

# An imaging analysis of $^{99m}\text{Tc}$ -UBI (29–41) uptake in *S. aureus* infected thighs of rabbits on ciprofloxacin treatment

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## Abstract

**Purpose** The uptake of  $^{99m}\text{Tc}$ -UBI (29–41) was evaluated at sites of bacterial infections in rabbits before and after treatment with ciprofloxacin.

**Methods** *Staphylococcus aureus* susceptible to ciprofloxacin was used to induce a focal infection in each rabbit of group 1 (G1), group 2 (G2), and group 3 (G3) with  $2 \times 10^4$ ,  $2 \times 10^6$ , and  $2 \times 10^8$  colony forming units (CFU), respectively. After 24 h, images of infected thighs (target: T) and contralateral thighs (nontarget: NT) were acquired. Animals then received ciprofloxacin intramuscularly for 5 days followed by imaging on the third and fifth days. The control group 4 (G4) was imaged at days 1, 3, and 5 under the same acquisition

parameters. Group 5 (G5) was employed to study biodistribution of the peptide.

**Results** Increases in (T/NT) ratios in G1, G2, and G3 were observed from 5 min onwards with maximum values at 60 min. G3 revealed the highest accumulation of the peptide. Growth of the same strain of *S. aureus* on blood agar medium was visualized after fine needle aspiration. After ciprofloxacin treatment, the images for G1–G3 resulted in significantly decreased ( $P < 0.05$ ) T/NT values on the third and fifth days that correlated with reduction in number of viable bacteria. No significant difference ( $P < 0.05$ ) in left to right thigh ratios in the control group (G4) was observed. Biodistribution of the peptide showed rapid removal of tracer from circulation through the kidneys.

**Conclusions**  $^{99m}\text{Tc}$ -UBI (29–41) accumulation directly correlates with the number of viable bacteria. This infection localization agent can be utilized for monitoring efficacy and duration of antibiotic treatment.

This study was conducted at Punjab Institute of Nuclear Medicine (PINUM), Faisalabad, in collaboration with the Isotope Production Division (IPD) of the Pakistan Institute of Nuclear Science and Technology (PINSTECH), Islamabad, Pakistan, and the Clinical Medicine & Surgery Department of the University of Agriculture, Faisalabad, Pakistan. There was no sponsor for this research activity. Dr. Muhammad Saeed Akhtar, the first author of this article, is a Ph.D. (Nuclear Medicine) fellow at the Pakistan Institute of Engineering and Applied Sciences (PIEAS) University, Islamabad, Pakistan.

**Keywords** Ubiquicidin · Synthetic peptide · Technetium · Infection detection · Ciprofloxacin · Monitoring therapy

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## Introduction

Infection is a global problem that needs prompt and accurate diagnosis for early management to avoid serious complications. However, the goal of precise localization of infective focus is difficult to achieve even in the modern era of technology. Broad-spectrum antibiotics are still blindly prescribed in medical and surgical scenarios, which lead to high costs of treatment. Gold standard detection tools for infection, like biopsy and culture of causative organisms, are not feasible in all conditions and carry the disadvantages of

being invasive, having inaccurate sampling, and having lengthy analysis times. To distinguish between infection and inflammation is the major hallmark for appropriate decision in patient management. Anatomical details are better visualized on ultrasound, CT, and MRI; however, each modality has certain limitations, and the leading question is still unanswered. Scintigraphy has the advantage of early elucidation of pathophysiological changes in the infective process; however, it is limited by poor resolution.

Recent advances in nuclear medicine technology resulted in commercially available instrumentation such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) that have markedly improved anatomical details. Autologous in vitro  $^{111}\text{In}$ - or  $^{99\text{m}}\text{Tc}$ -HMPAO-labeled leukocytes are still the gold standard for imaging infections. Planar images with gamma cameras are handicapped with limited resolution that is not sufficient for assessing the extent of disease. SPECT increases the sensitivity of the nuclear medicine procedures [1, 2], but precise anatomical localization of organs is still not possible. Hybrid SPECT/CT improves the diagnostic accuracy when subjected to  $^{99\text{m}}\text{Tc}$ -HMPAO-labeled leukocytes in patients with suspected osteomyelitis [3]. Marked improvement in sensitivity and definition of the extent of infection has been documented with SPECT/CT using  $^{67}\text{Ga}$ - and  $^{111}\text{In}$ -labeled leukocytes [4]. PET/CT with  $^{18}\text{F}$ -FDG-labeled autologous leukocytes has further improved the diagnosis and localization of infection lesions [5]; however, the technique is time consuming, demands a sterile environment, and carries the risk of transmission of blood-borne diseases.

