^{99m}Tc-Labeled Antimicrobial Peptides for Detection of Bacterial and *Candida albicans* Infections

TO THE EDITOR: We are compelled to respond to the criticism of Welling et al. (*1*) about ^{99m}Tc-ciprofloxacin (Infecton; Draximage Inc., Kirkland, Quebec, Canada) imaging because of their continued use of references that, as detailed analysis reveals, do not support their statements. We pointed these out in a previous letter to the editor of this journal (*2*) and in letters to the editor of the *European Journal of Nuclear Medicine* (*3,4*). No useful benefit will be served by repeating that information again in detail here; suffice it to say that, first, the amount of ciprofloxacin in Infecton is so tiny (2 mg, which is 1/200 of a single therapeutic intravenous dose of ciprofloxacin) that the risk of emergence of resistant bacteria is unlikely to be a clinical problem. Indeed, such emergence has not been observed in experiments in vitro or in patients who have undergone Infecton imaging, although we are monitoring the situation carefully. Second, mammalian topoisomerase has 100–1,000 times less affinity for binding to ciprofloxacin than does bacterial DNA gyrase (the basis of selective toxicity), and the binding is readily reversible. By comparison, Infecton remains bound to bacteria at sites of infection, giving a high target-to-background ratio, which is the basis of bacteria-specific imaging with Infecton. Third, our clinical experience with Infecton imaging has now extended to well over 1,300 patients and supports our previously published data showing that Infecton can detect a wide variety of bacterial infections with high specificity and distinguish reliably between infective and noninfective inflammation, if 24-h images are included, as previously reported (*2–4*).

We have a few other points to make regarding the article of Welling et al. (*1*). The authors used multidrug-resistant *Staphylococcus aureus* and *Klebsiella pneumoniae* in their experiments to induce infection and to study the uptake of various agents, including Infecton. Were these organisms also resistant to ciprofloxacin, and if so, what was the mechanism of resistance? If an organism does not take up ciprofloxacin, bacteria-specific imaging with Infecton will not occur.

Similarly, it is well known that ciprofloxacin is not taken up by and is inactive against fungal pathogens, including *Candida albicans*. This fact explains the experimental results of Welling et al. (*1*) for Infecton imaging of infection caused by this organism. In fact, *Candida albicans* acts as a good negative control for Infecton.

Some antibiotics, notably azithromycin and clindamycin, have been shown to affect the immune system, and such an effect may be beneficial by augmenting clearance of bacterial pathogens from the host. However, the clinical significance of this effect remains unknown. In addition, ciprofloxacin is unlikely to significantly affect the immune system at the tracer amount present in Infecton. The predicted peak serum concentration of ciprofloxacin after intravenous administration of Infecton is only 0.01–0.02 mg/L. The concentration of ciprofloxacin used by Riesbeck et al. (*5*) was far greater, ranging from 5 to 80 mg/L (i.e., 250–4,000 times greater), with the effect on interleukin-2 production being more pronounced at the higher concentrations of 20 and 80 mg/L. In this regard, it is relevant to note that the peak serum concentration after 1 h with a standard therapeutic intravenous dose of 400 mg is approximately 2–4 mg/L. One always has to be careful about extrapolating in vitro and, indeed, animal experimental data to humans. These experiments are useful as a guide to what may or may not occur in humans but by themselves cannot totally be relied on. Because of the very nature and function of the molecules, it is more likely that defensins such as antimicrobial peptides, even when designed to contain only the "antimicrobial" domains, will have a more pronounced and significant effect on the immune system than does ciprofloxacin or other antibiotics.

One must question whether Welling et al. (*1*) are preparing ^{99m}Tc-ciprofloxacin. They state that their preparation needs a further purification step because of 20%–40% free ^{99m}Tc. This is never seen with our ^{99m}Tc labeling of ciprofloxacin and suggests colloid formation, which may occur if the instructions are not followed precisely, such as when the reagents are combined in the wrong order.

