Imaging of Bacterial Infections with 99mTc-Labeled Human Neutrophil Peptide-1

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This study was undertaken to evaluate whether 99mTc-labeled human neutrophil peptide (HNP)-1 can be used as a tracer for rapid visualization of bacterial infections. Methods: Mice were injected intramuscularly with 1 million Staphylococcus aureus or Klebsiella pneumoniae organisms and 5 min later were injected intravenously with 0.4 µg (0.8 MBq) 99mTc-HNP-1. At various intervals, detailed information about clearance and accumulation of this tracer at sites of infection and in various organs was obtained by scintigraphy. 99mTc-labeled immunoglobulin G (IgG), an established marker of infection and inflammation, was used for comparison. Results: After injection into S. aureus– or K. pneumoniae–injected mice, 99mTc-HNP-1 was rapidly removed from the circulation, mainly through the kidneys and bladder, with half-lives of 170 and 55 min, respectively. Similar half-lives were observed for 99mTc-IgG in these animals. Visualization of foci with S. aureus or K. pneumoniae, as indicated by a ratio of 1.3 or higher between the targeted thigh muscle (containing bacteria) and the nontargeted (contralateral) thigh muscle (T/NT), was already achieved 5 min after injection of 99mTc-HNP-1. Similar T/NTs for 99mTc-IgG were obtained 4 h after injection of the tracer, indicating that imaging of foci of bacteria with 99mTc-HNP-1 is much faster than with 99mTc-IgG. To obtain insight into factors that contribute to accumulation of 99mTc-HNP-1 at sites of infection, the binding of this tracer to bacteria and leukocytes was assessed using a peritoneal infection model. Binding of 99mTc-HNP-1 to bacteria was approximately 1000 times higher than binding to leukocytes. Although the number of bacteria in the peritoneum was 1000-fold lower than the number of leukocytes, a significant correlation between binding of 99mTc-HNP-1 to bacteria on the one hand and accumulation of tracer on the other was still found, in contrast to 99mTc-IgG. Conclusion: 99mTc-HNP-1 allows rapid visualization of bacterial infections. Binding of this tracer to bacteria most likely contributes significantly to the accumulation of 99mTc-HNP-1 at sites of infection.

Key Words: infection imaging; bacteria; 99mTc labeling; human neutrophil peptide-1


Nuclear medicine techniques enable visualization of infection and inflammation (1–3). However, the present radiopharmaceuticals that can distinguish between infections and sterile inflammatory lesions suffer from major drawbacks. The low binding affinity of 99mTc-ciprofloxacin (4) to bacteria and the risk of emerging antibiotic-resistant microorganisms make this radiopharmaceutical unattractive for imaging bacterial infections (5). The specificity of radiolabeled monoclonal antibodies or their F(ab’)2 fragments can be expected to be useful for the detection of infections (6). However, Rubin et al. have reported that infection detection using F(ab’)2 fragments and nonspecific polyclonal immunoglobulin G (IgG) is similar. Clearly, a radiopharmaceutical that binds to a variety of microorganisms with little or no binding to host cells would be better.

