Skeletal Scintigraphy with Technetium-99m-Tetraphenyl Porphyrin Sulfonate for the Detection and Determination of Osteomyelitis in an Animal Model

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This article explores the accumulation of ^{99m}Tc-tetraphenyl porphyrin sulfonate ($TPPS_4$) at inflammatory sites, especially osteomyelitis, and compares the results with $^{111}In\ Cl_3$ and ^{111}In-WBC in an animal model. **Methods:** Osteomyelitis was induced in 12 New Zealand white rabbits by injecting staphylococcus aureus in the left tibia. Three weeks later, radiographs confirmed the disease. Two hours later, after injection of 74 MBq ^{99m}Tc-$TPPS_4$, scintiphotos of the lower extremities were acquired and repeat scintiphotos were obtained 24 hr after injection of 5.55 MBq $^{111}In\ Cl_3$. After these studies, 24- and 48-hr scintiphotos of the lower extremities were acquired after injecting 5.55 MBq ^{111}In-labeled WBC. **Results:** The left tibia averaged three times the uptake with ^{99m}Tc-$TPPS_4$ compared with right tibia; with $^{111}In\ Cl_3$ and ^{111}In WBC the ratios are two times. These three radiopharmaceuticals reveal positive images, but the image quality using ^{99m}Tc-$TPPS_4$ is better, as would be expected from the more favorable physical characteristics of ^{99m}Tc and the higher uptake. **Conclusion:** The traditional combination of three-phase bone and ^{67}Ga-citrate scintigraphy can be replaced by a single injection of ^{99m}Tc-$TPPS_4$ with imaging as early as 2 hr. Finally, the use ^{99m}Tc-$TPPS_4$ should result in a substantial reduction in radiopharmaceutical cost.

Key Words: osteomyelitis; technetium-99m-tetraphenyl porphyrin sulfonate; indium-111; white blood cells

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The most common organisms isolated from patients with osteomyelitis are methicillin-sensitive *Staphylococcus aureus* (*S. aureus*) and coagulase-negative *Staphylococci* (*1*). The accuracy of osteomyelitis diagnosis is essential for choosing a correct treatment regimen, and the diagnosis of osteomyelitis is initially made on the basis of clinical, laboratory and radiographic examinations. Under certain circumstances, such as a patient with chronic osteomyelitis or early acute hematogenous osteomyelitis, diagnosis may be problematic (*2*). Early and accurate diagnosis of acute osteomyelitis is required for prompt initiation of treatment to prevent both the progression to chronic osteomyelitis and the development of complications, which could result in serious morbidity.

For the detection and localization of osteomyelitis, a procedure should have high sensitivity and be applicable for whole-body use. Nuclear medicine imaging procedures typically have these characteristics. Further examinations by CT, MRI, biopsy and cultures often are necessary to delineate the extent and etiology of the process. The utility of three-phase ^{99m}Tc-methylene diphosphonate (MDP) scintigraphy in the initial evaluation of osteomyelitis has remained significantly high despite its lack of specificity because of its sensitivity. The addition of ^{67}Ga-citrate scintigraphy also has been helpful in defining the presence and intensity of the inflammation.

In many instances, the use of other techniques such as ^{111}In-labeled leukocytes (^{111}In-WBC) and ^{111}In-chloride (*3*), has replaced ^{67}Ga-citrate for imaging deep-seated infection (*4–6*) and inflammation (*7–9*). Indium-111-labeled leukocyte scintigraphy is accurate and specific, and the method of choice for diagnosing and localizing appendicular skeletal osteomyelitis (*10*). However, ^{111}In compounds have a comparatively long half-life, exposing the patient to a significant radiation dose. Another major disadvantage of ^{111}In leukocyte scintigraphy is that the labeling procedure, besides being time consuming, demands skilled personnel and requires an expensive facility to comply with regulatory constraints. In addition, the use of ^{111}In-labeled leukocytes for imaging has the disadvantage of a 24–48 hr delay.