Antimicrobial compounds that bind to the bacteria would be specific for infection localization if labeled with a suitable isotope because of their selective adhesion to the causative agents [6]. An early antibiotic radiopharmaceutical was  $^{99\text{m}}\text{Tc}$ -ciprofloxacin, which is an analog of a broad-spectrum quinolone antibiotic having the property of binding to DNA gyrase of bacteria and inhibiting DNA synthesis [7]. This  $^{99\text{m}}\text{Tc}$  agent showed encouraging results in various infections [8–10]; however, specificity was lower than expected, and its accumulation in noninfectious/inflammatory sites has also been reported [11]. Due to nonspecific accumulation in inflammatory sites, this agent has been proposed for identifying the presence and distribution of inflammation within joints [12]. Bacterial resistance to ciprofloxacin is another disadvantage, which results in false-negative results [13]. Antimicrobial peptide ubiquicidin UBI (29–41) in a freeze-dried kit form has been evaluated as a specific infection imaging agent with encouraging results in animal models [14]. This peptide preferentially binds to bacteria and fungi over mammalian cells and distinguishes bacterial and fungal infectious from sterile inflammation [15]. Accumulation of  $^{99\text{m}}\text{Tc}$ -UBI (29–41) has direct correlation with the number of viable bacteria in the focus, the lower

detection limit being  $10^3$  colony forming units [16]. A small human clinical trial of 18 patients searching for the ability of  $^{99\text{m}}\text{Tc}$ -UBI (29–41) as an infection localizing agent revealed overall sensitivity, specificity, and accuracy of 100%, 80%, and 94%, respectively [17]. Antibiotic treatment occasionally commences before confirming the site of infection and, on this promise, it was questioned whether  $^{99\text{m}}\text{Tc}$ -UBI (29–41) could localize such foci in the presence of concurrent antibiotic treatment. This study was designed to assess the accumulation of  $^{99\text{m}}\text{Tc}$ -UBI (29–41) peptide in bacterial foci of known concentrations in rabbit thighs before and after ciprofloxacin treatment. Ciprofloxacin was selected over cloxacillin and erythromycin, which were studied earlier [18], because it is usually employed as a first-line, broad-spectrum antibiotic in the current era.

## Materials and methods

Healthy immunocompetent rabbits were obtained from a farm house. The synopsis of the proposed research activity was approved by the Animal Ethics Committee of the Punjab Institute of Nuclear Medicine (PINUM) and the University of Agriculture, Faisalabad, Pakistan. The maximum upper limit of  $2 \times 10^8$  colony forming units (CFU) bacterial concentration per animal was allowed by the committee. Fifteen male rabbits, each weighing 1.2–1.8 kg (average 1.4 kg), were housed in animal housing facilities of the University of Agriculture, Faisalabad, Pakistan, at least 3 days before the start of the experiment and were fed and hydrated ad libitum.

### Grouping of animals

Fifteen rabbits were categorized into five groups, each composed of three animals:

- Group 1: Each rabbit was injected with  $2 \times 10^4$  CFU of viable *Staphylococcus aureus* into the left thigh muscle for induction of infection.
- Group 2: For injection with  $2 \times 10^6$  CFU of viable *S. aureus* in each animal.
- Group 3: For concentration of  $2 \times 10^8$  CFU of viable *S. aureus* in each rabbit.
- Group 4 (control): Neither bacteria nor ciprofloxacin was administered.
- Group 5 (biodistribution): For biodistribution study of  $^{99\text{m}}\text{Tc}$ -UBI (29–41).

### Microorganisms

A clinical isolate of *S. aureus* (6736153) susceptible to ciprofloxacin was obtained from the department of Clinical

Medicine and Surgery (CMS), University of Agriculture, Faisalabad. Overnight cultures of bacteria were prepared in brain heart infusion (CM 0225: Oxoid, Basingstoke, Hampshire, UK) on a horizontal shaker (Techno-Labs, Faisalabad, Pakistan) at a speed of 110 rpm at 37°C. The chosen numbers of bacteria ( $2 \times 10^4$ ,  $2 \times 10^6$ , and  $2 \times 10^8$  CFU) were prepared by counting their number spectrophotometrically. A laminar flow cabinet was used during microbiological work to prevent contamination.

#### Antibiotic activity against *S. aureus*

The susceptibility of *S. aureus* was determined by agar disc diffusion method (ADD, qualitative method) and minimum inhibitory concentration method (MIC, quantitative method) with E test [19]. The disc impregnated with ciprofloxacin was placed on the surface of a Muller–Hinton agar plate swabbed with a suspension of *S. aureus* corresponding to turbidity adjusted to one half of McFarland standard for 18–24 h at 37°C. The E test utilizes longitudinal paper strips impregnated with a predefined antimicrobial agent with serial concentrations starting from lowest concentration (0.064 µg/ml) from one end with gradual rise towards the other end (32 µg/ml). The E test strip (AB Biodisk, Solna, Sweden) impregnated with ciprofloxacin was placed in the agar plate previously inoculated with the organism. Following incubation, a symmetrical inhibition ellipse was noticed determining MIC of ciprofloxacin against *S. aureus* as 0.5 µg/ml.

#### Induction of experimental infection in rabbits

*Staphylococcus aureus* ( $2 \times 10^4$  CFU) in saline (0.5 ml) was administered as an intramuscular injection into the left thigh muscle of each rabbit of group 1 (G1). Similarly, each rabbit of G2 and G3 received intramuscular injections of  $2 \times 10^6$  and  $2 \times 10^8$  CFU (0.5 ml each). After 24 h, scintigraphic imaging commenced (first imaging).

