^{99m}Tc-PEG Liposomes for the Scintigraphic Detection of Infection and Inflammation: Clinical Evaluation

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Polyethyleneglycol (PEG) liposomes have been shown to be excellent vehicles for scintigraphic imaging of infection and inflammation in various experimental models. In this article we report on a series of patients with possible infectious and inflammatory disease in whom the performance of ^{99m}Tc-PEG liposomes was evaluated. The results of ^{99m}Tc-PEG liposome scintigraphy were directly compared with those of ^{111}In-immunoglobulin G (IgG) scintigraphy. **Methods:** Thirty-five patients (22 men, 13 women; mean age, 51 y; range, 20–76 y), suspected of having infectious or inflammatory disease, received 740 MBq ^{99m}Tc-PEG liposomes intravenously. Imaging was performed at 4 and 24 h after injection. Patients received 75 MBq ^{111}In-IgG 24 h after administration of the liposomes. The scintigraphic results were compared and verified by culture, biopsy, surgery, and follow-up of at least 6 mo. **Results:** Of the 16 proven infections and inflammations, 15 were detected by ^{99m}Tc-PEG liposome scintigraphy: soft-tissue infection (n = 3), septic arthritis (n = 3), autoimmune polyarthritis (n = 2), infected hip prosthesis (n = 1), infected osteosynthesis (n = 1), spondylodiscitis (n = 1), infected aortic prosthesis (n = 1), colitis (n = 1), abdominal abscess (n = 1), and pneumonia (n = 1). ^{99m}Tc-PEG liposome and ^{111}In-IgG scintigraphy both missed 1 case of endocarditis. In addition, an ^{111}In-IgG scan of a patient with mild soft-tissue infection was false-negative. Concordantly false-positive scans were recorded from 2 patients, both with uninfected pseudarthrosis and focal signs of sterile inflammation. During liposomal administration, 1 patient experienced flushing and chest tightness, which rapidly disappeared after lowering the infusion rate. No other adverse events were observed. **Conclusion:** This clinical evaluation of ^{99m}Tc-PEG liposomes shows that focal infection and inflammation can be adequately imaged with this new agent. The performance of ^{99m}Tc-PEG liposomes is at least as effective as that of ^{111}In-IgG. With the simple and safe preparation and the physical and logistic advantages of a ^{99m}Tc label, ^{99m}Tc-PEG liposomes could be an attractive agent for infection or inflammation imaging.

Key Words: ^{99m}Tc; liposomes, polyethyleneglycol; ^{111}In; immunoglobulin G; imaging; infection; inflammation

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Over the last decades several radiopharmaceuticals have been developed for the detection of infection and inflammation. Some have found their way into clinical practice and are routinely used for evaluation of infectious and inflammatory diseases (*1–9*). However, each of these agents has its limitations with regard to radiation dose, biodistribution, or preparation procedure, and thus the search for new and better agents for infection imaging continues. One of the newly developed and promising radiopharmaceuticals is radiolabeled liposomes. Liposomes are artificial phospholipid vesicles with an internal aqueous compartment. Since their discovery 30 y ago (*10*), they have been extensively investigated, mainly as potential carriers of therapeutic agents. The initial enthusiasm for liposomal carriers waned in the early 1980s because of the apparent limitations for radiopharmaceutical application (*11*). Rapid removal from the blood by cells of the mononuclear phagocyte system (MPS) compromised adequate targeting of non-MPS tissues. New insights in the recognition of liposomes by the MPS has led to the development of liposomes with long-circulating characteristics. Surface modification of liposomes with hydrophilic polymers such as polyethyleneglycol (PEG) resulted in decreased recognition by the MPS, and thus blood residence time was increased (*12,13*). As shown in both experimental and clinical studies, the enhanced circulatory half-life resulted in improved targeting potential of these PEG liposomes (*14–17*). Recently, we demonstrated the excellent performance of radiolabeled PEG liposomes in various animal models of infection and inflammation such as acute bacterial infection, colitis, arthritis, and chronic osteomyelitis (*18–20*). In addition, radiolabeled liposomes can be prepared relatively easily and can be labeled stably with ^{99m}Tc. Furthermore, widespread experience with PEG-liposomal drug formulations has shown minimal or no toxic side effects (*16,21–25*).

The encouraging results of our experimental studies and the apparent safety of liposomal administration to humans prompted us to perform a clinical study to investigate the potential role of ^{99m}Tc-labeled PEG liposomes in patients

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suspected of infectious or inflammatory disease. The results of ^{99m}Tc-PEG liposome scintigraphy were directly compared with those of ^{111}In-immunoglobulin G (IgG) scintigraphy, the standard procedure for infection imaging in our nuclear medicine department.

