In vivo monitoring of viable bacteria by SPECT using ^{99m}Tc -HYNIC(GH)₂-UBI 29-41 and ^{99m}Tc -HYNIC(Tricine)₂-UBI 29-41

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Abstract

Background: The number of bacterial infections that—for various reasons—are challenging to cure continues to increase. One such reason is persister cell infection. To investigate persister formation and persister infections, viable bacteria must be evaluated in the same animal over time. In this study, the feasibility of monitoring viable bacteria by SPECT using two labeled peptides was evaluated.

Results: Two types of ubiquicidin (UBI) 29-41 labeled with technetium-99m, $^{99m}\text{Tc-HYNIC}(\text{GH})_2\text{-UBI 29-41}$ and $^{99m}\text{Tc-HYNIC}(\text{Tricine})_2\text{-UBI 29-41}$, were synthesized. The *in vitro* binding of these labeled peptides to *Staphylococcus aureus* was measured. For the *in vivo* study, each labeled peptide was injected into *S. aureus* infected mouse thigh after treatment with various doses of ciprofloxacin (CPFX). Two hours after injection, the accumulation of each labeled peptide at the infection site was assessed by SPECT, and then the number of viable bacteria was determined from the accumulation detected. The peptide labeling was successful, and the radiochemical purity was $91 \pm 9\%$ (GH, $n=8$) and 100% (Tricine, $n=8$). The *in vitro* binding of the labeled peptides to *S. aureus* (5×10^8 cfu) without serum was 78.9% (GH) and 85.5% (Tricine) of the total ^{99m}Tc activity. With serum, the binding rate was 67.5% (GH) and 13.3% (Tricine). The accumulation of labeled peptide was calculated from the SPECT images, and that in the bacterial infection site (left thigh) was higher than that in the non-infection site (right thigh) for both peptides. Good correlation was found between the target-to-non-target (T/NT) ratios of each labeled peptide and the viable bacterial count at the infection site, and $^{99m}\text{Tc-HYNIC}(\text{Tricine})_2\text{-UBI 29-41}$ had a wider range than $^{99m}\text{Tc-HYNIC}(\text{GH})_2\text{-UBI 29-41}$.

Conclusion: Using the SPECT/labeled peptide method, it was possible to monitor viable bacterial count in the range 10^3 – 10^8 cfu, which is appropriate for tracking viable bacterial counts in the same animal over time.

Background

Bacterial infections continue to pose a serious global health threat. In addition to the development of bacterial resistance, there are many cases where infection-causing bacteria that are sensitive to antibiotics are not completely eradicated and therefore cause relapse. Some reports suggest that the bacteria in such cases include persisters. Maisonneuve *et al.* reported that there are multiple mechanisms that underlie the bacterial persister phenomenon, for example, environmental insults (i.e., starvation, oxidative and acid stress, and heat shock) provoke persister cell formation, biofilm microenvironments enhance persistence, and host-pathogen interactions, such as those of macrophages, induce the formation of persisters [1]. They also reported that when bacteria were treated with a bactericidal antibiotic, the bacterial count at persistent status was approximately 10^3 cfu [1]. The maximum growth of most bacteria *in vivo* is approximately 10^8 – 10^9 cfu. Based on these values, we believe that the ability to quantify 10^3 – 10^8 cfu is necessary for persister research.

To investigate persister formation and infections, bacterial behavior in the same animal must be traced over time. With conventional acute infection models it is only possible to count viable bacteria by euthanizing animals at each time point, making it difficult to trace persister formation in the same animal. In contrast, imaging technology is useful for evaluating the same animal over time.

There are two imaging methods that show potential for evaluating infections. One is optical imaging. van Oosten *et al.* reported using luciferase-engineered *S. aureus* and fluorescently labeled vancomycin (vanco-800CW) in a mouse myositis model [2]. The bioluminescence was used to indicate the localization of *S. aureus*, and allowed the overlap with vanco-800CW to be determined. The detected bacterial count in their report was 10^7 cfu/thigh. Ning *et al.* demonstrated *in vivo* detection of *Escherichia coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* using maltodextrin-based imaging probes (MDPs) in a rat myositis model [3]. The MDPs enabled the imaging of as few as 10^5 cfu/thigh. Tang *et al.* reported infection imaging using Concanavalin A (Con A) as a bacteria-targeting ligand, a nanoparticle carrier, and a near infrared fluorescent dye [4]. The detected count was 10^6 – 10^7 cfu/wound in a mouse wound model.