#### Validation of infection model

At 24 h, the infected thigh muscles were shaved of fur and the area was sanitized with pyodine solution (Brookes Pharmaceutical, Karachi, Pakistan). A 5-ml syringe with a 23-gauge needle was used to aspirate the swollen area, and an adequate sample was added to blood agar plates (0792-01-1, Difco, Detroit, MI, USA) for incubation at 37°C for 24–36 h. The sample was taken from those plates containing colonies of growth, fixed on a slide, gram-stained, and then observed under the microscope (Nikon, LABOPHOT-2, 465659, Tokyo, Japan) for the identification of strain.

#### Treatment of Infection with ciprofloxacin

After first imaging, all rabbits of G1, G2, and G3 received intramuscular ciprofloxacin (Ciprocin; Dae Sung Microbiological, Seoul, Korea) in the longissimus dorsi muscle (5 mg/kg, every 12 h for 5 days).

#### $^{99m}\text{Tc}$ -UBI Scintigraphy

##### *Radiopharmaceutical*

$^{99m}\text{Tc}$  pertechnetate was used to label antimicrobial peptide Ubiquicidin UBI (29–41) in a freeze-dried kit form supplied from the Pakistan Institute of Nuclear Science and Technology (PINSTECH), Islamabad, as manufactured and utilized in a previous study [14]. Each evacuated vial contained UBI (29–41) peptide, 400 µg dissolved in 10 µl 0.01 M acetic acid, stannous ions, 20 µl from pyrophosphate kit (10 mg sodium pyrophosphate and 2.5 mg stannous chloride dissolved in 5.7 ml of distilled water), and sodium borohydrate (8.0 µl; 0.7 mg/ml in 0.1 N NaOH). Each vial was reconstituted with sodium pertechnetate (370 MBq in 0.5 ml 0.9% saline) using an insulin syringe and then allowed to stand at room temperature for 30 min. The formulation was diluted with 1.5 ml 0.9% saline to make total volume of 2.0 ml. A dose of 0.1 ml  $^{99m}\text{Tc}$ -UBI (29–41) containing 20 µg of peptide and 18.5 MBq radioactivity was withdrawn in the insulin syringe for administration to each animal. Each kit was used within 6 h of reconstitution.

##### *Kit quality control*

$^{99m}\text{Tc}$ -pertechnetate in the reconstituted kit was determined by using Whatman No. 3 filter paper as a stationary phase and acetone as the mobile phase.  $^{99m}\text{Tc}$ -pertechnetate moved with the solvent front upwards, while labeled and hydrolyzed activity stayed at origin. Hydrolyzed activity was determined by using Instant Thin layer Chromatography (ITLC-SG; Gelman Sciences, Ann Arbor, MI, USA) as stationary phase and 85% ethanol at pH 3. Hydrolyzed activity stayed at the origin and all other forms; labeled and free pertechnetate moved upwards. For each vial, the procedure was done immediately after reconstitution and repeated after 6 h.

##### *Acquisition protocol*

A gamma camera (Siemens; E-Cam, Munich, Germany) equipped with a low-energy, all-purpose collimator was used for the acquisition. Each animal was placed on a flat, hard, wooden surface specially designed for rabbit body contour. Both hind legs of the animal were spread out and

all four legs were fixed with strings. The radiotracer was injected intravenously into the marginal ear vein. Static images of 500 K with both thighs in the camera's field of view were acquired at 5, 15, 30, 45, 60, and 120 min for G1–G4. For biodistribution study (G5), static, whole-body acquisition (each image of 500 K) was done at the same time intervals.

#### Quantitative analysis

To find out tracer accumulation in the infected thigh, anatomically adjusted region of interest (ROI) was drawn over the left thigh area (target; T) and a mirror region was created over the contralateral normal thigh (nontarget; NT). Accumulation of tracer at the site of infection was expressed as the ratio of counts in the target muscle to the counts in the nontarget muscle (T/NT ratio) using “region ratio” protocol of Siemens E-Cam gamma camera software. In the control group, left to right thigh ratios were determined in a similar fashion.

#### Biodistribution of $^{99m}\text{Tc}$ -UBI (29–41) in rabbits

Total body counts were determined by drawing ROI over the image of the entire animal. To find out the uptake (counts) of tracer in different organs, ROIs were drawn over the liver, both kidneys, and the urinary bladder in the images acquired at 5, 15, 30, 45, 60, and 120 min. Percent distribution of injected activity in these organs with respect to total body counts at various time intervals was determined as calculated in a previous study [14].

#### Statistical analysis of data

Duncan's multiple range (DMR) test was used to compare the means of T/NT ratios at days 1, 3 and 5 for statistical significant difference ( $P < 0.05$ ) of each group (1, 2, 3, and 4) at 60-min images. This test was developed by Duncan and was named as Duncan's new multiple range test (DMRT) [20]. It takes into account the number of means being compared. The set of critical values, least significant range (LSR), is determined taking into account the relative position of the means, the largest value being required for comparing extreme means, and the smallest for testing adjacent means. If the observed difference between the comparing means is greater than the LSR value, the two means will be significantly different from each other and denoted in a table by dissimilar letters in a row or column. Biodistribution data (G5) was obtained at 5, 15, 30, 45, 60, and 120 min after tracer injection. Mean values and standard error of percent-injected counts of liver, kidneys, and urinary bladder were calculated.