The captions of Figures 1 and 4 indicate that the studies were obtained at 1 h. The authors appear to still not understand that any small molecule diffuses into inflammation and out again in proportion to its size and the rate of the fall in blood level. Our studies on humans show that 4- and 24-h images are required to distinguish active inflammation, for example, in joints, from septic arthritis. Larikka et al. (*6*) showed an increase in specificity from 41% to 95% when a 24-h image was combined with a 4-h image to distinguish an infected hip prosthesis from noninfected inflammation.

In conclusion, successful imaging of bacterial infection stems from a combination of direct binding of ^{99m}Tc-ciprofloxacin to dividing bacteria and the dynamic specificity that occurs when uptake caused by bacterial infection persists while that caused by nonspecific inflammation fades. It appears difficult to mimic the reality of the situation in humans from animal models.

We welcome fair and scientifically valid criticism of our novel imaging technique. As a matter of good scientific principle, data from studies should be critically analyzed before they are used to support or refute anything. Sadly, Welling et al. (*1*) appear to be repeatedly neglecting this step when commenting on Infecton imaging.

REFERENCES

1. Welling MM, Lupetti A, Balter HS, et al. ^{99m}Tc-labeled antimicrobial peptides for detection of bacterial and *Candida albicans* infections. *J Nucl Med.* 2001;42:788–794.
2. Britton KE, Wareham D, Solanki KK, Das SS. Imaging of bacterial infections with ^{99m}Tc-labeled human neutrophil peptide-1 [letter]. *J Nucl Med.* 2000;41:2099–2100.
3. Das SS, Britton KE, Solanki KK, Wareham DW. Technetium-99m labelled antimicrobial peptides discriminate between bacterial infections and sterile inflammations [letter]. *Eur J Nucl Med.* 2000;27:1865–1868.
4. Britton KE, Wareham DW, Das SS. Concerns about ^{99m}Tc-labelled ciprofloxacin for infection detection [letter]. *Eur J Nucl Med.* 2001;28:779–781.
5. Riesbeck K, Andersson J, Gullberg M, Forsgren A. Fluorinated 4-quinolones induce hyperproduction of interleukin 2. *Proc Natl Acad Sci USA.* 1989;86:2809–2813.
6. Larikka M, Ahonen A, Onni N, et al. Comparison of ^{99m}Tc-ciprofloxacin (Infecton), ^{99m}Tc-white-blood cell and three phase bone imaging in the diagnosis of hip prosthesis infections: improved diagnostic accuracy with extended imaging time. *Nucl Med Commun.* 2002;23:655–661.

Satya S. Das
David W. Wareham
Keith E. Britton
Departments of Microbiology and Nuclear Medicine
St. Bartholomew's Hospital
London, United Kingdom

REPLY: In response to the letter of Das et al., we must first emphasize that the main point of our study was completely missed by these authors. Our study concerned the development and testing of a new class of radiolabeled compounds, namely antimicrobial peptides, for the detection of bacterial and *Candida albicans* infections (*1*). Our findings for ^{99m}Tc-ciprofloxacin (Infecton) were of importance but in the context of our article were used only as a reference.

Nevertheless, it was informative to take note of the 3 issues mentioned by Das et al. We were happy to read that the possible emergence of ciprofloxacin resistant bacteria in patients is being carefully monitored. We also agree with the statement that mammalian topoisomerase has 100–1,000 times less affinity for binding to unlabeled ciprofloxacin than does bacterial DNA gyrase and that the binding is readily reversible, but we wonder if these data are also true for radiolabeled ciprofloxacin. To explain the accumulation of ciprofloxacin in both bacterial and sterile inflamed tissues, we must emphasize that the affinity of radiolabeled ciprofloxacin for binding to bacterial as well as human DNA in vitro was equal (*2*). Besides our data concerning ^{99m}Tc-ciprofloxacin (*2*), we have never seen any radiochemical or preclinical data in the literature. Therefore, we assume that Das et al. will perform the experiments with radiolabeled ciprofloxacin in view of the possibility that this radiolabeled agent may show completely different behavior from the unlabeled molecules. In our hands, ^{99m}Tc-ciprofloxacin bound in vitro even better to human cells than to bacteria (*2*). The final issue that Das et al. mention is the clinical experience with Infecton in 1,300 patients and the increasing reliability of this agent to distinguish infection from sterile inflammation. Farther on, Das et al. mention that the specificity of Infecton increased from 40% to 96% when 24-h images were taken instead of 4-h images. We are surprised to hear that only 40% specificity was obtained using scintigraphic data after 4 h, because this information has never been included in reports by the London group and puts a different complexion on the reliability of previously published clinical data. In addition, one may wonder how it is possible that the abstract by Wareham et al. (*3*) from the same London group mentions 80% specificity whereas Das et al. mention 96% specificity.

