Myocardial Viability Assessment with FDG Imaging: Comparison of PET, SPECT, and Gamma-Camera Coincidence Detection

TO THE EDITOR: In the November 1999 issue of *The Journal of Nuclear Medicine*, Hasegawa et al. (*1*) reported a direct comparison among PET, SPECT, and dual-head gamma-camera coincidence detection imaging (DCD) to assess myocardial viability with FDG.

We would like to address three issues about the design and methods of the study.

1. The choice of performing the DCD tomography in 32 steps over 360° penalizes this modality by a factor of 2 in the examination duration, compared with 32 steps over 180° with ultra-high-energy general purpose collimators for SPECT. Furthermore, the gamma camera used in the study (Vertex Plus MCD; ADAC Laboratories, Milpitas, CA) is equipped with a 5/8-in. (15.9-mm) thick crystal, which means that the detection efficiency for the photopeak at 511 keV is approximately 21% for a single detector (*2*). Thus, the coincidence detection efficiency for DCD is only 21% × 21% = 4.41% (*2*), which would require more steps to obtain a similar count per image ratio and to allow for a more objective comparison between the 2 modalities. Finally, the 128 × 128-matrix reconstructed data obtained from DCD should have been reduced to a 64 × 64 matrix to obtain the same slice thickness as the SPECT images.

2. The DCD acquisition started 150 min after SPECT acquisition, occurring with 38.56% of the FDG activity that was initially available for SPECT imaging, because of FDG decay (half-life = 109.7 min).

3. The delayed DCD acquisition (210 min after injection and 150 min after SPECT) may be reasonable because of the risk of detector saturation by high counting rate, but it assumes that myocardial trapping of FDG is irreversible. Recently, Herrero et al. (*3*) demonstrated, on a canine model of myocardial glucose usage during hyperinsulinemic–euglycemic clamp, that a reversible myocardial trapping of FDG occurs within the first hour after tracer injection. This delay may therefore alter the myocardial distribution of FDG and consequently the DCD detection in an unpredictable manner.

All these factors acted to penalize DCD and could have influenced the final result of the study: a poor agreement of DCD imaging with PET and a better performance of SPECT. A randomized order for the first acquisition modality (DCD or SPECT) after PET imaging and an optimized DCD acquisition (duration and angle selection) would have been more adequate to sustain the published conclusion.

REFERENCES

1. Hasegawa S, Uehara T, Yamaguchi H, et al. Validity of ^{18}F-fluorodeoxyglucose imaging with a dual-head coincidence gamma camera for detection of myocardial viability. *J Nucl Med.* 1999;40:1884–1892.
2. Patton JA, Turkington TG. Coincidence imaging with a dual-head scintillation camera. *J Nucl Med*. 1999;40:432–442.
3. Herrero P, Sharp TL, Dence CS, et al. Demonstration of reversible myocardial trapping of F-18 fluorodeoxyglucose [abstract]. *Circulation.* 1999;100(suppl): I-89.

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Imaging of Bacterial Infections with ^{99m}Tc-Labeled Human Neutrophil Peptide-1

TO THE EDITOR: Although new techniques for imaging bacterial infections are needed, Welling et al.'s (*1*) criticisms of ^{99m}Tc-ciprofloxacin (Infecton) imaging lack substance and proof. Below, we explore, point by point, issues discussed by Welling et al.

"Low binding affinity of ^{99m}Tc-ciprofloxacin to bacteria": Where are the data to support this? Welling et al. (*1*) cited our article published in *The Lancet* (*2*), but the binding affinity of ^{99m}Tc-ciprofloxacin to bacteria is not discussed in our article. On the contrary, sites of bacterial infection can be imaged up to 24 h after the injection of 370 MBq (10 mCi) ^{99m}Tc ciprofloxacin. This agent shows specific binding to DNA gyrase, an essential enzyme for bacterial division, which is the basis of the therapeutic success of ciprofloxacin. Welling et al. (*1*) failed to mention the greater specificity of Infecton imaging for bacterial infection than radiolabeled white cells (*2*).