Because antimicrobial peptides preferentially bind to bacterial membranes (7), radiolabeling of these peptides would offer the medical community novel candidates for the development of bacteria-seeking radiopharmaceuticals (8,9). One of the best-studied antimicrobial peptides is human neutrophil peptide (HNP)-1, which is a member of the family of defensins (10,11). This antimicrobial peptide, which is stored in the granules of human neutrophils, contributes to bacterial killing during phagocytosis. HNP-1 displays antimicrobial activity against gram-positive and gram-negative bacteria, many fungi and some enveloped viruses (12–14). Defensins kill bacteria by a mechanism involving electrostatic interactions between the positively charged antimicrobial peptide and the negatively charged bacterial surface molecules. The bacterial phospholipid membranes then become permeable, resulting in metabolic injury (11). Therefore, one can imagine that small amounts of radiolabeled defensins may be seen at sites of bacterial infections. Initially, the potential use of HNP-1 for bactericidal therapy of experimental infections in mice was studied (8). In that study, a single injection of HNP-1 reduced the bacterial numbers significantly. This antibacterial effect was found to be associated with an increased influx of neutrophils into the infected area, a process that appeared to be a requirement for HNP-1 to have antibacterial activity in vivo. Radiolabeled HNP-1 was found to be trapped in the infected area, whereas unbound HNP-1 was rapidly removed from the circulation through renal excretion. Furthermore, it is believed that defensin-resistant bacteria may not emerge easily, because during evolution these peptides have been successful in protecting plants, animals and humans against the continuous threat of pathogens (7).
On the basis of these considerations, the present scintigraphic study was undertaken to investigate whether \( ^{99m} \text{Tc} \)-labeled HNP-1 can be used for rapid imaging of bacterial infections. Moreover, factors contributing to the accumulation of \( ^{99m} \text{Tc} \)-HNP-1 at sites of infection were assessed. We investigated two models. The first was a bolus bacterial injection 5 min after which the contribution of infiltrating leukocytes was negligible; the second was a full-blown peritoneal infection characterized by proliferating bacteria and recently immigrated leukocytes.

**MATERIALS AND METHODS**

**Proteins**

HNP-1 was purified from human neutrophils as described (15). Briefly, neutrophils were isolated from enriched leukocyte preparations obtained from a blood bank (Leiden University Medical Center, Leiden, The Netherlands). Next, purified neutrophils were disrupted by nitrogen cavitation, cellular debris was removed by centrifugation at 540g for 10 min at 4°C and the resulting supernatant was subsequently centrifuged at 27,000g for 20 min at 4°C to sediment granules. Granule proteins were extracted from this fraction using 5% (vol/vol) acetic acid and fractionated by gel filtration on a 2.5 x 250 mm C18 column (Vydac; The Separations Group, Hesperia, CA) using a linear water-acetonitrile gradient that contained 0.1% trifluoroacetic acid as an ion-pairing agent. The purity of the HNP-1 preparation was assessed by sodium dodecylsulfate-polycrylamide gel electrophoresis (PAGE), acid urea–PAGE and laser desorption mass spectrometry. The lipopolysaccharide content of a mixture was gently stirred for 30 min at room temperature. The purity of the HNP-1 preparation was assessed by instant thin-layer chromatography and was 95% for HNP-1 and 96% for IgG.

**Labeling Procedure and Quality Control**

HNP-1 and IgG were labeled with \( ^{99m} \text{Tc} \) as described (16). Briefly, 10 µL of a solution of 1 mg HNP-1 per milliliter of 10-mmol/L sodium phosphate buffer (Na-PB), pH 7.4, was added to 2 µL of an aseptic solution containing 0.5 mg stannous pyrophosphate per milliliter. Immediately, 4 µL of a solution containing 10 mg crystalline KBH4 (Sigma Chemical Co., St. Louis, MO) per milliliter of 0.1-mol/L NaOH was added. After the addition of 0.1 mL 200-MBq/mL \( ^{99m} \text{Tc} \)--sodium pertechnetate solution (Malinckrodt Medical BV, Petten, The Netherlands), the mixture was gently stirred for 30 min at room temperature.

To remove excess reactants (including free \( ^{99m} \text{Tc} \)), the mixture was applied to a Sep-Pak C18 cartridge (Waters, Milford, MA) previously flushed with 20 mL 0.01-mol/L acetic acid (pH 4.0). After being rinsed with 10 mL acetic acid, \( ^{99m} \text{Tc} \)-HNP-1 was eluted with 2 mL methanol (Sigma), and then the methanol was evaporated using hot air. IgG, used as the control tracer, was radiolabeled in an identical fashion without further purification. \( ^{99m} \text{Tc} \)-HNP-1 and \( ^{99m} \text{Tc} \)-IgG were resuspended in 100 µL Na-PB, and the peptide content was determined by a bicinchoninic acid kit (Pierce, Oud-Beijerland, The Netherlands). The recovery of HNP-1 after Sep-Pak C18 chromatography was 96%. Labeling yield was assessed by instant thin-layer chromatography and was 95% for HNP-1 and 96% for IgG. The stability of \( ^{99m} \text{Tc} \)-HNP-1 and \( ^{99m} \text{Tc} \)-IgG was tested by placing them in 20% human serum (vol/vol) for 4 h at 37°C, followed by 20% trichloroacetic acid precipitation.