Another aspect of late imaging with the ^{99m}Tc-labeled compound concerns, of course, the quality of the images after 4 half-lifetimes. It would be interesting to see routine clinical images and judge the readability of the scintigrams. In our experience, ^{99m}Tc-images at 24 h are difficult to interpret, and a recent report by the London group agrees (*4*). Also, the in vivo stability of the agent at 24 h should be considered, and one might want to see the radiochemical data on ^{99m}Tc-ciprofloxacin after incubation in human serum for 24 h.

We also must remark on the statement by Das et al. that "if an organism does not take up ciprofloxacin, bacteria-specific imaging with Infecton will not occur." Because ^{99m}Tc-ciprofloxacin was proposed as a sensitive and specific tracer for bacterial infections, one would expect that infections with ciprofloxacin-resistant bacteria, perhaps through an aspecific binding, are also detected. However, as reported in our study, ^{99m}Tc-ciprofloxacin accumulated in a similar way in both bacterial infections and sterile inflammation (*1*). In agreement with our results, Sarda et al. (*5*) recently reported the failure of ^{99m}Tc-ciprofloxacin to discriminate between infected and noninfected prosthetic joints in rabbits. In addition, although ^{99m}Tc-ciprofloxacin is not effective against *C. albicans*, accumulation of ^{99m}Tc-ciprofloxacin at sites of infection with *C. albicans* was even more pronounced than at sites of infection with ciprofloxacin-sensitive bacteria (*1*).

Another remark we must make involves the paragraph on the labeling and quality control of ^{99m}Tc-ciprofloxacin. We performed this labeling according to the method published by Vinjamuri et al. (*6*). The London group has used this method in at least 3 subsequent studies, for which successful imaging of infections in more than 240 clinical cases was reported. We understand that in the meantime this number has increased to well over 1,300 patients. In various communications by the group from London, we thoroughly searched for quality control data on the ^{99m}Tc-ciprofloxacin preparation. Unfortunately, we could not find relevant data. Even a recent article on ^{99m}Tc-ciprofloxacin did not report the results of quality control, except for mentioning a labeling efficiency of >92% (*4*). For such a new and important preparation, one would like to see data from high-performance liquid chromatography analysis of preparations containing ^{99m}Tc-ciprofloxacin, including ultraviolet chromatography and the identification of radioactivity peaks. It is, in our view, surprising that detailed information is still lacking, and it would only be logical and scientifically correct to provide such data as well as the relevant preclinical data in connection with successful clinical imaging. We had to remove 20%–40% of the total activity from the mixture containing radiolabeled ciprofloxacin (*1*)—an amount that agrees with the results published by the London group (*7*). Following the London procedure, we purified the preparation using diethylaminoethyl chromatography, which removes all colloids as well as free pertechnetate. Therefore, we consider our preparations colloid free, as confirmed by our routine reverse-phase high-performance liquid chromatography analysis for quality control of radiolabeled compounds. Therefore, it is encouraging that an independent research group using the Infecton kit, prepared according to the instructions of the London group, reported relevant quality control data showing lack of specificity for ^{99m}Tc-labeled ciprofloxacin in the detection of bacterial infections (*5*).

In their letter, Das et al. expressed some concern about the effect of antibiotics on the immune system. They caution the reader not to extrapolate in vitro and animal experimental data to humans. The London group is right, and we are happy that we did not speculate on this in our articles (*1*,*8*). We agree that antimicrobial peptides may interact with cells of the innate and acquired immune system (*9*), as we reported earlier for defensins (*10*). We have never observed side effects from (the low) concentrations of antimicrobial peptides that have been used so far for infection detection. These data agree with the statement by Das et al. that low concentrations of radiolabeled ciprofloxacin were used for detection of infections, in contrast to the higher concentrations used in therapy.

