01±0.12	2.99 ± 0.1	2.50 ± 0.7	0.95 ± 0.5
Lungs	2.29 ± 0.9	2.20 ± 0.8	1.85 ± 0.8	1.97±1.0	1.90±1.6	1.73±0.4	1.75±1.2	1.05±0.2	1.09±1.1	6.96 ± 1.7	1.05 ± 1.7	1.28 ± 0.1
Kidney	0.64 ± 0.1	0.99 ± 0.2	1.35 ± 0.65	0.58±1.3	0.5±0.2	0.96±0.6	0.20±0.2	0.5±0.1	1.40±0.3	0.25 ± 1.5	0.01 ± 0.1	0.54 ± 0.2
Bladder	7.28 ± 2.3	1.34 ± 0.6	3.56 ± 1.21	6.16±0.1	0.78±0.6	2.75±0.5	5.25±0.7	1.16±0.1	3.06±0.4	5.06 ± 0.2	0.26 ± 0.3	1.93 ± 0.3
Heart	0.18 ± 0.03	0.11 ± 0.05	0.07 ± 0.03	1.2±0.1	0.40±0.3	0.13±0.9	0.10±0.06	0.07±0.01	0.04±0.01	0.60 ± 0.4	0.30 ± 0.1	0.10 ± 0.1
Brain	0.06 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.03±0.5	0.09±0.5	1.04±0.2	0.02±0.1	0.42±1.0	0.03±0.01	0.90 ± 1.6	0.02 ± 0.6	1.57 ± 0.7
Blood	5.46 ± 1.5	2.71 ± 1.6	1.22 ± 0.48	4.95±0.01	1.97±0.5	1.10±1.6	1.79±0.5	1.01±0.1	1.09±0.2	3.30 ± 0.5	1.05 ± 0.2	0.80 ± 0.4
Body	0.91 ± 0.3	0.57 ± 0.09	0.38 ± 0.05	0.65 ± 0.4	0.54 ± 1.2	1.26 ± 1.6	1.15 ± 0.4	1.15 ± 0.4	1.15 ± 0.4	1.82 ± 0.5	2.41 ± 1.0	2.05 ± 1.4
Inflamedmuscle	0.66 ± 0.2	0.54 ± 0.01	0.36 ± 0.11	0.41±1.2	0.34±0.4	0.32±4.0	0.21±1.0	0.25±0.2	0.15±0.2	0.47 ± 0.02	0.42 ± 0.2	0.40 ± 0.4
Controlmuscle	0.25 ± 0.1	0.24 ± 0.03	0.19 ± 0.04	0.19±0.3	0.16±1.0	0.15±1.0	0.11±0.1	0.12±0.02	0.09±0.1	0.26 ± 0.8	0.18 ± 0.5	0.29 ± 0.9

[1]values represent the mean± standard deviation of data from 3 animals.

CONCLUSION

^{99m}Tc-vibramycin prepared by a direct method possesses high radiochemical purity of 95%. The ^{99m}Tc-vibramycin was stable and ≥95% labeling was maintained for up to 12 h and there was no need of post-labeling. The biological activity of ^{99m}Tc-vibramycin and ^{99m}Tc-ascorbic acid was comparable. The ^{99m}Tc-vibramycin was found to have greater ability to localize in bacterial infection sites induced by *S. aureus* in animal models. Thus data obtained from bio-evaluation studies showed that the prepared ^{99m}Tc-vibramycin is accumulated at the infectious site and may be a good bacterial infection imaging agent due to its specific binding to bacterial cells.

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БЕЛЯЗАНЕ, КАЧЕСТВЕН КОНТРОЛ И БИОЛОГИЧНА ОЦЕНКА НА ^{99m}Tc-ВИБРАМИЦИН ЗА ОПРЕДЕЛЯНЕ НА ИНФЕКТИРАНИ ЗОНИ

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(Резюме)

Използването на белязани антибиотици като диагностициращи агенти е развиваща се дейност в медицинските изследвания. Вибрамицинът е полу-синтетичен антибиотик от групата на тетрациклините, предписван за лечението на различни инфекции. В настоящата работа се представя нов вибрамицин, белязан с 99m-технеций и използващ $SnCl_2.2H_2O$ като редуктор. Стабилността на белязания вибрамицин е оценена върху човешки серум при 37^0C. Изследвано е биологичното разпределение на ^{99m}Tc-вибрамицина върху Sprague-Dawley плъхове, заразени с бактериална инфекция. *In vitro*-изследванията са извършени, за да се определи свързващото взаимодействие на белязания антибиотик с бактериите и неговата стабилност. Извършено е сцинтиграфско изследване с γ-camera един, четири и двадесет и четири часа след инжектирането на радиотрейсера в плъхове с инфекциозни поражения на мускулите. Потвърдено е чрез моментна тънкослойна хроматография, че ^{99m}Tc-вибрамицинът притежава висок добив на белязане (95%). Пробите показват добро свързване с *S. aureus*. Сцинтиграфията при зайци показва поглъщане на ^{99m}Tc-вибрамицина в инфектирани тъкани един, четири и двадесет и четири часа след инжектирането. Биоразпределението на ^{99m}Tc-вибрамицин показва, че радиоактивният препарат значително се натрупва в инфектираните места и се изхвърля чрез бъбреците. Отлагането при целево към нецелево използване на ^{99m}Tc-вибрамицина в засегнатите места и в контролните мускули се различават значително. Изследването показва, че ^{99m}Tc-вибрамицинът се свързва предимно с живи бактерии. Биологичната активност *in vitro* на ^{99m}Tc-вибрамицина е изследвана при оптимизирани параметри и е установено качеството му на добър агент за изобразяването на инфекции.