"The risk of emerging antibiotic resistant organisms": This is unlikely to be a significant clinical problem because only a tracer amount of ciprofloxacin is present in Infecton (i.e., 2 mg ciprofloxacin, which, being 1/100 to 1/200 of the intravenous therapeutic dose of 200–400 mg, is extremely small). Indeed, no such problems have been encountered in our multicenter study comprising 879 patients worldwide, and no drug toxicity has been observed in that study (*3*).

Reference 5 cited by Welling et al. (*1*) does not address the risk of emerging antibiotic-resistant microorganisms. The cited chapter gives an excellent account of how microbes, particularly those that are intracellular pathogens, have developed strategies to overcome or evade mechanisms that are used by macrophages and polymorphs to kill microorganisms. The chapter includes a discussion about the resistance to defensins (antibacterial peptides, a member of which is human neutrophil peptide-1 [HNP-1]) in *Salmonella typhimurium*. Similarly, other articles have been published that deal with microbial resistance–susceptibility to defensins. It is naive, therefore, to suggest that defensin-resistant bacteria may not emerge easily. The host–microbe interaction is not static but constantly evolving, with each trying to outdo the other. Successful pathogens, many of which are intracellular, have developed systems that enable them to resist and overcome host defense mechanisms, including antibacterial peptides. It would be interest-

ing to study whether infections with these microbes are amenable to imaging with ^{99m}Tc-labeled HNP-1.

HNPs may destroy human cells because the mechanisms involved in killing of microorganisms are not specific to this activity. Indeed, some of these proteins may subserve multiple roles in inflammation. In an editorial, Lehrer (*4*) raised many unanswered but important questions about defensins (e.g., whether defensins, which are small peptides, are rapidly degraded and whether there is penetration of extracellular defensins into tissues such as the lung and the gut). Condon et al. (*5*) showed that the peritoneal cavity is a poor model for binding studies. It is also an assumption to state that antibacterial activity equates to the level of binding. Welling et al. (*1*) stated that labeling was undertaken under acidic conditions but did not state the postlabeling pH, nor did they state if they assayed for acetic acid. In our experience, acidity may affect bacterial binding. Clearly, there is still a lot to be learned about these important compounds.

In regards to the study of Welling et al. (*1*), it remains to be determined whether their results are reproducible. As they have only studied 2 bacterial species, *S. aureus* and *Klebsiella pneumoniae*, it may be premature at this stage to extrapolate the results, as promising as they are, to cover all bacterial infections. In their article, Table 2 shows variable levels of bacterial numbers per gram of thigh muscle, and no data are given as to how their model was standardized.

It remains to be seen whether HNP-1 is truly infection specific, let alone bacteria specific. It is relevant that HNP-1 may be also active against fungi and some enveloped viruses. This peptide is a larger molecule than ciprofloxacin and more likely to be retained nonspecifically at sites of inflammation and slower to diffuse out of such sites than Infecton.

Welling et al. (*1*) have undertaken no human clinical trials to date. To suggest that because a product is from human origin, it is safe is seriously misleading. It is known that HNPs are cationic, cytotoxic, corticostatic, chemotactic, opsonic peptides. No toxicology is presented. Infecton has a significant advantage over other potential bacterial-specific imaging agents, because its bacterial specificity has been validated in a large international multicenter trial (*3*) and other publications (*2,6*). There are nearly 2 decades of experience concerning the safety of ciprofloxacin in humans.

In conclusion, in trying to undermine ^{99m}Tc-ciprofloxacin imaging, Welling et al. (*1*) have made the elementary but grave mistake of not checking their references correctly. References 4 and 5 in their article do not lend any support to their argument against ^{99m}Tc-ciprofloxacin imaging for bacterial infection. Not only have the authors misled an internationally reputable journal and its readers, false statements and inaccurate citations are damaging to the spirit of good scientific research. We, therefore, expect an apology from Welling et al. (*1*) regarding their false statements. However, we do wish them well in their further studies on HNP-1. Healthy but fair competition is good for research and development!