**Bacteria**

*Staphylococcus aureus* 25923 and *Klebsiella pneumoniae* 43816 (American Type Culture Collection, Rockville, MD) were prepared in a brain-heart infusion broth (Oxoid Ltd., Basingstoke, UK) kept overnight in a shaking water bath at 37°C. The resulting cultures, containing approximately 1 billion viable bacteria per milliliter, were stored at −70°C. Immediately before the experiment, an aliquot of this suspension was rapidly thawed in a water bath at 37°C and diluted in pyrogen-free saline.

**Assay for Antibacterial Activity**

An in vitro assay was used to determine whether the labeling procedure affected the antibacterial activity of HNP-1. Briefly, 0.1 mL Na-PB containing various concentrations of HNP-1 or \( ^{99m} \text{Tc} \)-HNP-1 was added to 0.1 mL Na-PB supplemented with 2% (vol/vol) tryptic soybean broth (TSB) (Difco Laboratories, Detroit, MI) and 200,000 *K. pneumoniae* organisms. At the onset and after 2 and 3 h of incubation at 37°C in a shaking water bath, the number of viable bacteria in the samples was determined microbiologically using diagnostic-sensitivity-test agar (DST) plates (Oxoid). As a control, Na-PB supplemented with 2% TSB instead of HNP-1 was used.

**Mice**

Specific, pathogen-free male Swiss mice weighing 20–25 g (Broekman Institute, Someren, The Netherlands) were used throughout the study. The mice were housed in the central animal housing facilities of the Leiden University Medical Center for at least 1 week before the experiments began. Food and water were given ad libitum.

**Experimental Infections and Injection of Radiopharmaceuticals**

The animal studies complied with the ethical committee of the Leiden University Medical Center and with national laws relating to the conduct of animal experiments.

**Thigh Muscle Model.** Mice were anesthetized with a single intraperitoneal injection of 0.1 mL saline containing 1 mg fluani
dine and 0.03 mg fentanyl citrate (Hynpnom; Janssen Pharmaceutics, Tilburg, The Netherlands). Immediately afterward, a bolus of approximately 1 million colony-forming units (CFUs) *S. aureus* or *K. pneumoniae* in 0.1 mL saline was injected into the right thigh muscle (17,18). Five minutes later, 0.2 mL saline containing either 0.4 µg \( ^{99m} \text{Tc} \)-HNP-1 (0.58 µmol/L, amounting to 0.8 MBq) or 10 µg \( ^{99m} \text{Tc} \)-IgG (0.33 µmol/L, amounting to 0.8 MBq) was injected intravenously. This technique was termed “injection.” In additional studies, scintigraphy was performed with a nonmicrobicidal synthetic peptide (KVAKQEKKKKKT), i.e., a residue of ubiqui
tinin, a new antimicrobial peptide (19). The mice were then killed at intervals by intraperitoneal injection of 0.25 mL saline containing 12 mg sodium pentobarbital, and the number of viable bacteria per gram of thigh muscle was determined microbiologically. Some experiments were performed on mice that were injected with the tracer 18 h after peritoneal injection with *S. aureus*. This technique was termed “infection.” The infected thigh muscles were removed and, after weighing, homogenized in 4 mL phosphate-buffered saline (PBS), pH 7.4. Appropriate dilutions of the homogenate were plated onto DST plates (Oxoid), and the number of colonies
was counted after overnight incubation at 37°C. The results were expressed as the number of CFUs per gram of infected tissue. All cultures with negative findings were assigned the value 100 CFU/mL, the lower limit of detection.