## Results

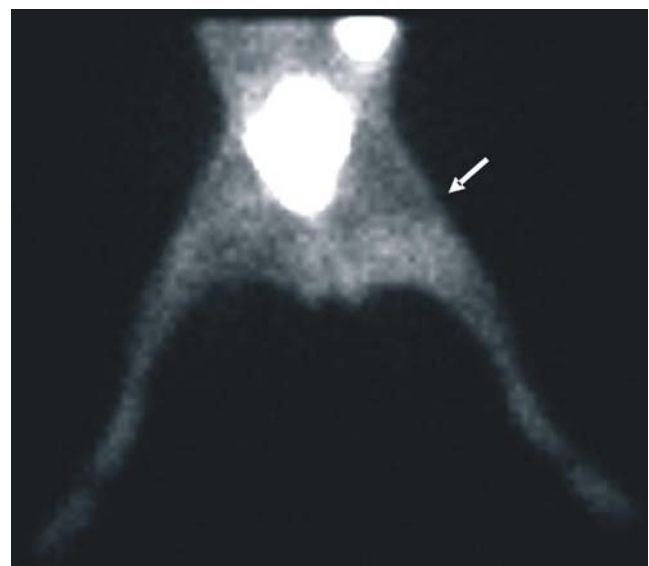
#### Kit quality control

Free technetium remained  $< 0.1\%$  and overall labeling efficiency of the labeled peptide was  $> 95\%$  in each case. Bound activity values remained unchanged up to 6 h after reconstitution of kit.

#### Accumulation of $^{99m}\text{Tc}$ -UBI (29–41) at the site of bacterial infection

##### After 24 h of induction of infection (before ciprofloxacin)

T/NT ratios increased gradually from 5-min images, and maximum ratios were observed at 60-min images in all three groups (Fig. 1 of an animal in group 3). Mean T/NT  $\pm$  SE values at 5, 15, 30, 45, and 60 min of G1 were  $1.70 \pm 0.07$ ,  $1.88 \pm 0.06$ ,  $1.91 \pm 0.06$ ,  $2.10 \pm 0.05$ , and  $2.19 \pm 0.10$ , respectively. Values for G2 were  $1.78 \pm 0.05$ ,  $1.80 \pm 0.13$ ,  $1.93 \pm 0.08$ ,  $2.18 \pm 0.11$ , and  $2.25 \pm 0.23$  at the same time points, respectively. Group 3 showed values of  $1.88 \pm 0.11$ ,  $2.27 \pm 0.41$ ,  $2.45 \pm 0.09$ ,  $2.76 \pm 0.24$ , and  $2.89 \pm 0.11$  at the same time points, respectively. Gradual decline of T/NT ratios was observed at 120 min, as is apparent from the values of  $1.89 \pm 0.05$ ,  $1.81 \pm 0.10$ , and  $2.12 \pm 0.12$  for G1, G2, and G3, respectively (Table 1). Figure 2 shows graphical representation of mean T/NT ratios of groups 1, 2, and 3 at 24 h of infection induction, from 5 to 120 min after injection of  $^{99m}\text{Tc}$ -UBI.



**Fig. 1** Significant  $^{99m}\text{Tc}$  UBI (29–41) accumulation in left thigh muscle of a rabbit in group 3 at 60 min of tracer injection (arrow)

**Table 1** Mean T/NT  $\pm$  SE of group 1 (G1), group 2 (G2), and group 3 (G3) before and after treatment with ciprofloxacin at various time intervals

Minutes		Days		
		1st imaging (day 1)	2nd imaging (day 3)	3rd imaging (day 5)
5	G1	1.70 $\pm$ 0.078	1.39 $\pm$ 0.047	1.11 $\pm$ 0.035
	G2	1.78 $\pm$ 0.052	1.36 $\pm$ 0.058	1.11 $\pm$ 0.030
	G3	1.88 $\pm$ 0.118	1.45 $\pm$ 0.040	1.12 $\pm$ 0.045
15	G1	1.88 $\pm$ 0.062	1.52 $\pm$ 0.007	1.21 $\pm$ 0.035
	G2	1.80 $\pm$ 0.13	1.42 $\pm$ 0.044	1.20 $\pm$ 0.023
	G3	2.27 $\pm$ 0.141	1.99 $\pm$ 0.195	1.26 $\pm$ 0.138
30	G1	1.91 $\pm$ 0.064	1.65 $\pm$ 0.054	1.29 $\pm$ 0.023
	G2	1.93 $\pm$ 0.085	1.59 $\pm$ 0.070	1.31 $\pm$ 0.042
	G3	2.45 $\pm$ 0.092	1.97 $\pm$ 0.208	1.44 $\pm$ 0.095
45	G1	2.10 $\pm$ 0.052	1.66 $\pm$ 0.047	1.28 $\pm$ 0.010
	G2	2.18 $\pm$ 0.113	1.60 $\pm$ 0.089	1.28 $\pm$ 0.042
	G3	2.76 $\pm$ 0.248	2.20 $\pm$ 0.195	1.38 $\pm$ 0.092
60	G1	2.19 $\pm$ 0.102	1.48 $\pm$ 0.121	1.23 $\pm$ 0.078
	G2	2.25 $\pm$ 0.231	1.55 $\pm$ 0.135	1.20 $\pm$ 0.024
	G3	2.89 $\pm$ 0.113	1.79 $\pm$ 0.217	1.30 $\pm$ 0.055
120	G1	1.89 $\pm$ 0.059	1.33 $\pm$ 0.072	1.09 $\pm$ 0.022
	G2	1.81 $\pm$ 0.104	1.31 $\pm$ 0.055	1.11 $\pm$ 0.009
	G3	2.12 $\pm$ 0.122	1.69 $\pm$ 0.116	1.11 $\pm$ 0.032