REFERENCES

1. Welling MM, Nibbering PH, Paulusma-Annema A, et al. Imaging of bacterial infections with ^{99m}Tc-labeled human neutrophil peptide-1. *J Nucl Med.* 1999;40:2073–2080.
2. 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.
3. International Atomic Energy Agency. *Nuclear Imaging for Infection and Inflammation: a Coordinated Research Project* [E1.30.14 technical document]. Vienna, Austria: International Atomic Energy Agency; 2000.
4. Lehrer RI. Editorial response: Questions and answers about defensins. *Clin Infect Dis.* 1997;25:1141–1142.
5. Condon MR, Viera A, D'Alessio M, Diamond G. Induction of a rat enteric defensin gene by hemorrhagic shock. *Infect Immun.* 1999;67:4787–4793.
6. Hall AV, Solanki KK, Vinjamuri S, Britton KE, Das SS. Evaluation of the efficacy of ^{99m}Tc-Infecton, a novel agent for detecting sites of infection. *J Clin Pathol.* 1998;51:215–219.

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REPLY: This contribution is a reply to the comments of Britton et al. on our recent article in *The Journal of Nuclear Medicine* describing the potential use of ^{99m}Tc-labeled human defensins 1–3 for discrimination between infections and sterile inflammatory processes in laboratory animals (*1*). The major criticism deals with our remarks concerning ^{99m}Tc-labeled ciprofloxacin, which is mentioned in a single sentence in the introductory section of our publication. When submitting our manuscript, we were already involved in experiments comparing the possibilities and limitations of antimicrobial agents, including antimicrobial peptides (*1,2*) and ciprofloxacin (*3*), for the specific detection of experimental infections in mice. The results available to us at that time contributed to our creating this now disputed sentence. The publication by Vinjamuri et al. (*3*) was intended to acknowledge the work with ^{99m}Tc-labeled ciprofloxacin, and the reference to the publication by Bäumler and Heffron (*4*) in this sentence should have been placed immediately after the mentioning of antibiotic-resistant microorganisms. As Britton et al. indicated in their letter, the reference to Vinjamuri et al. (*3*) was not optimal in the context of our remarks, and we apologize for this shortcoming. We do want to stress that it was never our intention to undermine ^{99m}Tc-labeled ciprofloxacin for imaging but to make some critical remarks on the use of this pharmaceutical for discrimination between infections and sterile inflammatory processes.

We disagree with Britton et al.'s statement that we misled *The Journal of Nuclear Medicine* and its readers with our critical remarks. In agreement with data reported by others (*5*), we have found that this radiolabeled compound binds equally well to purified DNA from gram-positive and gram-negative bacteria (12 ± 2 μg of labeled ciprofloxacin/mg of DNA) and human leukocytes activated in vitro by bacterial products (13 ± 5 μg of labeled ciprofloxacin/mg of DNA), with the respective values for radiolabeled human defensins 1–3 being 0.07 ± 0.02 μg of labeled peptide/mg of DNA and 0.05 ± 0.02 μg of labeled peptide/mg of DNA ($n = 3$). In addition, in vitro binding studies revealed more binding of ^{99m}Tc-labeled ciprofloxacin to activated human leukocytes and cytokine-activated human endothelial cells compared with *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans* (Table 1). In contrast, radiolabeled human defensins 1–3 bind to human cells less well than to these microorganisms (Table 1). These data indicate that binding of radiolabeled defensins to bacteria and *C. albicans* involves other binding sites than DNA. Indeed, it has been reported that defensins bind to negatively charged residues in the plasma membrane and possibly to specific receptors as well (*2*). Because radiolabeled human defensins 1–3 preferentially bind to microorganisms, the suggestion by Britton et al. that, because of their size (approximately 4 kD) defensins will

TABLE 1
In Vitro Binding of ^{99m}Tc-Labeled Defensins 1–3 and Ciprofloxacin to Microorganisms and Activated Human Cells