**Peritoneum Model.** A peritoneal *K. pneumoniae* infection model was used to quantify the binding of tracers to both bacteria and leukocytes from the site of infection. In short, 0.1 mL saline containing approximately 1 million CFUs *K. pneumoniae* was injected into the peritoneal cavity of anesthetized mice (8,20), and 5 min later, 0.2 mL saline containing 0.4 µg 99mTc-HNP-1 or 10 µg 99mTc-IgG was injected intravenously as a control. The mice were then killed at intervals by an intraperitoneal injection of sodium pentobarbital, and the peritoneal cavity was lavaged by intraperitoneal administration of 4 mL ice-cold PBS supplemented with heparin (50 IU/mL; Leo Pharmaceutical Products BV, Weesp, The Netherlands). The abdomen was gently shaken for 60 s, after which the peritoneal leukocytes and bacteria were harvested and centrifuged for 5 min at 110g at 4°C. The radioactivity and the number of cells in the pellet, i.e., the leukocytes, were counted. Subsequently, the supernatant, containing the bacteria and the unbound 99mTc-peptide, was centrifuged for 5 min at 2000g at 4°C. The radioactivity in the pellet, i.e., of the bacteria, was counted, and the number of CFUs was determined as described. The remaining supernatant, containing the unbound peptide, was also counted for radioactivity.

For analysis of the relationship between the binding of tracers to the various populations of leukocytes and the accumulation of tracers in the peritoneal cavity, monocytes, lymphocytes and granulocytes were purified from the leukocyte fraction. In short, leukocytes in the pellet were resuspended and then adjusted above isotonic Percoll (Sigma) with three layers having density grades of 1.070, 1.087 and 1.100 (55%, 70% and 81%, respectively) and centrifuged at 1500g for 5 min at 4°C. Next, the various fractions were collected, and the radioactivity associated with the cells was determined (21,22).

**Scintigraphy**

The pharmacokinetics of 99mTc-HNP-1, or of 99mTc-IgG as a control, in *S. aureus*– or *K. pneumoniae*–injected mice were assessed using planar scintigraphy. Before the scintigraphy, a subcutaneous injection of 0.1 mL saline containing 0.2 mg diazepam (Valium; Hoffmann-La Roche, Mijdrecht, The Netherlands) was administered. Then the mice were placed supine on a single-head, planar gamma camera (GCA 7100/UI; Toshiba, Tokyo, Japan) equipped with a low-energy general-purpose parallel-hole collimator. Both hind legs of each mouse were spread out and fixed with surgical tape, and whole-body images were acquired every 60 s during the first hour after tracer injection. Four hours after tracer injection, a 5-min image was acquired. The camera was connected to a computer (GMS 5500/UI; Toshiba), and high-resolution images were obtained in a 256 × 256 matrix. The energy peak was set at 140 keV, with a 20% window.

On the scintigraphic images, anatomically rendered regions of interest (ROIs) were drawn on the heart, liver, kidneys, bladder, stomach, lungs and both thighs to provide data about the uptake of 99mTc-HNP-1. An ROI of 5 × 5 pixels was drawn over the focus of infection in the right thigh muscle (targeted). The region was copied to the same location of the contralateral thigh muscle (nontargeted), and after the radioactivity in both regions was counted, the targeted-to-nontargeted ratio (T/NT) was calculated (21). Accumulation of 99mTc-labeled tracers at sites of infection was expressed as the ratio of the counts in the ROI drawn over the targeted and nontargeted thighs of each mouse. Clearance of tracers from the circulation was assessed by determining their half-life in ROIs drawn over the heart at various intervals. The radioactivity in the heart was corrected for decay and subsequently expressed as the percentage injected dose (%ID).