### Confirmation of infection

Bacteria cultured on the growth medium were screened and identified as *S. aureus* (6736153), identical to the species that was injected.

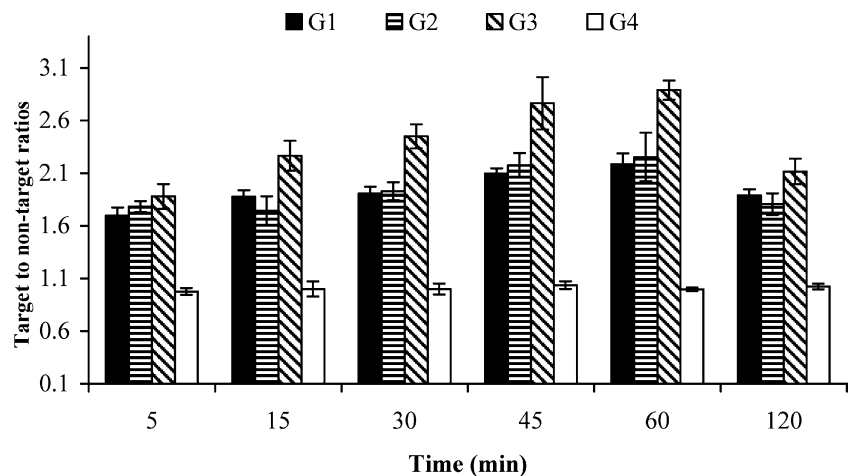
### After treatment with ciprofloxacin

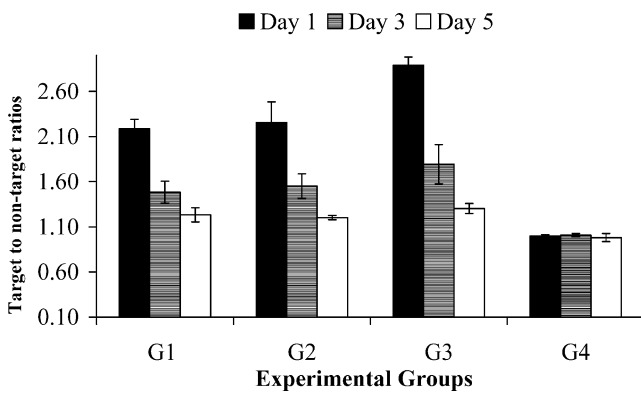
Ciprofloxacin 5 mg/kg, every 12 h, was started in infection models (G1–G3). Second imaging was conducted at the end of the third day of treatment under the same acquisition parameters. Mean T/NT ratios  $\pm$  SE of G1 at 5, 15, 30, 45, and 60 min were 1.39 $\pm$ 0.04, 1.52 $\pm$ 0.007, 1.65 $\pm$ 0.05, 1.66 $\pm$ 0.04, and 1.48 $\pm$ 0.12, respectively. Values of G2 were 1.36 $\pm$ 0.05, 1.42 $\pm$ 0.04, 1.59 $\pm$ 0.07, 1.60 $\pm$ 0.08, and 1.55 $\pm$ 0.13, respectively. Group three revealed ratios of 1.45 $\pm$

0.04, 1.99 $\pm$ 0.19, 1.97 $\pm$ 0.20, 2.20 $\pm$ 0.19, and 1.79 $\pm$ 0.21, respectively. Values of G1, G2, and G3 at 120 min were 1.33 $\pm$ 0.07, 1.31 $\pm$ 0.05, and 1.69 $\pm$ 0.11, which indicate gradual decline in the respective groups.