In recent years, efforts have been made to find either more suitable radiopharmaceuticals for leukocyte labeling (*11,12*) or other ^{99m}Tc-labeled agents that demonstrate adequate affinity for sites of bone infection and/or inflammation (*13*). During investigations on the mechanisms of tumor localization of certain synthetic porphyrins (*14*), it was found that these porphyrins accumulated at histamine receptors, unlike ^{67}Ga-citrate and ^{111}In-chloride which localize at transferrin sites (*15*). It is well known that there are histamine receptors in the basophils, neutrophils, mast cells and eosinophils, and all these cells are involved in the acute inflammatory process. It was reasoned that porphyrins localize in areas of infection and inflammation (*14*).

This study explores the possibility that ^{99m}Tc-$TPPS_4$ would accumulate at the site of an infectious or inflammatory process, such as osteomyelitis. In addition, we compared ^{99m}Tc-$TPPS_4$ with ^{111}In-chloride and ^{111}In-WBC scintiphotos in an osteomyelitic animal model.

MATERIALS AND METHODS

Preparation and Labeling of Porphyrin

All the work reported in this paper has been performed with meso-$TPPS_4$ (Porphyrin Products, Logan, UT) and labeled with ^{99m}Tc using stannous reduction techniques widely used for radiolabeling of ^{99m}Tc radiopharmaceuticals (*16*).

Technetium-99m-$TPPS_4$ was prepared by the following method: in a vial containing 1 mg $TPPS_4$, 20 mg para-amino-benzoic acid per 1 ml and 1 mg $SnCl_2.2H_2O$/0.1 ml HCl, 5 ml ^{99m}Tc-sodium pertechnetate in citrate-saline buffer (5.9 mg sodium chloride and 11.4 mg sodium citrate/ml) containing 30–40 mCi were added.

Labeling efficiency was determined by ITLC using aluminum-

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TABLE 1
Criteria for Visually Grading the Severity of *Staphylococcus aureus* Osteomyelitis in Rabbits

Score	Gross pathology	Radiographic findings
0	Normal	Normal
1+	No bone involvement; soft-tissue swelling at proximal tibial metaphysis	Elevation or disruption of periosteum, soft-tissue swelling, or both
2+	Soft-tissue abscess; <10% widening of proximal tibial metaphysis	<10% disruption of normal bone architecture
3+	>10% widening of proximal tibial metaphysis	10%–40% disruption of normal bone architecture
4+	Disruption or pitting of normal bone architecture	>40% disruption of normal bone architecture

backed silica gel plates and developed with acetone. The radiolabeled ^{99m}Tc-TPPS$_4$ remains at the origin (R_f = 0.0) while free ^{99m}Tc-pertechnetate migrates all the way to the solvent front (R_f = 1.0). The labeling efficiency was ≥ 98%.

Indium-111-Chloride and Indium-111-WBC

All work reported in this article was performed with ^{111}In-Cl$_3$ (Amersham-Mediphysics, Arlington Heights, IL). Indium-111-chloride was used in the detection of osteomyelitis (*17*). Indium-111-WBC was prepared using the widely used technique to radiolabel WBC with ^{111}In (*18*).

Organism and Osteomyelitis Production

S. aureus obtained from a patient with osteomyelitis was lyophilized at −70°C in trypticase soy broth, reconstituted with cation-supplemented Mueller Hinton broth and used as needed. A colony of this strain was grown overnight in tryptic soy broth, counted through a Petroff-Hausser Counting chamber and diluted in 0.85% saline to a concentration of 10^7 colony-forming units (CFU) per milliliter.

New Zealand white rabbits (Ray Nicholl's Rabbitry, Lumberton, TX), 8–12 wk and weighing 2.0–3.5 kg, were used for the study. Rabbits were anesthetized using an intramuscular injection of 30 mg/kg Ketaset (Fort Dodge Laboratories, Inc., Fort Dodge, IA), 10 mg/kg Acepromazine (Fort Dodge Laboratories, Inc., Fort Dodge, IA), and 1 mg/kg Xylazine (Rugby Laboratories, Inc., Rockville Center, NY). An 18-gauge needle was then inserted percutaneously through the lateral aspect of the left tibial metaphysis into the intramedullary cavity. One-tenth milliliter 5% sodium morrhuate (Eli Lilly, Indianapolis, IN), 0.1 ml *S. aureus* (10^7 CFU/ml) and 0.2 ml sterile 0.85% saline were injected sequentially (*22–24*). The needle was then removed and the animal was returned to its cage.