**Statistical Analyses**

Differences between the values for 99mTc-HNP-1 and 99mTc-IgG in the various infection models were analyzed using the Mann-Whitney test. The relationship between accumulation of tracers in the peritoneal cavity on the one hand and radioactivity associated with bacteria or leukocytes on the other was calculated using multiple-regression analysis. The level of significance was set at $P < 0.05$.

**RESULTS**

**Quality Control**

After incubation of 200,000 CFUs *K. pneumoniae* with unlabeled HNP-1 or 99mTc-HNP-1 at a concentration of 50 µg/mL for 2 and 3 h in vitro, no viable bacteria could be recovered, indicating that the labeling procedure did not affect the antimicrobial activity of HNP-1. Neither unlabeled IgG nor 99mTc-IgG affected the number of viable bacteria. In addition, more than 95% of the radioactivity associated with 99mTc-HNP-1 or 99mTc-IgG remained after incubation in Na-PB supplemented with 20% serum for 4 h at 37°C.

**Pharmacokinetics**

In mice injected with a bolus of *S. aureus* or *K. pneumoniae*, 99mTc-HNP-1 was rapidly removed from the circulation, with half-lives of 175 ± 28 min (mean ± SEM) and 55 ± 9 min, respectively ($n = 4$), indicating that elimination of this tracer in *K. pneumoniae*–injected mice is significantly ($P < 0.05$) faster than in *S. aureus*–injected mice. Moreover, 99mTc-HNP-1 rapidly accumulated in the kidneys, bladder and liver (Figs. 1A and B, Table 1) and, to a lesser extent, in the spleen (5–7% ID) and lungs (3–5% ID), whereas no radioactivity was observed in the thyroid and stomach of these mice. Furthermore, only minor differences were seen in half-life and biodistribution of 99mTc-HNP-1 between mice that received an *S. aureus* bolus injection and mice with *S. aureus* infection (Tables 1 and 2). The half-lives for 99mTc-IgG in the circulation of *S. aureus*– or *K. pneumoniae*–injected mice were 250 ± 35 and 70 ± 11 min, respectively ($n = 4$), showing that the clearance rate of this tracer from the circulation did not differ from that of 99mTc-HNP-1. 99mTc-IgG was rapidly taken up by the liver and kidneys and was excreted through the bladder (Table 1).

**Accumulation at Sites of Infection**

Within 5 min after injection of 99mTc-HNP-1 into mice that had received a bolus injection of *S. aureus* or *K. pneumoniae*, the site of infection had already been localized, as indicated by a significant T/NT in comparison with that after 99mTc-IgG injection. Maximum values were seen 15–60 min after injection of 99mTc-HNP-1 (Fig. 2). Furthermore, the T/NTs 5 and 15 min after injection of the tracers were significantly ($P < 0.05$) higher for 99mTc-HNP-1 than for
Figure 1. Representative scintigraphic images between 1 and 60 min after injection of $^{99m}$Tc-HNP-1 in mice with 

$^{99m}$Tc-IgG (Fig. 2), whereas the T/NTs observed 4 h after injection were similar for $^{99m}$Tc-HNP-1 and $^{99m}$Tc-IgG. In contrast to the T/NTs observed for $^{99m}$Tc-HNP-1, those for $^{99m}$Tc-IgG increased significantly ($P < 0.05$) during the 4-h period. Interestingly, the T/NTs for $^{99m}$Tc-HNP, but not for $^{99m}$Tc-IgG, were significantly ($P < 0.05$) higher in mice with an S. aureus infection than in mice with a bolus injection of S. aureus (Fig. 3). In additional studies with the cationic synthetic peptide (KVAKQEKKKKKT) derived from ubiqui-

Effect on Number of Viable Bacteria

The possibility was considered that the T/NTs for $^{99m}$Tc-HNP-1 do not increase over time because the peptide