The third imaging at the end of 5 days of treatment showed further decline in T/NT ratios as compared to ratios of second imaging. T/NT ratios  $\pm$  SE of G1 were 1.11 $\pm$ 0.04, 1.21 $\pm$ 0.04, 1.29 $\pm$ 0.02, 1.28 $\pm$ 0.01, and 1.23 $\pm$ 0.07, respectively. G2 showed values of 1.11 $\pm$ 0.03, 1.20 $\pm$ 0.02, 1.31 $\pm$ 0.04, 1.28 $\pm$ 0.04, and 1.20 $\pm$ 0.02, respectively. Ratios of G3 were 1.12 $\pm$ 0.04, 1.26 $\pm$ 0.13, 1.44 $\pm$ 0.09, 1.38 $\pm$ 0.09, and 1.30 $\pm$ 0.05, respectively. T/NT ratios of G1, G2, and G3 at 120 min were 1.09 $\pm$ 0.02, 1.11 $\pm$ 0.009, and 1.11 $\pm$ 0.03, respectively (Table 1). Figure 3 shows graphical comparison of mean T/NT ratios of G1, G2, and G3 before and after ciprofloxacin treatment. Figure 4 shows compar-

**Fig. 2** Comparison of mean T/NT ratios of group 1 (G1), group 2 (G2), and group 3 (G3) on first imaging with mean left to right ratios of group 4 (G4) (control) from 5–120 min





**Fig. 3** Comparison of mean T/NT ratios at 60-min images of group 1 ( $2 \times 10^4$  CFU), group 2 ( $2 \times 10^6$  CFU), and group 3 ( $2 \times 10^8$  CFU) acquired before treatment with ciprofloxacin (*day 1*) with the same parameter after treatment with antibiotic (*days 3 and 5*). Group 4 (control) mean left to right thigh ratios at 60 min reveal insignificant difference

ison of tracer accumulation at infection site before and after treatment with antibiotic.

#### Accumulation of $^{99m}\text{Tc}$ -UBI (29–41) in control group

Group 4 ( $n=3$ ) served as the control group, to which neither bacteria nor ciprofloxacin was given. The first imaging on day 1 under the same acquisition parameters showed mean left to right thigh ratios of  $0.98 \pm 0.03$ ,  $1.00 \pm 0.07$ ,  $1.00 \pm 0.05$ ,  $1.04 \pm 0.03$ ,  $1.00 \pm 0.01$ , and  $1.02 \pm 0.02$  at 5, 15, 30, 45, 60, and 120 min, respectively. The second imaging at day 3 revealed left to right thigh ratios of  $1.05 \pm 0.01$ ,  $0.95 \pm 0.05$ ,  $0.97 \pm 0.07$ ,  $1.00 \pm 0.03$ ,  $1.01 \pm 0.04$ , and  $1.06 \pm 0.05$ . Similarly, the third imaging on day 5 showed left to right thigh ratios of  $0.94 \pm 0.02$ ,  $1.02 \pm 0.01$ ,  $1.02 \pm 0.08$ ,  $0.94 \pm 0.03$ ,  $0.98 \pm 0.00$ , and  $1.00 \pm 0.06$ . No significant difference was noted ( $P < 0.05$ ) in all imaging values. Values of G1, G2, and G3 on day 1 are presented with those of the control

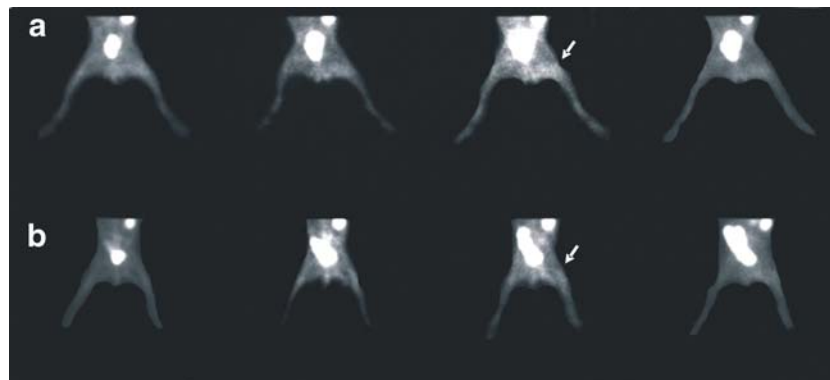
group in Fig. 2. Similarly, for days 1, 3, and 5, values of the control group at 60 min are presented with those of G1, G2, and G3 in Fig. 3 for comparison.

#### Image analysis of $^{99m}\text{Tc}$ -UBI (29–41)

Biodistribution of labeled UBI (29–41) in various organs of rabbits was determined scintigraphically by drawing regions of interest on kidneys, liver, urinary bladder, and the whole body at 5-, 15-, 30-, 45-, 60-, and 120-min images. One tenth CC of radiotracer containing 18.5 MBq of  $^{99m}\text{Tc}$  and 20  $\mu\text{g}$  of peptide was injected intravenously into each rabbit of group 5. Kidneys displayed gradual excretion of radioactivity with mean percent uptake values of  $7.8\% \pm 1.1\%$ ,  $6.0\% \pm 1.0\%$ ,  $5.5\% \pm 0.9\%$ ,  $4.3\% \pm 0.1\%$ ,  $3.8\% \pm 0.2\%$ , and  $3.4\% \pm 0.4\%$ . Liver also showed gradual decline in uptake values with the passage of time with mean percent uptake values of  $9.5\% \pm 1.0\%$ ,  $8.1\% \pm 0.8\%$ ,  $8.9\% \pm 0.5\%$ ,  $7.8\% \pm 1.0\%$ ,  $7.5\% \pm 1.2\%$ , and  $5.4\% \pm 1.6\%$ . Rapid accumulation of radioactivity was visualized in the urinary bladder with  $75.9\% \pm 6.6\%$  of injected tracer present at 120 min. Mean values of three data points and standard errors are shown in Table 2.