MATERIALS AND METHODS

Radiopharmaceuticals

^{99m}Tc-PEG-Hexamethyl Propyleneamine Oxime Liposomes. Glutathione-containing PEG liposomes (hexamethyl propyleneamine [HMPAO] liposomes) were prepared as described previously (*20*). Each batch was checked for sterility and for absence of pyrogens. The mean size of the liposome preparations as determined by dynamic light scattering measurements was 80–100 nm, with a polydispersity index <0.1. Preformed glutathione-containing liposomes were labeled with ^{99m}Tc, essentially as described previously (*26*). Briefly, the liposomes (70 μmol phospholipid/mL) were incubated for 15 min at room temperature with freshly prepared ^{99m}Tc-HMPAO (30 MBq/μmol phospholipid). Labeling efficiency was 60%–85%. Removal of unencapsulated ^{99m}Tc-HMPAO was achieved by gel filtration on an extensively prerinsed PD-10 column (Pharmacia, Woerden, The Netherlands) with 5% glucose as the eluent. A dose of 35–40 μmol PEG liposomes labeled with 740 MBq ^{99m}Tc was administered intravenously.

^{111}In-IgG. HIV and HBsAg-negative, human, nonspecific, polyclonal IgG (Gammagard; Baxter/Hyland, Lessines, Belgium) was conjugated with diethylenetriaminepentaacetic acid (DTPA) as described previously (*7*). Two or three DTPA-chelates were conjugated per IgG molecule. The conjugate was radiolabeled with ^{111}In (^{111}In-chloride; Mallinckrodt, Inc., Petten, The Netherlands) by citrate transchelation. Labeling efficiency was always >95%, as determined by instant thin-layer chromatography on silica gel strips (Gelman, Ann Arbor, MI) using 0.15 mol/L sodium citrate (pH 6.0) as the mobile phase. A dose of approximately 2 mg IgG labeled with 75 MBq ^{111}In was injected intravenously.

^{111}In-Leukocytes. First, total white blood cell (WBC) count and differentiation were determined from a blood sample from each patient. Then 50 mL blood were drawn by venapuncture in a syringe containing 10 mL acid citrate dextrose (ACD). Under strict sterile conditions, 6 mL 6% hydroxyethyl starch were added to 50 mL ACD-blood. The erythrocytes were allowed to sediment during 1 h. The supernatant was taken and centrifuged for 10 min at 150*g*. The cell pellet was washed with 5 mL 1% human serum albumin (HSA) solution in phosphate-buffered saline ([PBS] pH 7.4) and centrifuged once more for 10 min at 150*g*. The cell pellet was resuspended in 1.5 mL 1% HSA solution in PBS. ^{111}In-oxine (~25 MBq; Mallinckrodt) in 0.2 mol/L tris(hydroxymethyl)aminomethane (pH 8.0) was added to the cell suspension. Incubation at room temperature continued for 30 min. After centrifuging a third time for 10 min at 150*g*, the supernatant was discarded and the cell pellet was resuspended in 5 mL 1% HSA solution in PBS. Labeling efficiency, determined by measuring cell-associated and supernatant activity in a sample of the labeled WBC suspension, was always >95%. A dose of 25 MBq ^{111}In-WBC was injected intravenously.

Patients

Thirty-five patients (22 men, 13 women; mean age, 51 y; range, 20–76 y) referred to the nuclear medicine department for imaging of infection or inflammation were studied prospectively. Informed consent was obtained from each patient. Patients younger than 18 y and pregnant or lactating female patients were excluded from the study. The study protocol was approved by the ethical review board of the University Hospital Nijmegen, The Netherlands. Twenty-two patients were suspected of having infection or inflammation of the musculoskeletal system. Five patients had bacteremia and were referred either to localize the original focus or to investigate whether there was dissemination. Three patients had fever of unknown origin; 3 patients had possible abdominal pathologies; 1 patient was suspected of having endocarditis; and 1 patient was suspected of having an infected thrombus.

All 35 patients underwent ^{99m}Tc-PEG liposome scintigraphy, and 34 also underwent ^{111}In-DTPA-IgG scintigraphy. In 1 patient, who had a possibly infected aortic prosthesis, ^{111}In-leukocytes scanning was performed as standard scintigraphic procedure. The scintigraphic results were verified by culture (n = 14), biopsy (n = 1), surgery (n = 11), radiography (n = 17), or clinical follow-up of at least 6 mo (n = 22). Follow-up implied the absence of signs or symptoms of infection or inflammation in patients, confirming negative scintigraphic findings, or a favorable response to initiation of treatment, confirming positive scintigraphic results.