<table>
<thead>
<tr>
<th>Interval after injection (min)</th>
<th>$^{99m}$Tc-protein</th>
<th>K. pneumoniae--</th>
<th>S. aureus--</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HNP-1</td>
<td>IgG</td>
<td>injected mice</td>
</tr>
<tr>
<td>5</td>
<td>15 ± 4†</td>
<td>17 ± 7†</td>
<td>18 ± 5†</td>
</tr>
<tr>
<td>15</td>
<td>23 ± 4†</td>
<td>15 ± 1†</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>60</td>
<td>16 ± 5*</td>
<td>16 ± 9</td>
<td>11 ± 6*</td>
</tr>
<tr>
<td>240</td>
<td>27 ± 7†</td>
<td>7 ± 5</td>
<td>19 ± 5</td>
</tr>
<tr>
<td>1440</td>
<td>16 ± 7*</td>
<td>20 ± 4*</td>
<td>6 ± 6*</td>
</tr>
</tbody>
</table>

* P < 0.05, HNP-1 compared with IgG.
† P < 0.05, K. pneumoniae injection compared with S. aureus infection.
‡ P < 0.05, S. aureus injection compared with S. aureus infection.

Data are mean (± SD) activity per organ, expressed as percentage injected dose, for 8–14 animals from three experiments.
reduces the number of viable bacteria (8). Therefore, the number of viable bacteria in the thigh muscle was assessed at intervals after injection of $^{99m}$Tc-HNP-1 or $^{99m}$Tc-IgG. In mice that received a bolus injection of S. aureus or K. pneumoniae, $^{99m}$Tc-HNP-1, in contrast to $^{99m}$Tc-IgG, greatly reduced the number of viable bacteria 4 and 24 h after injection (Table 2). The antibacterial effect of HNP-1 proved to be considerably less in S. aureus–infected mice than in K. pneumoniae–infected mice. Furthermore, the number of viable organisms in mice with an S. aureus infection was significantly ($P < 0.05$) higher than in mice that had received an S. aureus bolus injection.

### TABLE 2

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Experimental condition</th>
<th>$^{99m}$Tc-HNP-1 0 h</th>
<th>$^{99m}$Tc-HNP-1 4 h</th>
<th>$^{99m}$Tc-HNP-1 24 h</th>
<th>$^{99m}$Tc-IgG 4 h</th>
<th>$^{99m}$Tc-IgG 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>Injection</td>
<td>1.7 ± 0.5 × 10^5</td>
<td>6.9 ± 5.2 × 10^4</td>
<td>7.1 ± 2.7 × 10^4 *</td>
<td>2.2 ± 0.9 × 10^5</td>
<td>1.0 ± 1.6 × 10^6</td>
</tr>
<tr>
<td></td>
<td>Infection</td>
<td>4.7 ± 1.5 × 10^7</td>
<td>3.1 ± 0.3 × 10^7</td>
<td>6.7 ± 4.0 × 10^6 *</td>
<td>3.5 ± 0.5 × 10^7</td>
<td>1.5 ± 0.4 × 10^8</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>Injection</td>
<td>9.2 ± 0.5 × 10^5</td>
<td>8.2 ± 2.7 × 10^4 *</td>
<td>1.4 ± 2.1 × 10^4 *</td>
<td>7.9 ± 8.9 × 10^5</td>
<td>2.8 ± 5.8 × 10^7</td>
</tr>
</tbody>
</table>

* $P < 0.05$, compared with number of viable bacteria recovered from mice injected with IgG.

Data mean (± SD) of at least 6 animals.

![FIGURE 2. Target-to-nontarget ratios for either $^{99m}$Tc-HNP-1 (white bars) or $^{99m}$Tc-IgG (vertically hatched bars) in mice that received bolus injection of K. pneumoniae and for $^{99m}$Tc-HNP-1 (black bars) and $^{99m}$Tc-IgG (horizontally hatched bars) after administration of tracer into mice injected with bolus of S. aureus. Results are mean (± SD) of at least 9 animals from three series of experiments. * $P < 0.05$, compared with $^{99m}$Tc-IgG; # $P < 0.05$ for HNP-1 in S. aureus infections compared with HNP-1 in K. pneumoniae infections according to Mann-Whitney test.](image-url)
Binding to K. pneumoniae and Leukocytes in Peritoneal Cavity