With regard to Figures 1 and 4, Das et al. suggested that we do not understand the basic principles of the accumulation of radiopharmaceuticals in tissues and organs. This suggestion is disturbing, because the London group apparently fails to remember that in several of our publications we have provided data showing the increasing accumulation of ^{99m}Tc-ubiquicidin 29–41 at infection sites (*1*,*8*). The same publications also provide data showing that ^{99m}Tc-ciprofloxacin, but not ^{99m}Tc-labeled antimicrobial peptides, accumulated at the site of sterile inflammation. On the basis of these findings we concluded that accumulation of ^{99m}Tc-labeled antimicrobial peptides at sites of infection could not be attributed merely to "increased perfusion."

We hope to read more evidence on the specificity of ^{99m}Tc-ciprofloxacin as a marker for bacterial infections. The London group may remember that a report by Sarda et al. (*11*) and a recent publication in *The Journal of Nuclear Medicine* (*5*) pointed out a lack of specificity of ^{99m}Tc-ciprofloxacin for the detection of a *Staphylococcus aureus* infection in a rabbit model as well as infections in patients (*12*). On

the basis of these data and our results (*1,8*), we cast doubt on the conclusion that successful imaging of bacterial infection is caused by direct binding of ^{99m}Tc-ciprofloxacin to dividing bacteria. In this connection, we wonder what the authors have in mind when stating that "there might be additional localization mechanisms for Infecton" (*13*). Also, we welcome scientifically valid criticism on our findings, and we suggest that the London group should perhaps submit their findings with additional details as a regular manuscript to peer review.

REFERENCES

1. Welling MM, Lupetti A, Balter HS, et al. ^{99m}Tc-labeled antimicrobial peptides for detection of bacterial and *Candida albicans* infections. *J Nucl Med.* 2001;42:788–794.
2. Welling MM, Nibbering PH, Paulusma-Annema A, Hiemstra PS, Pauwels EKJ, Calame W. Imaging of bacterial infections with ^{99m}Tc-labeled human neutrophil peptide-1 [reply to letter]. *J Nucl Med.* 2000;41:2100–2102.
3. Wareham DW, Das S, Solanki K, et al. Imaging intra-abdominal infection with ^{99m}technetium labelled ciprofloxacin (Infecton). In: *Abstracts Book of the 41st ICAAC, Chicago, IL, 16–19 December 2001.* Herndon, VA: ASM Press; 2001:444.
4. De Winter F, van de Wiele C, Dumont F, et al. Biodistribution and dosimetry of ^{99m}Tc-ciprofloxacin, a promising agent for the diagnosis of bacterial infection. *Eur J Nucl Med.* 2001;28:570–574.
5. Sarda L, Saleh-Mghir A, Peker C, et al. Evaluation of ^{99m}Tc-ciprofloxacin scintigraphy in a rabbit model of *Staphylococcus aureus* prosthetic joint infection. *J Nucl Med.* 2002;43:239–245.
6. Vinjamuri S, Hall AV, Solanki KK, et al. Comparison of ^{99m}Tc Infecton imaging with radiolabelled white-cell imaging in the evaluation of bacterial infection. *Lancet.* 1996;347:233–235.
7. Solanki KK, Bomanji J, Siraj Q, et al. Tc-99m-Infecton: a new class of radiopharmaceutical for imaging infection [abstract]. *J Nucl Med.* 1993;34(suppl):119P.
8. Welling MM, Paulusma-Annema A, Balter HS, et al. Technetium-99m labelled antimicrobial peptides discriminate between bacterial infections and sterile inflammations. *Eur J Nucl Med.* 2000;27:292–301.
9. Hancock REW, Diamond G. The role of cationic antimicrobial peptides in innate host defences. *Trends Microbiol.* 2000;8:402–410.
10. Welling MM, Hiemstra PS, van den Barselaar MT, et al. Antibacterial activity of human neutrophil defensins in experimental infections in mice is accompanied by increased leukocyte accumulation. *J Clin Invest.* 1998;102:1583–1590.
11. Sarda L, Saleh-Mghir A, Peker C, et al. Evaluation of Infecton imaging in a rabbit model of prosthetic joint infection due to *Staphylococcus aureus*: preliminary results [abstract]. *Eur J Nucl Med.* 1999;26:1015.
12. Sarda L, Lebtahi R, Genin R, et al. Value of Infecton scintigraphy for the detection of post-operative osteo-articular infection: preliminary results of a clinical study [abstract]. *Eur J Nucl Med.* 2000;27:970.
13. Sonmezoglu K, Sonmezoglu M, Halac M, et al. Usefulness of ^{99m}Tc-ciprofloxacin (Infecton) scan in diagnosis of chronic orthopedic infections: comparative study with ^{99m}Tc-HMPAO leukocyte scan. *J Nucl Med.* 2001;42:567–574.