Optical imaging is considered useful for detecting bacterial infection; however, it can only be used if the infection model is close to the surface, such as in subcutaneous and thigh infections. Furthermore, the optical imaging sensitivity of the bacterial count was reported to be approximately 10^5 – 10^7 cfu at the infection site; therefore, the sensitivity was insufficient to detect persistent status.

The other imaging technology is based on radioactivity. Radioactivity-based methods can be used to evaluate deep infections such as lung infections, and they have high sensitivity. We therefore selected a radioactivity-based method so that the 10^3 cfu expected lower limit of persisters could be detected. To monitor the same animal using radioactivity, we used single photon emission computed tomography (SPECT) and ^{99m}Tc -labeled probes.

Many radiolabeled agents, such as antibodies, antibiotics, and peptides, have been evaluated for imaging infections [5]. Antimicrobial peptides were designed to kill a broad spectrum of Gram-positive and Gram-negative bacteria, and fungi. The most widely investigated peptide is ubiquicidin (UBI) 29-41. UBI 29-41 is a cationic human antimicrobial peptide fragment with six positively charged residues (5 Arg + 1 Lys) that accumulates at the negatively charged surfaces of microorganisms. Nibbering *et al.* reported *in vivo* studies using UBI 29-41 in mice and rats infected with *S. aureus* [6]. They monitored the efficacy of antibiotics in mice and rats with *S. aureus* infections and reported good correlation between the accumulation of UBI 29-41 at the infection site and the dose of antibiotics administered.

There are many reports describing the use of the bifunctional chelator hydrazine nicotinamide (HYNIC) as an intermediary with UBI [7, 8]. The HYNIC moiety allows the use of a variety of coligands. It has been reported that the nature of the coligand affects the biodistribution of ^{99m}Tc -labeled HYNIC-chemotactic peptide [9]. We selected α -D-glucosaminic acid (GH) and tricine as coligands. GH is more hydrophobic than tricine and shows high distribution in organs [10]. Therefore, high SPECT detection sensitivity was

expected for GH-containing labels. Tricine has been reported to show low distribution in organs, but has been confirmed to show high accumulation at the infection site in animal models [10]. Using these two labeled peptides, we investigated the correlation between the accumulation of labeled peptide and the viable bacterial count at the infection site and determined which peptide is most suitable for detection by SPECT.

Methods

Antibiotics and Synthetic Antimicrobial Peptide

Ciprofloxacin (CPFX; Hydrochloride, $\geq 98\%$ activity) was purchased from LKT Laboratories, Inc. (MN, USA). UBI 29-41 denotes TGRAKRRMQYNRR (1693 Da). 6-Hydrazinonicotinic acid (HYNIC)-UBI 29-41 was synthesized at Toray Research Center (Tokyo, Japan, Fig. 1).

Labeling Procedure and Quality Control

^{99m}Tc -HYNIC(GH)₂-UBI 29-41 was labeled as follows: 2 mg of GH kit (freeze-dried α -D-glucosuccinic acid [GH] 2.0 mg and $\text{SnCl}_2/2\text{H}_2\text{O}$ 1.2 μg) was dissolved in 1 mL of $^{99m}\text{TcO}_4^-$ (185 MBq/mL, Nihon Medi-Physics Co. Ltd.) and the sample was incubated for 10 min at room temperature to synthesize ^{99m}Tc -GH. Then, 200 μL of the ^{99m}Tc -GH solution and 200 μL of 400 μM HYNIC-UBI aqueous solution were mixed and incubated for 60 min at room temperature. ^{99m}Tc -HYNIC(Tricine)₂-UBI 29-41 was labeled as follows: 125 μL of $^{99m}\text{TcO}_4^-$ was mixed with 125 μL of 40 mg/mL tricine in 10 mM acetate buffer (pH 4). Then, 10 μL of 25 μM HYNIC-UBI aqueous solution and 6 μL of 1 mg/mL $\text{SnCl}_2/2\text{H}_2\text{O}$ in 0.1 N HCl were added, and the sample was incubated for 10 min at 90°C [11]. Following labeling, each reaction mixture was analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC). The sample was applied to an XBridge® C18 5 μm column (4.6 $\times$ 150 mm, Waters) attached to a chromatograph equipped with an on-line UV set at 254 nm and a NaI (TI) crystal gamma detection system (Gabi star, Raytest, Straubenhardt, Germany). ^{99m}Tc -HYNIC(GH)₂-UBI 29-41 was detected using a linear gradient of two eluents, 0.1% (v/v) trifluoroacetic acid (TFA)/water (solvent A) and 0.1% TFA/acetonitrile (solvent B), at a flow rate of 1.2 mL/min. The gradient was applied as follows: 95%–20% A in 15 min and 95% A for 5 min. ^{99m}Tc -HYNIC(Tricine)₂-UBI 29-41 was detected using a linear gradient of two eluents, 0.01% (v/v) trifluoroacetic acid (TFA)/water (solvent A) and 0.01% TFA/acetonitrile (solvent B), at a flow rate of 1.0 mL/min. The gradient was applied as follows: 95%–70% A in 15 min and 95% A for 5 min.