Study Design

To avoid crossover of activity, ^{99m}Tc-PEG liposome scintigraphy was performed 24 h before the standard scintigraphy with ^{111}In-labeled agents (IgG or leukocytes). ^{99m}Tc-PEG liposomes were administered as a bolus injection or slowly infused over a period of 10 min (3–4 μmol/min). Vital signs were recorded up to 1 h after injection. Blood samples were taken from all patients before and after ^{99m}Tc-PEG liposome scintigraphy for hematologic and biochemical evaluation. In 7 patients blood samples were taken for measurement of the blood clearance of ^{99m}Tc-PEG liposomes. In addition, urine was collected during a 24-h period to estimate the excretion of the ^{99m}Tc label.

Imaging Protocol and Image Interpretation

Scintigraphic images were obtained with a Siemens Orbiter connected to a Scintiview image processor or a MultiSpect 2 γ camera connected to an ICON computer system (Siemens Inc., Hoffman Estates, IL). All images were collected in digital format in a 256 × 256 or a 256 × 1024 matrix. Whole-body scans of ^{99m}Tc-liposomes were recorded at 4 h (scan speed, 15 cm/min) and 24 h after injection (scan speed, 6 cm/min). Additional SPECT images were acquired if abdominal abnormalities were suspected. If indicated, spot views of ^{99m}Tc-PEG liposomes were acquired at 4 and 24 h after injection for a preset time of 5 and 15 min, respectively, using a low-energy, high-resolution, parallel-hole collimator (140-keV photopeak, 15% symmetric window).

^{111}In-labeled IgG or leukocytes were administered 24 h after the administration of ^{99m}Tc-PEG liposomes, and spot views were acquired at 4, 24, and 48 h after injection, with a preset time of 5, 7.5, and 10 min, respectively, using a medium-energy, parallel-hole collimator (15% symmetric window for both the 173- and the 247-keV energy peaks). We have previously shown that in such settings the scatter of ^{99m}Tc in the ^{111}In images at 4 h after injection is <1% (*27*).

Images were read by 2 nuclear medicine physicians who were unaware of the results of the verification procedures. Both ^{99m}Tc-PEG liposome and ^{111}In-IgG scans were considered positive for inflammation or infection if focal and, with time, increasing accumulation of tracer was observed.

Biodistribution and Dosimetry

To calculate the uptake of ^{99m}Tc-PEG liposomes in various organs, 9 patients underwent additional imaging, including whole-body images at 15 min after injection (scan speed, 15 cm/min) and SPECT images of the abdominal organs. Regions of interest (ROIs) were drawn around the whole body, lungs, liver, spleen, kidneys, and testes. Estimates of absolute activity uptake in these organs were made by calculating the geometric mean of activity in an organ ROI, corrected for radioactive decay. Organ radioactivity as percentage injected dose (%ID) was estimated by using the whole-body ROI of the first scan as 100% of the administered dose. The absorbed doses to the organs and the effective dose were calculated using the MIRD scheme.

Blood and urine samples were collected and the content of radioactivity was determined. To correct for physical decay and to calculate activity in blood and urine as a fraction of the injected dose, injection standards were counted simultaneously. Whole-blood data were analyzed by nonlinear least-squares fit using a biexponential model.

RESULTS

Thirty-five patients with 49 possible infectious and inflammatory foci were investigated. The individual clinical characteristics and the results of the scintigraphic and verification procedures are summarized in Table 1. Figure 1 shows a representative whole-body image 24 h after injection of ^{99m}Tc-PEG liposomes. This image clearly illustrates the physiologic uptake of the agent in the cardiac blood pool, larger blood vessels, liver and spleen, and, to a lesser extent, the kidneys. Uptake in lung, bone, and gastrointestinal tract was low. The prolonged circulation time of PEG liposomes is apparent from the blood clearance curve (Fig. 2) showing a $t_{1/2}\alpha$ of 0.31 ± 0.09 h and a $t_{1/2}\beta$ of 58.1 ± 17.0 h. The mean whole-body retention at 4 and 24 h after injection was 87.0 ± 4.8 %ID and 79.8 ± 10.9 %ID, respectively. The radioactivity was excreted mainly through the kidneys (19.3 ± 6.3 %ID in the urine in the first 24 h). The mean organ uptakes of labeled liposomes at various time points are shown in Table 2. After initial uptake of activity, no further accumulation or release of activity from the source organs was found. The mean absorbed doses to the organs are presented in Table 3. The mean effective dose was 7.6 ± 0.5 μSv/MBq. For a typical administered activity of 740 MBq ^{99m}Tc-PEG liposomes, the dose to the red marrow was 3.0 mGy, the dose to the testes was 9.6 mGy, and the effective dose was 5.6 mSv.