^{99m}Tc-labeled compound	*S. aureus**	*K. pneumoniae*	*C. albicans*†	Leukocytes‡	Endothelial cells§
Defensins 1–3	48 ± 10	37 ± 6	17 ± 1	5 ± 1	3 ± 1
Ciprofloxacin	5 ± 1	6 ± 2	6 ± 1	17 ± 2	20 ± 5

Binding of ^{99m}Tc-labeled defensins 1–3 and ciprofloxacin to microorganisms was assessed at 4°C as described (*1,2*). In short, 1×10^7 CFU of microorganisms in 10-mmol/L sodium phosphate buffer (pH, 7.4; NaPB) was added to a mixture containing ^{99m}Tc-labeled ciprofloxacin or defensins 1–3 (1 nmol) in NaPB supplemented with 50% v/v 0.01 mol/L acetic acid and 0.01% v/v between −80 (final pH, 5) and incubated for 1 h. Next, microorganisms were washed twice and radioactivity in cell pellet was measured in dose calibrator. Radioactivity associated with microorganisms is expressed as percentage of added radioactivity bound to 2×10^7 microorganisms. Binding of ^{99m}Tc-labeled defensins 1–3 and ciprofloxacin to activated human leukocytes or endothelial cells was determined under similar conditions, except that NaPB was supplemented with 50 IU of heparin/mL. Values (means and SEMs) are expressed as percentage of radioactivity bound to 2×10^7 CFU of microorganisms or 2×10^7 human cells. Results are from 6 independent experiments.

*Multi–drug-resistant *S. aureus* type 2141 is a clinical isolate (Dept. of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands).

†Fluconazole-resistant *C. albicans* Y-01 was obtained from Pfizer Inc., New York, New York.

‡Monocytes and granulocytes were isolated from blood, mixed, and then incubated with 100 nmol/L of synthetic bacterial breakdown product formyl-MLP and 100 ng LPS/mL.

§Human umbilical cord endothelial cells were incubated with 10 U/mL of recombinant IL-1β.

be retained more likely in a nonspecific fashion at the site of infections than ciprofloxacin, is not solid.

Furthermore, studies in mice have demonstrated similar values for the accumulation of radiolabeled ciprofloxacin in foci of infection and in sterile inflammatory lesions in mice, again in contrast to our results with human defensins 1–3 (Table 2). On the basis of the values found for ciprofloxacin in mice with an inflammation induced by lipopolysaccharides (LPS) and in mice having an inflammatory process induced by heat-killed bacteria, we concluded that radiolabeled ciprofloxacin most likely binds to infiltrating leukocytes and bacteria in the inflamed/infected area. Accumulation of ciprofloxacin in sterile inflammatory lesions has also been reported by others (*6*). Other disadvantages of (radiolabeled) ciprofloxacin are its ability to stimulate cytokine production in human cells (*7*) and the emergence of antibiotic-resistant bacteria (*8*). In connection to the latter, the (frequent) use of suboptimal concentrations of antibiotics as used by the group of Britton et al. is certainly a risk factor because many bacteria survive and, on contact at the site of infection, have the opportunity to become resistant to the antibiotics. No data on the development of ciprofloxacin-resistant bacteria in the studies by Britton et al. have been reported yet.

The second line of criticism by Britton et al. focuses on limitations of ^{99m}Tc-labeled human defensins 1–3 for infection detection. Defensins are small cationic antimicrobial peptides with broad-spectrum antimicrobial activity against a variety of bacteria, fungi, and enveloped viruses. The possibility that defensins (as well as various other antimicrobial peptides) can leave the circulation to reach the site of infection where they preferentially interact with bacteria (and fungi) makes these peptides our candidates of choice for the development of new radiopharmaceuticals for infection detection (*1,2*). Indeed, we demonstrated that imaging of infections can be achieved with ^{99m}Tc-labeled defensins (*1*). However, we realize that because of their tertiary structure, it is difficult to prepare defensins in large amounts under good manufacturing practice conditions. For this reason and because immunologic side effects could not be excluded (*9*), we concluded that defensins are not optimal for infection detection (*2*). This conclusion led us to study the possibilities and limitations of other antimicrobial peptides for imaging of infections (*2*). The occurrence of bacteria that is resistant to antimicrobial peptides, defensins in particular, has been reported (*10*). On the other hand, these peptides have been successful in protecting plants, animals, and humans against the continuous threat by a variety of pathogens (*11*), which argues against the suggestion by Britton et al. that resistance to antimicrobial peptides may develop easily. We do not agree with these authors that information about the behavior of defensins 1–3 in experimental animals is lacking (*9*). Moreover, we have found substantial accumulation of radiolabeled defensins 1–3 in infectious foci caused by an injection with various gram-positive bacteria (e.g., multi–drug-resistant *S. aureus, Listeria monocytogenes*), gram-negative bacteria (*K. pneumoniae, Escherichia coli, Salmonella typhimurium*), and the fluconazole-resistant *C. albicans* (Table 2).