Ernest K.J. Pauwels
Mick M. Welling
Peter H. Nibbering
Leiden University Medical Center
Leiden, The Netherlands

Antonella Lupetti
Università degli Studi di Pisa
Pisa, Italy

Henia S. Balter
Centro de Investigaciones Nucleares
Montevideo, Uruguay

Evaluation and Localization of Thyroid Tissue Using an Intraoperative-Type Solid-State Radiation Probe

TO THE EDITOR: We would like to describe a recent clinical case that we think illustrates a simple and apparently new approach to solving what traditionally has been a difficult and time-consuming clinical problem. A full-term 59-d-old male infant presented to the Department of Nuclear Medicine for a thyroid scan as part of a work-up for possible ectopic thyroid tissue. The baby was born with several abnormalities of the oral cavity and nasopharynx, including a large cleft palate, a multilocular nasopharyngeal mass protruding into the cleft, a bifid tongue, and a 1×1.5 cm midline mass between the 2 lingual processes. Because of airway compromise, the palatal mass had been removed 1 wk earlier and was found to be a teratoma with elements of neurofibroma. The etiology of the lower mass remained unknown, and lingual thyroid tissue was within the differential diagnosis. A thyroid scan was ordered to confirm the presence of an orthotopically placed thyroid gland within the neck and to evaluate for the presence of iodide-concentrating tissue within the mass.

The baby was fed formula mixed with 2.8 MBq ^{123}I NaI. Twenty-four hours later, radioactive iodide uptake over the neck, using an adult-sized phantom, was calculated to be 12.7%. Pinhole imaging was performed on the sleeping child in anterior and lateral projections. A single focus of uptake was noted and, after re-imaging with markers, was thought to be localized in the neck and not the pharynx. The location of the activity was confirmed using an intraoperative cadmium–tellurium probe (Navigator GPS and Standard Lymphatic Mapping Probe; United States Surgical, Norwalk, CT), which was placed on the upper anterior neck, above the thyroid cartilage. Through rotation of the collimated detector, all the detectable counts were shown to originate inferiorly, from the usual location of the thyroid, and not posteriorly, from the region of the lingual mass. The probe was then invaginated into a glove and placed in the baby's mouth, directly abutting the mass. No discernible increase in counts above the background level was noted. The patient went to surgery 1 wk later, during which the mass was removed and found to consist of ectopic salivary gland tissue without elements of thyroid tissue. The child has remained euthyroid after surgery.

In the clinical evaluation of an ectopic thyroid gland, we believe that use of a nonimaging collimated intraoperative probe can quickly and elegantly evaluate whether a normal orthotopic thyroid is present and, second, whether iodide concentration is present within the mass. Although thyroid imaging has traditionally been used for these indications, differentiating lingual from normally placed thyroid can still be a difficult and time-consuming challenge, especially in newborns, who often require sedation and multiple views. In contrast, with use of the handheld probe, no sedation was required to rapidly confirm the presence of functional thyroid tissue within the neck, and the entire procedure took only seconds to complete. We have not addressed the minimal amount of uptake necessary for detection of ectopic thyroid tissue; however, the extremely low concentration of ^{123}I in tissues other than thyroid and the high sensitivity of the intraoperative probe suggest that even a small amount of ectopic activity above the background level can be detected. It is also possible that for the current indication, the dose of ^{123}I NaI can be reduced if imaging is not performed.