#### Statistical analysis

DMRT was used to observe statistical significant difference ( $P < 0.05$ ) between “before treatment” mean T/NT values and “after treatment” mean T/NT values at 60 min of tracer injection in rabbits of G1, G2, and G3. Day-3 mean T/NT values of three groups significantly decreased ( $P < 0.05$ ) as compared to day-1 values. Similarly, day-5 mean T/NT values further decreased as compared to day-3 values but were more marked in the G3 group. There was no statistically significant difference between days 1, 3, and 5 mean left to right thigh ratios in rabbits of the control group (G4) (Table 3).



**Fig. 4** **a** Left to right, images at 5, 30, 60, and 120 min of  $^{99m}\text{Tc}$ -UBI (29–41) tracer injection (before ciprofloxacin treatment); arrow indicates significant tracer uptake at the infection site in 60-min

image. **b** Left to right, same parameters at the end of 5 days of ciprofloxacin treatment. Decreased tracer accumulation is visible at infection site (arrow)

**Table 2** Biodistribution of  $^{99m}\text{Tc}$ -UBI (29–41) in rabbits of group 5 (G5)

% of injected dose per organ																			
Sr No	Liver						Kidneys						Urinary bladder						
	5 min	15 min	30 min	45 min	60 min	120 min	5 Min	15 min	30 min	45 min	60 min	120 min	5 min	15 min	30 min	45 min	60 min	120 min	
1	11.4	9.8	9.8	6.3	5.8	3.5	10.0	7.7	6.9	4.5	4.1	3.6	6.4	21.4	38.1	48.9	56.9	80.5	
2	9.3	9.3	8.4	7.2	6.9	4.0	6.7	6.1	5.7	4.3	3.5	4.0	22.4	37.7	45.0	61.1	67.3	84.3	
3	7.7	7.3	8.5	9.9	9.9	8.7	6.5	4.2	4.0	4.0	3.8	2.6	6.0	25.6	44.6	52.1	56.4	62.8	
Mean	9.5	8.1	8.9	7.8	7.5	5.4	7.8	6.0	5.5	4.3	3.8	3.4	11.6	28.1	42.6	54.0	60.2	75.9	
(±SE)	(1.0)	(0.8)	(0.5)	(1.0)	(1.2)	(1.6)	(1.1)	(1.0)	(0.9)	(0.1)	(0.2)	(0.4)	(5.4)	(5.0)	(2.2)	(3.7)	(3.6)	(6.6)	

Almost 76% of tracer is visualized in urinary bladder at 120 min of injection

### Side effects

No anaphylactic reaction was observed with  $^{99m}\text{Tc}$ -UBI (29–41) in all rabbits studied in the infection model, control group, and biodistribution group. All animals tolerated the discomfort of injections and imaging procedures. No animal died during the acquisition protocol and after 7 days of completion of the experiments.

### Discussion

Use of hybrid SPECT/CT with  $^{99m}\text{Tc}$ -HMPAO-labeled leukocyte scintigraphy [3] and  $^{67}\text{Ga}$ ,  $^{111}\text{In}$  labeled leukocyte scintigraphy [4] has improved diagnosis of infection by providing accurate localization and precise definitions of pathology. Similarly,  $^{18}\text{F}$ -FDG-WBC PET/CT is a promising technique with high sensitivity, having major advantages over conventional nuclear medicine and radiological methods [5]. However, highly sophisticated and costly technology limits mainstream application as a commonly available modality. The limitations of autologous labeled leukocytes are that the radiolabeling step is time-consuming and carries the risk of infection to the operator, particularly

in critical situations. There is still the need for a simpler diagnosis of focal infection for the referring clinician. Antimicrobial peptide, ubiquicidin (29–41) in a freeze-dried kit formulation, previously showed encouraging results as an infection imaging agent in animals [14], as well as in humans [17]. Nibbering et al. [16] demonstrated that the binding of this antimicrobial peptide to viable cell membranes of bacteria is also directly related to their number in the infective focus. Similarly, in another study by the same group,  $^{99m}\text{Tc}$ -UBI (29–41) was recommended for monitoring the efficacy of the antibacterial agents cloxacillin and erythromycin in animals [18]. Ciprofloxacin is a commonly employed broad-spectrum antibiotic that is occasionally started blindly to treat patients for infection before confirming the infectious lesion. Evaluation of its effects on  $^{99m}\text{Tc}$ -UBI (29–41) scintigraphy is important regarding sensitivity and specificity of the technique. Based on these considerations, a study was designed to investigate the relationship between the number of viable bacteria and accumulation of  $^{99m}\text{Tc}$ -UBI (29–41) in focal muscular infections of rabbits before and after treatment with ciprofloxacin. Furthermore, the possibility of monitoring efficacy of antibacterial therapy as a future prospect was evaluated.