In contrast to the statements of Britton et al., but on the basis of our results (showing small standard errors time after time), we have no doubts about the reliability of our results with radiolabeled peptides. In several articles, we already described in detail our method of labeling peptides with ^{99m}Tc, including quality controls and stability of the radiolabeled peptides (*2*). In addition, to exclude the possibility that the labeling methods affect the ability to identify infections, we copied precisely the protocol of Vinjamuri et al. (*3*) for labeling of ciprofloxacin with ^{99m}Tc and analyzed its capacity to accumulate at sites of infection in mice. The results for ciprofloxacin radiolabeled by our method and the method of Vinjamuri et al. with respect to infection detection were identical. Furthermore, ^{99m}Tc-labeled defensins 1–3 rapidly accumulated in the kidneys, bladder, and liver, and no activity was observed in the thyroid and stomach of the mice (*1*), indicating little or no free pertechnetate in our radiolabeled antimicrobial peptide preparations. Currently, we are investigating the half-life of antimicrobial peptides (*1*) and their degradation in bacterially infected mice.

We were surprised by the comment of Britton et al. that the peritoneal cavity model is not useful to study the binding of antimicrobial agents to bacteria and leukocytes. The publication cited by these authors does not deal with binding studies but describes the inducible expression of rat enteric defensin genes.

TABLE 2
Accumulation of ^{99m}Tc-Labeled Defensins 1–3 and Ciprofloxacin in Infections and Inflammatory Lesions

^{99m}Tc-labeled compound	*S. aureus**	*K. pneumoniae*	*C. albicans*†	LPS	Heat-killed microorganisms
Defensins 1–3	2.9 ± 0.2	1.9 ± 0.1	2.9 ± 0.2	1.0 ± 0.1	1.4 ± 0.2
Ciprofloxacin	1.8 ± 0.1	1.7 ± 0.1	2.5 ± 0.1	1.9 ± 0.1	1.7 ± 0.2

Anesthetized mice were injected into right thigh muscle with 1×10^7 CFU of microorganisms, and 18 h thereafter, 0.2 mL saline containing ^{99m}Tc-labeled defensins or ciprofloxacin was injected intravenously (*2*). Sterile inflammatory process was induced in mice by injection with either 50 μg LPS or 1×10^8 heat-killed microorganisms 24-h prior to injection of tracer. Next, accumulation of tracer in infected and inflamed muscle was assessed by planar scintigraphy. On scintigrams, anatomically adjusted regions of interest were drawn over infected/inflamed (target) and contralateral (nontarget) thigh muscle, providing reasonable estimation of accumulation of radiolabeled tracer in infected/inflamed sites. Results are expressed as target to nontarget ratio (T/NT) (*1,2*). Values represent mean (±SEM) T/NT for ^{99m}Tc-labeled defensins 1–3 and ciprofloxacin observed between 30 min and 120 min after injection of the tracer. Results are from at least 8 mice in 3 independent experiments.

*Multi–drug-resistant *S. aureus* type 2141 is a clinical isolate (Dept. of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands).

†Fluconazole-resistant *C. albicans* Y-01 was obtained from Pfizer Inc., New York, New York.