During injection of the labeled liposomes, 1 patient, with a history of hyperventilation, experienced mild flushing and tightness of the chest, which subsided when the injection was temporarily stopped. On the scintigram acquired 4 h later, increased uptake in liver and spleen and decreased activity in the heart region were noted, indicating enhanced blood clearance of the agent in this patient. None of the other patients experienced any side effects, nor were there any significant changes in hematologic or biochemical values.

Sixteen of the 35 investigated patients had proven infections or inflammations (Table 1), with a total of 28 foci. ^{99m}Tc-liposome scintigraphy correctly identified 27 of these foci in 15 patients. ^{111}In-IgG scintigraphy, performed in 15 of the 16 positive patients, visualized 25 of 27 foci in 13 patients. The sixteenth patient underwent ^{111}In-leukocytes scintigraphy as the second procedure, which correctly identified the infectious lesion. Nine patients, including 1 patient with a spondylodiscitis, had infections of the locomotor system; 2 patients had noninfectious polyarthritis; 1 had an infected aortic prosthesis; 1 had a pneumonia; 1 patient had endocarditis; 1 had colitis; and 1 patient had an abdominal abscess. Examples of abnormal ^{99m}Tc-liposome and ^{111}In-IgG scintigrams are shown in Figures 3–5.

The results of ^{99m}Tc-liposome and ^{111}In-IgG scintigraphy were discordant in 1 patient. This patient, a 40-y-old man, was referred to our department to exclude a diagnosis of osteomyelitis because of swelling and redness at the level of an old tibial fracture. Increased focal uptake was seen with the liposomes but not with ^{111}In-IgG (Fig. 3). Although no cultures were acquired in this patient, the findings on physical examination and the rapid improvement with antibiotic treatment were consistent with soft-tissue infection, and the patient was classified as true-positive for ^{99m}Tc-liposome scintigraphy and false-negative for ^{111}In-IgG. In 2 other patients, both scans were classified as true-positive, but the extent and the severity of the disease were more clearly visualized on the ^{99m}Tc-liposome scintigram (Fig. 4).

The results of ^{99m}Tc-liposome and ^{111}In-leukocyte scintigraphy in patient 16 were classified as concordantly true-positive. This 75-y-old man was referred because of persistent fever and possibly infected aortic graft. Both scans showed focal uptake of the radiolabel around the prosthesis, consistent with findings on MRI. Surgery was not performed because of severe cardiac disease. Although cultures remained negative, clinical improvement on antibiotic treatment and the absence of other apparent foci were found to be consistent with a diagnosis of an infected aortic prosthesis.

A concordantly false-negative result of ^{99m}Tc-liposomes and ^{111}In-IgG was obtained in a 43-y-old patient with a 3-wk history of fever and back pain. He was suspected of having endocarditis and was referred for infection scanning to exclude spondylodiscitis. Both scans gave negative results in accordance with radiographic findings. Subsequent blood cultures grew *S. sanguis,* and the patient was started on antibiotic treatment. Initially, echocardiography gave negative results, but a repeated study 1 mo later showed thickening of the mitral valve and regurgitation, consistent with a diagnosis of endocarditis. Although this had not been the reason for referral, and the clinician had been aware of the limited usefulness of the standard scintigraphic procedure in visualizing endocarditis, both scans were classified as false-negative.

False-positive scans were recorded in 2 patients with pseudoarthroses, both with ^{99m}Tc-liposome and ^{111}In-IgG scintigraphy. In both cases, focal signs of inflammation were noted at surgery, but cultures remained negative.