Three weeks later, radiographs of both tibias were taken, and severity of the infection by radiographic appearance was graded by a system previously reported (Table 1) (*1*). Only rabbits that developed Grade IV osteomyelitis after 2 wk were included in the study. The rabbits were monitored daily for stool character, weight loss, caloric intake, tibial tenderness and overall general health.

Three-week infected rabbits then were injected with 74 MBq ^{99m}Tc-TPPS$_4$. Serial imaging studies were performed at 30 min interval up to 3 hr. The optimum uptake was 2 hr postinjection. Whole-body scintiphotos were acquired 2 hr postinjection using the ADAC Vertex or Genesys (Milpitas, CA) dual-head gamma camera followed by planar scintiphotos of 500 K counts of the lower extremities. After imaging with ^{99m}Tc-TPPS$_4$, the rabbits were injected with 5.55 MBq ^{111}In-chloride. Twenty-four hours later, a whole- body scintigram and planar scintiphotos of the lower extremities were acquired for 20 min. Another group of 3-wk infected rabbits had blood samples drawn and the WBC were radiolabeled with ^{111}In oxine using a widely used technique (*18*). The infected rabbits were injected with 5.55 MBq ^{111}In WBC. Twenty-four and 48 hr after injection of ^{111}In leukocytes, whole-body and planar scintiphotos of the lower extremities were acquired for 20 min. Corresponding regions of interest (ROIs) of the infected and noninfected tibias were defined on the images and quantified.

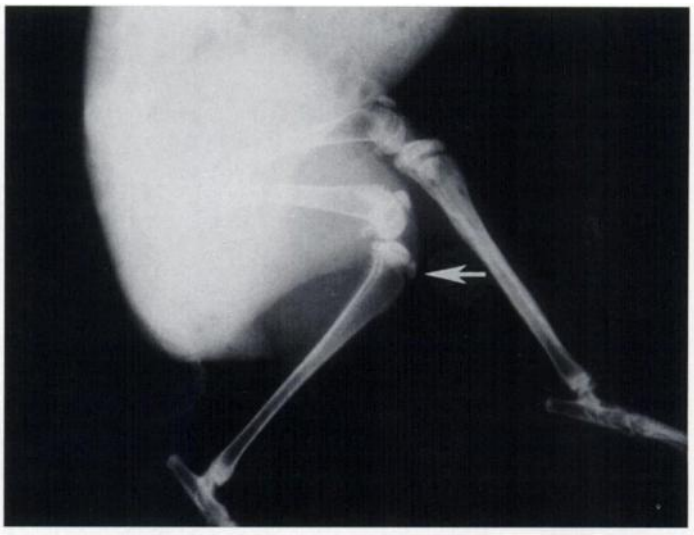

FIGURE 1. The radiograph of both tibias of a typically infected rabbit revealing normal right and cortical, and sclerotic changes, in the left consistent with osteomyelitis.

RESULTS

Twelve of 15 rabbits developed Grade IV left tibia infection as confirmed radiologically. The remaining three rabbits did not develop infection and were not imaged. Two control rabbits were imaged for determination of the normal uptake and biodistribution of the radiotracer. Figure 1 shows the radiograph of both tibias of a typically infected rabbit revealing normal right lower extremities and cortical and sclerotic changes in the left tibia consistent with osteomyelitis.

Figure 2 shows the scintiphotos of 2-hr post ^{99m}Tc-TPPS$_4$, 24-hr post ^{111}In-chloride and 24- and 48-hr post ^{111}In leukocyte injections, respectively. All show increased uptake in the left tibia reflecting the obvious inflammatory process. However, the 2-hr ^{99m}Tc-TPPS$_4$ image demonstrates a more focal uptake in the left tibia as compared to the ^{111}In-chloride and ^{111}In-WBC images. Also, quantification of the radiotracer uptake shows the left tibia has three times more uptake than background with ^{99m}Tc-TPPS$_4$ while ^{111}In-chloride and ^{111}In-WBC images have approximately two times the uptake of background (Table 2). Therefore, the target-to-background ratio is significantly higher in the ^{99m}Tc-TPPS$_4$ scintiphotos ($p < 0.05$). Although the three radiopharmaceuticals reveal positive osteomyelitic scintiphotos, the image quality using ^{99m}Tc-TPPS$_4$ appears to be better.