Microorganism

Staphylococcus aureus 25923 (American Type Culture Collection) susceptible to CPFX (minimum inhibitory concentration [MIC] < 1 $\mu\text{g}/\text{mL}$) was used. *S. aureus* 25923 was cultured on Brain–Heart Infusion Agar (BHIA) for 24 h at 37°C. The suspension of colony was washed, counted by optical density, and used in the *in vitro* binding assay. For the *in vivo* assay, a stock solution of *S. aureus* 25923 stored at –80°C was used.

In Vitro* Binding to *S. aureus

Ten microliters of the preparation containing each labeled peptide and 10 μL of suspension containing 4×10^{11} cfu/mL *S. aureus* were added to 80 μL of a binding buffer (20 mM phosphate buffered saline [PBS] containing 0.01% Tween80 and 5 mM acetic acid, pH=5). For the serum conditions, instead of 10 μL of the binding buffer, 10 μL of mouse serum were added. The suspensions were gently mixed using a vortex mixer and incubated at 37°C for 1 h. After incubation, the tubes were centrifuged at 5000 $\times g$ for 10 min. The supernatant was removed, and the pellet was resuspended in 100 μL of binding buffer and centrifuged again using the conditions described above. The supernatant was again removed and the radioactivity of the pellet was counted using a γ -counter. The radioactivity associated with the bacteria pellet was expressed as percentage of the total $^{99\text{m}}\text{Tc}$ activity added.

Animals

All of the procedures for the animal studies were approved by the Institutional Animal Care and Use Committee of Shionogi & Co., Ltd. (Osaka, Japan). Specific-pathogen-free (SPF) male ICR mice (CLEA Japan Inc., 5 weeks) were used in the infection and SPECT studies.

Biodistribution

A 0.2-mL solution containing 30 kBq of $^{99\text{m}}\text{Tc}$ -HYNIC(Tricine)₂-UBI 29-41 or 10 kBq of $^{99\text{m}}\text{Tc}$ -HYNIC(GH)₂-UBI 29-41 was administered via the tail vein of mice. Animals were euthanized by exsanguination and opening of the thoracic cavity at 0.5, 2, and 3 h post injection. Organs were excised and weighed, and their activity was counted using a gamma counter. The organ uptake was calculated as a percentage of the injected dose per gram of wet tissue (%ID/organ, Table 1).

Treatment of Animal Infections with Antibacterial Agents

Mice were anesthetized with isoflurane, and 6.0×10^6 – 2.0×10^7 cfu of bacteria in 0.1 mL saline were aseptically injected into the left thigh muscle of each mouse. Mice were initially subcutaneously administered CPF_X 100 mg/kg q.d. or t.i.d. for 1 or 2 days. To evaluate the correlation between the accumulation of each labeled peptide and the viable bacterial count, mice received 0–100 mg/kg of CPF_X for 2 days after infection q.d. (2, 24 h) or t.i.d. (2, 4, 6, 24, 26, 28 h) (Fig. 3).