Lionel S. Zuckier, MD
Kayvon Haghighi, MD
Kathryn A. Skuza, MD
New Jersey Medical School
Newark, New Jersey

Labeling Method Largely Affects the Imaging Potential of Interleukin-8

TO THE EDITOR: We read with interest the study by Gross et al. entitled "Imaging of Human Infection with ^{131}I-Labeled Recombinant Human Interleukin-8" (*1*). The authors conducted a pilot study with ^{131}I-labeled IL-8 for detection of infection in patients. The data nicely showed that this IL-8 preparation could visualize infectious foci in patients with osteomyelitis and cellulitis. Furthermore, the agent appeared to be safe, since no significant side effects were observed on intravenous administration. Apparently, the study was performed years before the present publication (*2*). In the meantime we studied several aspects related to labeling IL-8 for detection of infection.

We have shown that the labeling method used in this study is rather suboptimal for IL-8 and that substantial improvements can be obtained using alternative labeling strategies. IL-8 was radioiodinated according to the chloramine-T method (*3*). This method results in iodination of the tyrosine residues similar to the IODO-GEN method. We pointed out that the radioiodination method clearly affected the in vivo biodistribution of IL-8 (*4*). IL-8 radioiodinated by the Bolton–Hunter method showed superior characteristics for infection imaging compared with IL-8 radioiodinated by the IODO-GEN method. In a rabbit model of *E. coli*-induced soft-tissue infection, abscess-to-background ratios as determined by region-of-interest analysis of the images were 3 for ^{123}I-IL-8 (IODO-GEN method) versus 13 for ^{123}I-IL-8 (Bolton–Hunter method) at 8 h after injection. As pointed out by the authors themselves, labeling IL-8 with ^{123}I would have been a better choice instead of labeling with ^{131}I. The physical properties of ^{131}I limit spatial resolution.

The authors made the suggestion that IL-8 labeled with a radiometal instead of iodine may be preferable as an infection imaging agent. Indeed, preclinical studies in our laboratory with ^{99m}Tc-labeled IL-8 showed excellent imaging characteristics for this preparation, with abscess-to-background ratios of up to 22 at 8 h after injection (*5*). Studies in patients using ^{99m}Tc-labeled IL-8 are planned to evaluate the efficacy of this particular preparation in visualizing inflammatory foci in humans.

REFERENCES

1. Gross MD, Shapiro B, Fig LM, Steventon R, Skinner RWS, Hay RV. Imaging of human infection with ^{131}I-labeled recombinant human interleukin-8. *J Nucl Med.* 2001;42:1656–1659.
2. Gross MD, Shapiro B, Skinner RS, Shreve P, Fig LM, Hay RV. Scintigraphy of osteomyelitis in man with human recombinant interleukin-8 [abstract]. *J Nucl Med.* 1996;37(suppl):25P.
3. Hay RV, Skinner RS, Newman DC, et al. Scintigraphy of acute inflammatory lesions in rats with radiolabelled recombinant human interleukin-8. *Nucl Med Commun.* 1997;18:367–378.
4. Van der Laken CJ, Boerman OC, Oyen WJG, van de Ven MTP, van der Meer JWM, Corstens FHM. Radiolabeled interleukin-8: scintigraphic detection of infection within a few hours. *J Nucl Med.* 2000;41:463–469.
5. Rennen HJJM, Boerman OC, Oyen WJG, van der Laken CJ, Corstens FHM. Specific and rapid scintigraphic detection of infection with ^{99m}Tc-labeled interleukin-8. *J Nucl Med.* 2001;42:117–123.

Huub Rennen, MSc
Otto Boerman, PhD
Wim Oyen, MD
Frans Corstens, MD
University Medical Center Nijmegen
Nijmegen, The Netherlands