DISCUSSION

A new radiopharmaceutical was used for the detection of osteomyelitis by incorporating a radionuclide with highly desirable imaging characteristics (^{99m}Tc) in a nonbiological pharmaceutical (tetraphenyl sulfonate porphyrin). Our results reveal a preliminary specificity and sensitivity of 100% in the rabbit osteomyelitis model. Although all of the three radiopharmaceu-

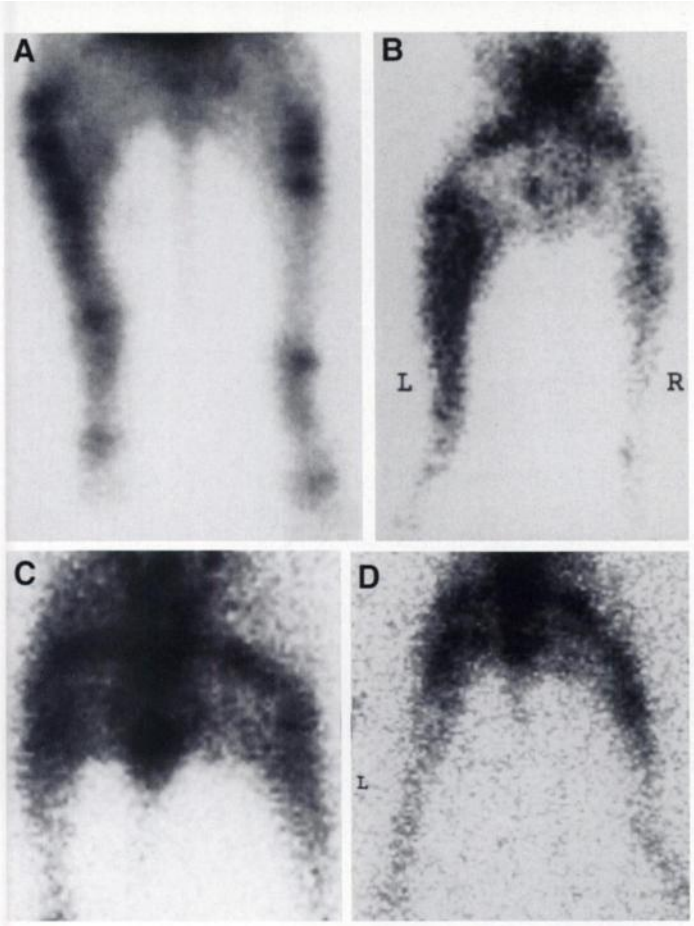

FIGURE 2. (A) Rabbits 3 wk postinfection injected with 74 MBq ^{99m}Tc-TPPS$_4$. Scintiphoto reveals focal radiotracer uptake in the left tibia compared to the right tibia. This is more focal than ^{111}In-Cl$_3$ and ^{111}In-WBC. (B) After imaging with ^{99m}Tc-TPPS$_4$, the rabbits were injected with 5.55 MBq ^{111}In-chloride. Twenty-four hours later, spot scintiphotos of the lower extremities were obtained. These reveal diffuse radiotracer uptake of two times in the left (L) tibia compared to the right (R) tibia. Another group of rabbits 3 wk postinfection were imaged at 24 hr (C) and 48 hr (D) after the intravenous injection of 5.55 MBq ^{111}In-WBC. Twenty-four hour scintiphotos reveal diffuse radiotracer uptake of two times as much in the left tibia compared to the right tibia.

ticals reveal positive osteomyelitic scintiphotos, the image quality using ^{99m}Tc-TPPS$_4$ appeared to be better. The improved visual characteristics of the ^{99m}Tc image would be expected from the more favorable physical and radioactive properties of the ^{99m}Tc and the higher uptake, potentially allowing earlier identification and more precise localization of the osteomyelitis.