SPECT imaging

At 46 h after infection, 0.2 mL of solution containing 10–30 MBq of each labeled peptide was administered via the tail vein of mice. The accumulation of each labeled peptide in the bacteria-infected site in mice was assessed by SPECT/CT (Triumph II SPECT 2H/XO SRI CT, TriFoil Imaging). Two hours after the labeled peptide injection, mice were anesthetized with isoflurane. The mice were then arranged lying face down on a SPECT/CT bed with both hind legs spread out and fixed with surgical tape. Whole body images were acquired under the following conditions; energy window: 20% at 140 keV, projection

limit: 20 s, projection count: 64, rotation angle: 360 degrees. After whole body imaging, the mice were euthanized and the infected (left) and normal (right) legs were extracted. Leg-only images were then acquired using the same conditions. For the image processing, adjusted regions of interest (ROI) were drawn over the entire infected muscle (target [T]) and contralateral muscle (nontarget [NT]). The accumulation of each labeled peptide at the infection site was expressed as the ratio of the counts in the target and the nontarget muscles (T/NT).

Determining the Number of Viable Bacteria

After SPECT imaging, the entire infected thigh muscles were removed and individually homogenized in Mueller–Hinton broth. Serial dilutions of the thigh homogenate were plated on Brain–Heart Infusion Agar. The plates were then incubated for 24 h at 37°C, and the number of cfu was counted.

Statistical Analysis

The differences between log cfu before and after treatment of mice with CPFY were evaluated using the Student t test. The P values were calculated, and statistical significance was accepted within 95% confidence limits. All results were reported as means and SD. The Pearson correlation coefficient (r) was used to assess the correlation between the accumulation of each labeled peptide and the viable bacterial count.

Results

Labeling and Quality Control

RP-HPLC analysis of ^{99m}Tc -HYNIC(GH)₂-UBI 29-41 showed two major peaks (4.0 and 4.5 min) and that of ^{99m}Tc -HYNIC(Tricine)₂-UBI 29-41 showed a single peak (4.2 min). Leading and trailing shoulders were detected for both peptides; however, a previous report indicated that derivatives with GH and tricine as coligands existed in many isomeric forms [12]. The radiochemical purity of ^{99m}Tc -HYNIC(GH)₂-UBI 29-41 was 91±9% (n=8), and that of ^{99m}Tc -HYNIC(Tricine)₂-UBI 29-41 was 100% (n=8).

In Vitro* Binding to *S. aureus

The binding of ^{99m}Tc -HYNIC(GH)₂-UBI 29-41 to *S. aureus* (5×10⁸ cfu) without serum was 78.9% of the total ^{99m}Tc activity, and in the presence of serum the binding was 67.5%. The binding of ^{99m}Tc -HYNIC(Tricine)₂-UBI 29-41 was 85.5% without serum, while binding was reduced to 13.3% in the presence of serum (Fig. 2).

Biodistribution

The biodistributions of ^{99m}Tc -HYNIC(GH)₂-UBI 29-41 and ^{99m}Tc -HYNIC(Tricine)₂-UBI 29-41 in normal mice at 0.5, 2, and 3 h are summarized Table 1. The data show that the highest concentrations of radioactivity

were measured in the kidney for both peptides. As was found for different peptides in a previous report [10], $^{99m}\text{Tc-HYNIC}(\text{GH})_2\text{-UBI 29-41}$ showed a high organ distribution and was 2–10 times more distributed than $^{99m}\text{Tc-HYNIC}(\text{Tricine})_2\text{-UBI 29-41}$ in all organs at all time points. This difference in organ distribution is attributed to the difference in coligand. The activity of the thigh—which was the infection site—was found to show no further increase after 2 h; therefore, the SPECT images were acquired 2 h after peptide injection.

Effect of Antibiotic Administration

For 1-day administration the viable bacteria count reached a minimum of 10^4 cfu/thigh; however, for 2-day administration the number reached 10^3 cfu/thigh, which was the desired target (Fig. 4). Therefore, 2-day treatments were used to evaluate the correlation between the accumulation of the labeled peptide and the viable bacterial count.

Effect of Antibiotics on Viable Bacterial Counts

To establish a variety of bacterial counts, 10–100 mg/kg of CPFEX was administrated to the mice. The decrease in viable bacteria count was found to correlate with the CPFEX dose (Fig. 5).