Therefore, this comment is of no concern to our study. Moreover, the reliability of the peritoneal model for binding studies of radiolabeled compounds has been described before (*12*). Our approach using radiolabeled antimicrobial peptides for infection detection is reproducible, because the standard errors of the mean in our results were rather small (*1*,*2*) and identical results were found by an independent research group (*2*). Furthermore, the peptides specifically identified infections, as we found no target: nontarget (T–NT) ratio values >1.5 in mice having a sterile inflammations (i.e., that had been injected with LPS and heat-killed microorganisms) (Table 2) or serum and phorbol myristate acetate (data not shown). In addition, we found a positive correlation between the number of viable bacteria in infected thighs and the T–NT values for defensins (*1*), as we also observed earlier for IgG. These data imply that the efficacy of the antibiotic treatment of infections can be monitored with radiolabeled antimicrobial peptides. It should also be noted that we never observed cytotoxic effects of the amounts of radiolabeled antimicrobial peptides we used, including defensins 1–3, in our animal experiments. Nevertheless, we are still studying new peptides for infection detection and taking possible side effects into account. Of course, extensive toxicity studies have to be performed with selected peptides before they can be taken into clinical studies.

Together, the major limitations of 2 new radiopharmaceuticals—ciprofloxacin and defensins 1–3—for infection identification are discussed in this reply. Our final goal is to develop optimal radiopharmaceuticals that discriminate between infections and sterile inflammatory processes. Therefore, we thank Britton et al. for their comments, which will enable us to provide more information on the possibilities and limitations of radiolabeled ciprofloxacin and human defensins 1–3 for infection detection. We remain open for further discussion on this important issue.

REFERENCES

1. Welling MM, Nibbering PH, Paulusma-Annema A, et al. Imaging of bacterial infections with a ^{99m}Tc-labeled antimicrobial human neutrophil peptide-1. *J Nucl Med*. 1999;40:2073–2080.
2. Welling MM, Paulusma-Annema A, Balter HS, Pauwels EKJ, Nibbering PH. ^{99m}Tc-labelled antimicrobial peptides discriminate between bacterial infections and sterile inflammations. *Eur J Nucl Med*. 2000;27:292–301.
3. 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.
4. Bäumler AJ, Heffron F. Microbial resistance to macrophage effector functions: strategies for evading microbicidal mechanisms and scavenging nutrients within mononuclear phagocytes. In: Brogden KA, Minion FC, Wannemuehler MJ, Roth JA, Bolin CA, eds. *Virulence Mechanisms of Bacterial Pathogens*. 2nd ed. Washington, DC: American Society for Microbiology;1995:115–131.
5. Bryant RE, Mazza JA. Effect of the abscess environment on the antimicrobial activity of ciprofloxacin. *Am J Med*. 1989;87:23–27.
6. Sarda L, Salegh-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.
7. Riesbeck K, Forsgren A, Henriksson A, Bredberg A. Ciprofloxacin induces an immunomodulatory stress response in human T lymphocytes. *Antimicrob Agents Chemother*. 1998;42:1923–1930.
8. Dax SL. Quinolone antibacterials. In: *Antibacterial Chemotherapeutic Agents*. 1st ed. London: Blackie Academic & Professional;1997:298–345.
9. 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.
10. Shafer WM, Qu X, Waring AJ, Lehrer RI. Modulation of *N. gonorrhoea* susceptibility to vertebrate antibacterial peptides due to a member of the resistance/modulation/division efflux pump family. *Proc Natl Acad Sci USA*. 1998;95:1829–1833.
11. Hutmark D, Steiner H, Rasmuson T, Boman HG. Insect immunity: purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of *Hyalophora cecropia*. *Eur J Biochem*. 1980;106:7–16.
12. Calame W, Welling MM, Feitsma RIJ, Goedemans WT, Pauwels EKJ. Contribution of phagocytic cells and bacteria to the accumulation of technetium-99m labelled polyclonal human immunoglobulin at sites of inflammation. *Eur J Nucl Med*. 1995;22:638–644.

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