The peritoneum model was used to obtain insight into binding of 99mTc-HNP-1 to bacteria and leukocytes at sites of infection. The results for 99mTc-HNP-1 revealed that approximately 37% of radioactivity in the peritoneal washings could be recovered in the fraction containing leukocytes, approximately 23%, in the fraction containing free bacteria and the remaining 40% in the washing fluid. The respective values for 99mTc-IgG were approximately 10%, 10% and 80%. After corrections based on these findings were applied (Fig. 4), 99mTc-HNP-1 binding to bacteria proved to be approximately 1000 times higher than to leukocytes, whereas binding of 99mTc-IgG was roughly the same for leukocytes and bacteria.

In agreement with the results in the thigh muscle model, the number of viable bacteria found in peritoneal washings was much lower for K. pneumoniae–infected mice injected with 99mTc-HNP-1 than for those injected with 99mTc-IgG (8). Furthermore, the number of leukocytes in peritoneal washings was higher \( P < 0.05 \) for mice injected with 99mTc-HNP-1 than for mice injected with 99mTc-IgG.

In addition, 4 and 24 h after injection, multiple-regression analysis revealed that binding of 99mTc-HNP-1 to bacteria \( (r = 0.81, P < 0.05) \), macrophages \( (r = 0.97, P < 0.04) \), granulocytes \( (r = 0.76, P < 0.05) \) and lymphocytes \( (r = 0.64, P < 0.05) \) contributed to accumulation of this tracer at the site of infection. Interestingly, for 99mTc-IgG, only the binding to macrophages \( (r = 0.93, P < 0.05) \) and granulocytes \( (r = 0.64, P < 0.05) \), not to bacteria, correlated with accumulation of this tracer in the infected peritoneal cavity.

DISCUSSION

The main conclusion from this study is that 99mTc-HNP-1 can be used for rapid visualization of bacterial infections. This conclusion is based on the observation that T/NTs higher than 1.3 for 99mTc-HNP-1 had already occurred within 5 min after administration of the tracer to mice that had been injected intramuscularly with either gram-negative bacteria.
or gram-negative bacteria. This rate is considerably faster than that for $^{99m}$Tc-IgG, an established marker of infection and inflammation \((1,18)\), for which similar T/NTs were observed 4 h after administration of the tracer. The observation that the T/NT for $^{99m}$Tc-HNP-1 remained roughly constant over the 4-h postinjection period contrasts with the rises in T/NT for $^{99m}$Tc-IgG. An explanation for the finding that the T/NT for $^{99m}$Tc-HNP-1 does not increase after 30 min could be elimination of bacteria in the thigh muscle on introduction of the peptide. Agreeing with this view is an earlier report that the number of viable bacteria in the thigh muscle of mice decreased after injection of $^{99m}$Tc-HNP-1 but increased after injection of $^{99m}$Tc-IgG \((8)\).

The suggestion that binding of $^{99m}$Tc-HNP-1 to bacteria is the major factor contributing to accumulation of this tracer in bacterial infections is based on the following findings. First, in mice with an infected peritoneal cavity, binding of $^{99m}$Tc-HNP-1, but not $^{99m}$Tc-IgG, to bacteria was found to correlate positively with accumulation of tracer at the site of infection. Moreover, binding of $^{99m}$Tc-HNP-1 to bacteria was at least 1000 times higher than to leukocytes in vivo. However, 2 h after injection of $^{99m}$Tc-HNP-1, the number of leukocytes in the infected peritoneal cavity of mice was already more than 100 times higher than the number of bacteria. Poor binding of $^{99m}$Tc-HNP-1 to relatively high numbers of leukocytes could explain why binding of $^{99m}$Tc-HNP-1 to leukocytes was also found to correlate well with accumulation of this tracer in the peritoneal cavity. Part of the $^{99m}$Tc-HNP-1 activity related to leukocytes can likely be attributed to binding of $^{99m}$Tc-HNP-1 to phagocytosed bacteria. Unfortunately, binding of tracers to leukocytes could not be distinguished from binding of tracers to bacteria associated with these leukocytes. In addition, not all activity in the peritoneal washings could be attributed to cells. Other possible modes of action, such as aspecific leakage of protein into the site of infection, binding of the tracer to proteins \((22)\) or other noncellular components and reduction of blood flow, may also contribute to accumulation in the peritoneal cavity.