Although the ^{99m}Tc-containing compounds presently used are very sensitive in the detection of osteomyelitis, false-positive readings have resulted from cases of new bone formation, fracture healing, heterotopic ossification, arthritis and soft-tissue infections. However, the specificity of bone scintigraphy in detecting osteomyelitis is improved with three-phase studies, especially in differentiating bone from soft-tissue infection. In addition, the three-phase scintigraphy is usually followed by ^{67}Ga-citrate imaging for confirmation of osteomyelitis, but uncertainty will remain in some cases as to whether the increased localization of ^{99m}Tc-MDP or ^{67}Ga-citrate is due to excessive new bone formation or sepsis (*19*). Gallium-67-citrate does not have good imaging characteristics and has the disadvantage of a 24–48 hr delay for localization (*20,21*).

Technetium-99m-TPPS$_4$ concentrates in areas of acute inflammation such as an osteomyelitis. Acute inflammation is characterized by hyperemia, increased vascular permeability with exudation of proteins, leukocyte migration leading to increased histamine concentrations, and upregulation of histamine receptor production (*22,23*). The accumulation of ^{99m}Tc-TPPS$_4$ at these sites of inflammation is due to the ability of the porphyrins to attach to the cellular histamine receptors (*14*).

The use of ^{99m}Tc-TPPS$_4$ eliminates some of the problems associated with these radiopharmaceuticals. It does not require in vitro manipulation of autologous blood, the radiation dose is substantially reduced, and the short half-life of ^{99m}Tc means that reimaging can be performed at intervals of 2 days. In addition, rapid localization was demonstrated when ^{99m}Tc-labeled chelates detected inflammation even before labeled granulocytes had migrated to the involved foci (*24*). The first two phases of a scan, reflecting hypermia and increased vascular permeability, can also be performed with ^{99m}Tc-TPPS$_4$.

TABLE 2
Mean Target-to-Background Ratios for All Rabbits Tested

Indium-111-Cl$_3$	Indium-111-WBC	Technetium-99m-TPPS$_4$*
1.67	1.73	3.35

*Ratio of ^{99m}Tc-TPPS$_4$ is significantly higher than other radiopharmaceuticals ($p < 0.05$).

CONCLUSION

The use of ^{99m}Tc-TPPS$_4$ should result in a substantial reduction in radiopharmaceutical and imaging costs. Therefore, the traditional combination of three-phase bone scintigraphy and ^{67}Ga-citrate imaging for the detection of osteomyelitis may be replaced by a single injection of ^{99m}Tc-TPPS$_4$ with imaging as early as 2 hr.

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Erratum

Due to a production error, Figure 2 in the article "Regional Methionine and Glucose Uptake in High-Grade Gliomas: A Comparative Study on PET-Guided Stereotactic Biopsy" by Goldman et al. (*JNM* 1997;38:1459–1462) was printed incorrectly. The figure is reprinted correctly below.

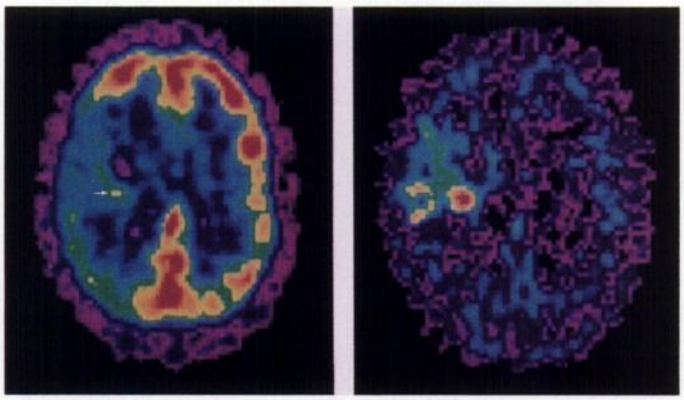

FIGURE 2. Fluorine-18-FDG (left) and ^{11}C-MET (right) PET images of an anaplastic astrocytoma in the temporal lobe. The uptake of ^{18}F-FDG is globally reduced in the tumor area, but a spot of high ^{18}F-FDG uptake is found where stereotactic histologic samples demonstrate anaplasia (arrow). The uptake of ^{11}C-MET is the most pronounced at the level of this anaplastic area, but increased uptake of this tracer is also found in nonanaplastic zones of the tumor.