In the current experiment, a freeze-dried kit of UBI (29–41) was utilized as investigated in a previous study [14]; however, keeping the same reconstituted volume (2 ml), a higher specific activity of pertechnetate was added (740 MBq/ml instead of 200 MBq/ml) in each vial. Quality control revealed <1% free pertechnetate and labeling efficiency of >95%, which correlated well with the previous experiments. Known concentrations of viable bacteria *S. aureus* (6736153) were administered in rabbits to induce a focal infection. MIC of ciprofloxacin for this strain was 0.5 µg/ml. Three groups of rabbits ( $n=3$  in each group), designated as G1, G2, and G3, received bacterial concentrations of  $2 \times 10^4$  CFU,  $2 \times 10^6$  CFU, and  $2 \times 10^8$  CFU in thigh muscles, respectively. At the end of 24 h of bacterial injection, when local redness and swelling were

**Table 3** Comparison of mean T/NT ratios of group 1 (G1), group 2 (G2), group 3 (G3) and mean left to right thigh ratios of group 4 (G4) at 60 min of  $^{99m}\text{Tc}$ -UBI (29–41) injection

Groups	Before ciprofloxacin treatment/(day 1)	After ciprofloxacin treatment/(day 3)	After ciprofloxacin treatment/(day 5)
G1	2.19 a	1.48 b	1.23 bc
G2	2.25 a	1.55 b	1.20 bc
G3	2.89 a	1.79 b	1.30 c
G4	1.00 d	1.01 d	0.98 d

Means sharing similar letters in a row are statistically non-significant by Duncan's Multiple Range test ( $P<0.05$ )

apparent, the first scintigraphy was performed (day 1). T/NT ratios increased gradually from 5-min images, with maximum values at 60 min in all the three groups (Fig. 1), followed by a decline at 120-min images. Comparison of T/NT ratios of the three infected groups revealed the highest values for G3, which may be due to the fact that this group was injected with the highest concentration of *S. aureus*. Maximum uptake values noted at 60-min images correlated well with the findings of a previous study by Akhtar et al. [14]. Therefore, we selected 60-min images as a reference point for comparison with similar pictures of second and third imagings. Decline of ratios at 120 min could be explained on the basis that the antimicrobial peptide UBI (29–41) interacts electrostatically with the membrane lipids of bacteria. After entering the cell, the radiopharmaceutical could be bound to a cytoplasmic specific site on a target bacterial protein, causing a fast cell death with the subsequent bacterial removal [21]. After the first imaging, all the rabbits in infected groups received intramuscular ciprofloxacin, 5 mg/kg every 12 h, for 5 days. The second imaging performed under the same acquisition parameters at the end of the third day of treatment revealed significantly reduced ( $P < 0.05$ ) mean T/NT ratios for G1, G2, and G3 at 60 min as compared to the first imaging. Ciprofloxacin was continued for two more days, followed by the third imaging, which showed further decline in T/NT ratios in the respective groups.

G4 consisting of three rabbits that served as control, to which neither bacteria nor ciprofloxacin was given. First, second, and third imagings at a similar protocol showed insignificant differences ( $P < 0.05$ ) in all imaging values.

The study indicates that a direct relation exists between the number of viable bacteria and accumulation of  $^{99m}\text{Tc}$ -UBI at the site of bacterial infection. The highest concentration of bacteria utilized in group 3 as compared to group 2 and group 1 revealed maximum tracer accumulation, which provides evidence that the higher the number of bacteria, the greater is the binding of  $^{99m}\text{Tc}$ -UBI. T/NT ratios of the three infected groups were higher before ciprofloxacin therapy and declined after institution of antibiotic, which decreased the number of bacteria. Some of the bacteria might have been killed with the peptide used for imaging. Ideally, the number of bacteria should have been counted in infected thighs by microbiological techniques, but this was not possible in our setting. This finding correlates well with the previous study by Nibbering et al. [16]. Prior use of an effective antibacterial therapy would reduce the sensitivity of localizing an infective site with  $^{99m}\text{Tc}$ -UBI (29–41). In addition to aiding early diagnosis, serial imaging may prove very useful in monitoring the response to and determining the optimum duration of anti-infective therapy, which would not only reduce the incidence of resistance emergence but would also minimize

the cost. However, further studies in this regard with different antibiotics and microorganisms are needed before considering the use of  $^{99m}\text{Tc}$ -UBI (29–41) for its capability of monitoring the efficacy of antibiotics in human beings. This study indicated that almost 76% of tracer was excreted through kidneys in the urinary bladder at 120 min of injection. This finding correlated well with a previous experiment [14]; however, currently, higher specific activity of pertechnetate (740 vs 200 MBq/ml) and low dose of peptide in each rabbit (20 vs 40  $\mu\text{g}$ ) was utilized. Good clearance characteristics of this radiotracer are a favorable asset regarding relatively low whole-body radiation dose.

## Conclusion

Accumulation of  $^{99m}\text{Tc}$ -UBI (29–41) antimicrobial peptide, being directly related to viable number of bacteria, declined after ciprofloxacin treatment. Use of this radiopharmaceutical as an infection localization agent would face the problem of reduced sensitivity in the presence of effective antimicrobial treatment. However, it can be utilized for monitoring efficacy and duration of antibiotic treatment.

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