Second, the observation that the T/NTs for $^{99m}$Tc-HNP-1 in mice infected with \emph{S. aureus} are much higher than in mice injected with \emph{S. aureus} may be attributed to the higher

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Binding of $^{99m}$Tc-labeled human neutrophil peptide (HNP-1) and $^{99m}$Tc-immunoglobulin G (IgG) to 1 million bacteria or peritoneal leukocytes after injection into mice infected with \emph{K. pneumoniae}. At intervals after this injection, peritoneal cavity was lavaged and, after selective centrifugation, fractions containing free bacteria (black bars) and leukocytes (white bars) were counted for numbers and for radioactivity.}
\end{figure}
numbers of bacteria in mice with an infection. Alternatively, binding of 99mTc-HNP-1 to cytokine-activated leukocytes or other cell types present at the site of infection, e.g., endothelial cells, may explain the difference in T/NT. This possibility is less likely because in vitro binding studies have shown that cytokine-activated human leukocytes and endothelial cells from the umbilical cord vein bind negligible amounts of HNP-1 (MM Welling et al., unpublished data, 1999). In addition, 4 h after injection of HNP-1 in mice that received a bolus injection of S. aureus, similar T/NTs were observed for HNP-1, despite a influx of leukocytes during this period.

Another important finding pertains to differences in 99mTc-HNP-1 pharmacokinetics in mice infected with S. aureus or K. pneumoniae. Elimination of 99mTc-HNP-1 from the circulation and uptake of this tracer by various organs and the infected thigh muscle are faster in mice infected with K. pneumoniae than in mice infected with S. aureus. Although a definitive explanation cannot be offered, the severity of the K. pneumoniae infection, which is indicated by the high number of circulating leukocytes in these mice, may well be responsible for these results. Other possible explanations, such as differences in the binding capacity of HNP-1 to S. aureus or K. pneumoniae, cannot be ruled out. 99mTc-HNP-1 has several advantages over currently used tracers for detection of bacterial infections. The small molecular size of HNP-1 enables rapid entrance of the tracer at the site of infection, as illustrated in this study. Adverse effects in patients, e.g., triggering of antibodies or allergic reactions against this peptide, are not likely because HNP-1 is of human origin. Furthermore, the highest concentration of HNP-1 used in this study (400 ng per mouse; i.e., 260 ng/mL blood) is not toxic to human cells. During infection in humans, the level of HNP-1 in blood has been reported to increase from 40 ng/mL to 1 µg/mL (23,24). Rapid uptake of 99mTc-HNP-1 by the kidneys and bladder, followed by excretion through the urine, reduces the radiation burden in organs. When considering the possibility that bacteria become resistant to this antimicrobial peptide, one should remember that HNP-1 eliminates bacteria by causing bacterial plasma membranes to become permeable and, subsequently, subject to metabolic injury (7). This fact makes unlikely the development of bacteria that are resistant against HNP-1. Besides, during evolution, defenses have been successful in protecting plants, animals and humans against the continuous threat of pathogenic microorganisms (25).

CONCLUSION

99mTc-HNP-1 allows rapid visualization of bacterial infections, in part because of binding of this tracer to the microorganisms at these sites.

ACKNOWLEDGMENTS

The authors appreciate the technical assistance of Petra Dibbets-Schneider and Bram Sinon of the Department of Radiology, Division of Nuclear Medicine; Maria van den Barselaar of the Department of Infectious Diseases; and Sandra van Wetering of the Department of Pulmonology (Leiden University Medical Center).

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