Detection of Accumulation at the Infection Site using SPECT

The bacterial infection site could be imaged 2 h after the injection of each labeled peptide. Typical images for each labeled peptide in *S. aureus* infected mice are shown in Fig. 6. For $^{99m}\text{Tc-HYNIC}(\text{GH})_2\text{-UBI 29-41}$, when the viable bacterial count was 10^7 cfu/thigh, T/NT was 2.4, while when the viable bacterial count was 10^3 cfu/thigh, T/NT decreased to 1.2. For $^{99m}\text{Tc-HYNIC}(\text{Tricine})_2\text{-UBI 29-41}$ T/NT was significantly higher when the viable bacterial count was 10^8 cfu/thigh, with a value of 10.0; however, T/NT was 1.3 at 10^3 cfu/thigh, which is similar to the value for $^{99m}\text{Tc-HYNIC}(\text{GH})_2\text{-UBI 29-41}$ with the same bacterial count.

The correlations between the accumulation of each labeled peptide and the bacterial counts are shown in Fig. 7. The accumulation of each labeled peptide showed good correlation with the viable bacterial count; for $^{99m}\text{Tc-HYNIC}(\text{GH})_2\text{-UBI 29-41}$ $r = 0.906$, $P = 0.002$; for $^{99m}\text{Tc-HYNIC}(\text{Tricine})_2\text{-UBI 29-41}$ $r = 0.857$, $P = 0.001$.

Discussion

The *in vitro* binding study indicated that both peptides showed high binding in the absence of serum. In the presence of serum, the binding of $^{99m}\text{Tc-HYNIC}(\text{Tricine})_2\text{-UBI 29-41}$ was significantly lower than that of $^{99m}\text{Tc-HYNIC}(\text{GH})_2\text{-UBI 29-41}$. Several research groups have reported that small biomolecules labeled with tricine as a coligand were unstable and existed as multiple species in solution; and that they may react with the imidazole group of the histidine residues of circulating blood proteins such as albumin [13,

14]. The decrease in ^{99m}Tc -HYNIC(Tricine) $_2$ -UBI 29–41 binding in the presence of serum is therefore attributed to similar interactions. Further investigation of the behavior and properties of the labeled peptide in the presence of serum will form part of future work.

The biodistribution of ^{99m}Tc -HYNIC(Tricine) $_2$ -UBI 29–41 showed fast elimination from the blood and poor distribution in all organs. The clearance of ^{99m}Tc -HYNIC(GH) $_2$ -UBI 29–41 was slower than that of ^{99m}Tc -HYNIC(Tricine) $_2$ -UBI 29–41, and the distribution of ^{99m}Tc -HYNIC(GH) $_2$ -UBI 29–41 in organs was higher than that of the other peptide. The distributions of both peptides were in the following order: kidney > liver > lung > left thigh. The distributions in muscle were the lowest of the organs measured in this study. One reason for the low distribution of ^{99m}Tc -HYNIC(Tricine) $_2$ -UBI 29–41 in organs could be that it is trapped by blood proteins as described above. As detection by SPECT requires a certain amount of accumulation owing to the detection limit of the device, the higher organ distribution of ^{99m}Tc -HYNIC(GH) $_2$ -UBI 29–41 is expected to make it easier to detect than ^{99m}Tc -HYNIC(Tricine) $_2$ -UBI 29–41.

Finally, we aimed to quantify the viable bacteria by measuring the amount of labeled peptide accumulated by the bacteria using SPECT. To do this we investigated the correlation between the accumulation of each labeled peptide and the viable bacterial count. To the best of our knowledge, this correlation has only been investigated by Lupetti *et al.* using ^{99m}Tc labeled fluconazole with *Candida albicans* at 10^6 – 10^8 cfu/g tissue [15], for which good correlation was observed. In this study, we set a dynamic range of bacterial count of 10^3 – 10^8 cfu/thigh. In light of a previous report that quantified the bacterial count at persistent status [1], a lower limit of 10^3 cfu was our target for demonstrating the feasibility of the labeled peptide/SPECT system for future investigation of bacterial persisters. To achieve this bacterial count we used CPFX. Following 1-day administration the bacterial count reached a minimum of 10^4 cfu/thigh; however, after 2-day administration the count reached our target of 10^3 cfu/thigh (Fig. 4, 5).

The accumulation of each labeled peptide was first evaluated at high bacterial count (10^7 – 10^8 cfu/thigh) using SPECT. The T/NT value of ^{99m}Tc -HYNIC(GH) $_2$ -UBI 29–41 was 2.4 and that of ^{99m}Tc -HYNIC(Tricine) $_2$ -UBI 29–41 was 10.0 as shown in Fig. 6. We then investigated peptide accumulation at the detection target of 10^3 cfu/thigh using the same method and determined T/NT values of 1.2 and 1.3 for ^{99m}Tc -HYNIC(GH) $_2$ -UBI 29–41 and ^{99m}Tc -HYNIC(Tricine) $_2$ -UBI 29–41, respectively. The T/NT range of ^{99m}Tc -HYNIC(GH) $_2$ -UBI 29–41 was 1.2–3.3 and that of ^{99m}Tc -HYNIC(Tricine) $_2$ -UBI 29–41 was 1.2–10.0. This difference is thought to be due to the differences in the biodistributions of each peptide and their binding activity to the bacteria. Based on this thigh infection system, ^{99m}Tc -HYNIC(Tricine) $_2$ -UBI 29–41 is more suitable for quantifying bacteria than ^{99m}Tc -HYNIC(GH) $_2$ -UBI 29–41 owing to its wide operation range.

Our results show that SPECT imaging is able to quantify viable bacteria in the range 10^3 – 10^8 cfu by measuring the accumulation of labeled peptides. This is the most important finding of this study as it

demonstrates the feasibility of monitoring viable bacterial counts in the same animal over time. Using this method, we will elucidate about persistent bacterial infections and persister cells, and search for ways to reduce such infections.

Conclusions

SPECT imaging can be used to quantify viable bacterial counts ranging from 10^3 to 10^8 cfu by measuring the accumulation of labeled antimicrobial peptides. This will enable the monitoring of viable bacterial count in the same animal over time, which required for the investigation of persistent infection.

Declarations

Ethics approval

All of the procedures for the animal studies were approved by the Institutional Animal Care and Use Committee of Shionogi & Co., Ltd. (Osaka, Japan).

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions

TU participated in the design of the labeled peptide and drafted the manuscript. KO and HM participated in the design of the infection study and drafted the manuscript. KA and YA were the supervisors of the study. SM participated in the study coordination, the design of the study, and drafted the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Biodistribution of ^{99m}Tc-HYNIC(Tricine)₂-UBI 29-41 and ^{99m}Tc-HYNIC(GH)₂-UBI 29-41 in mice

sue	Time after injection					
	0.5 h (Mean ± SD)		2 h (Mean ± SD)		3 h (Mean ± SD)	
	Tricine	GH	Tricine	GH	Tricine	GH
g	1.77±0.16	4.07±0.50	0.55±0.04	3.55±0.43	0.36±0.03	3.75±0.24
er	2.11±0.12	5.07±0.51	1.96±0.02	7.37±0.77	2.08±0.26	8.18±0.07
ney	83.89±5.83	46.16±1.46	84.88±16.08	56.53±9.20	73.07±9.81	65.45±4.33
thigh	0.84±0.06	1.41±0.18	0.22±0.01	1.17±0.25	0.18±0.03	1.11±0.24
od	2.01±0.19	3.43±0.11	0.40±0.05	2.41±0.29	0.22±0.03	2.34±0.11

Expressed as % injected dose per gram. Each value represents mean ± SD for three animals at each interval.

Figures

Figure 1

Structural formula of HYNIC-UBI 29-41.

Figure 2

In vitro bacterial binding of 99mTc-HYNIC(Tricine)2-UBI 29-41 and 99mTc-HYNIC(GH)2-UBI 29-41 to *S. aureus*.

Figure 3

Timeline of SPECT imaging of t.i.d. administrations.

Figure 4

Viable bacterial count in thigh infected with *S. aureus* for mice treated with CPFX for 1 or 2 days. Results are expressed as the mean $\pm$ SD for three animals. *Values that were significantly ($P < 0.05$) different to the control.

Figure 5

Viable bacterial count in thigh infected with *S. aureus* for mice treated with CPFX for 2 days. Results are expressed as the mean $\pm$ SD for three animals. *Values that were significantly ($P < 0.05$) different to the control.

Figure 6

SPECT images of mouse legs infected in the left thigh (red circle) with *S. aureus*, acquired 2 h after the administration of 99mTc-HYNIC(Tricine)₂-UBI 29-41 or 99mTc-HYNIC(GH)₂-UBI 29-41.

Figure 7

Correlation between viable *S. aureus* counts and the accumulation of 99mTc-HYNIC(Tricine)2-UBI 29-41 (circle) and 99mTc-HYNIC(GH)2-UBI 29-41 (square), expressed as the target-to-non-target (T/NT) ratio.