TABLE 1
Clinical Characteristics, Results of Scintigraphy, and Verification Procedures

Patient no.	Sex	Age (y)	Duration	Suspected focus	Scintigraphy		Verification	
					^{99m}Tc-liposome	^{111}In-IgG	Procedure	Result
1	M	40	2 wk	Tibia	+	−	Bo, FU	Soft-tissue infection
2	M	20	1 mo	Colon	+	+	E, B	Colitis
3	M	62	4 wk	Knee	+	+	Bo, FU	Bursitis
4	F	46	3 d	Shoulder, elbow, wrist, ankles, knee	+	+	C, FU	Autoimmune polyarthritis
5	M	76	2 wk	Treated SP bacteremia; hip	+	+	R, C, FU	SP septic arthritis
6	M	42	4 wk	Hip	+	+	S	SA septic arthritis
7	M	58	2 mo	Shoulders, hips, wrists, small hand joints	+	+	C, FU	Rheumatoid arthritis
8	M	24	1 wk	Abdomen	+	+	S	BF abscess
9	F	75	2 wk	Spine	+	+	S	SA spondylodiscitis
10	M	29	4 d	SP bacteremia; lung, shoulder	+	+	R, C, FU	SP pneumonia No arthritis
11	M	65	1 wk	SP bacteremia; hip	+	+	C, FU	SP septic arthritis
12	M	63	3 mo	Total hip prosthesis	+	+	R, FU	Bursitis
13	F	66	2 mo	Total hip prosthesis	+	+	R, C, FU	SA-infected prosthesis
14	M	21	1 mo	Tibia	+	+	S	SA-infected osteosynthesis
15	M	43	4 wk	Endocarditis; spine	−	−	R, C	SS endocarditis No spondylodiscitis
16	M	75	2 mo	Fever of unknown origin	+	+ (^{111}In-WBC)	R, FU	Infected aortic prosthesis
17	F	72	4 mo	Tibia	+	+	S	Pseudoarthrosis
18	F	28	1 y	Ankle	+	+	S	Pseudoarthrosis
19	M	44	18 mo	Girdlestone	−	−	S	No infection
20	F	58	1 y	Total knee prosthesis	−	−	R, FU	No infection
21	M	45	3 wk	Treated SH bacteremia; persisting fever	−	−	R, C, FU	No cause identified Fever subsided
22	M	55	1 y	Girdlestone	−	−	C, FU	No infection
23	F	71	18 mo	Total knee prosthesis	−	−	S	Loosening prosthesis
24	M	21	14 mo	Ankle	−	−	S	Noninfected osteosynthesis
25	M	58	1 y	Total hip prosthesis	−	−	S	Loosening prosthesis
26	F	71	18 mo	Total hip prosthesis	−	−	S	Pseudoarthrosis
27	F	54	1 y	Spine	−	−	R, C, FU	Noninfected osteosynthesis
28	F	65	2 y	Total knee prosthesis	−	−	R, FU	No infection
29	M	42	1 y	Total knee prosthesis	−	−	R, C, FU	Radicular syndrome
30	F	35	5 y	Fever of unknown origin	−	−	R, C, FU	Factitious fever
31	M	56	4 y	Fever of unknown origin	−	−	R, C, FU	No cause identified
32	M	29	1 wk	Colon	−	−	R, C, FU	Resolved diverticulitis
33	F	63	2 wk	Treated SE bacteremia; total knee prosthesis	−	−	R, FU	No infection
34	M	24	1 wk	Iliacal vessel	−	−	R, FU	No infection
35	M	75	2 mo	Crista	−	−	R, FU	No infection

Bo = bone scan; FU = clinical follow-up of at least 6 mo; E = endoscopy; B = biopsy; C = bacterial culture; SP = *Streptococcus pneumoniae;* R = radiologic procedures; S = surgery; SA = *Staphylococcus aureus;* BF = *Bacteroides fragilis;* SS = *Streptococcus sanguis;* SH = *Streptococcus hemolyticus;* SE = *Staphylococcus epidermis.*

The 17 patients with true-negative ^{99m}Tc-liposome scans included 2 patients with bacteremia. Patient 21, a 45-y-old man, had been admitted with a *S. hemolyticus* sepsis and was referred because of persisting subfebrile temperature despite adequate antibiotic treatment. The negative scintigraphic findings were classified as true-negative because cultures remained sterile, whereas the temperature normalized spontaneously. Patient 33 was a 63-y-old woman who had been treated for *S. epidermidis* bacteremia. She had temporarily complained of a painful knee and was referred to exclude bacterial infection of her knee prosthesis. The negative scintigraphic results were in accordance with the clinical follow-up and were therefore considered to be true-negative. Two patients with fever of unknown origin were also classified as true-negative. Patient 30, a 35-y-old woman, appeared to have factitious fever. In patient 31, the fever subsided, and the verification procedures remained negative.

The sensitivity and specificity of ^{99m}Tc-liposome scintigraphy in this group of patients were 94% and 89%, respectively. For ^{111}In-IgG, these figures were 87% and 89%, respectively.

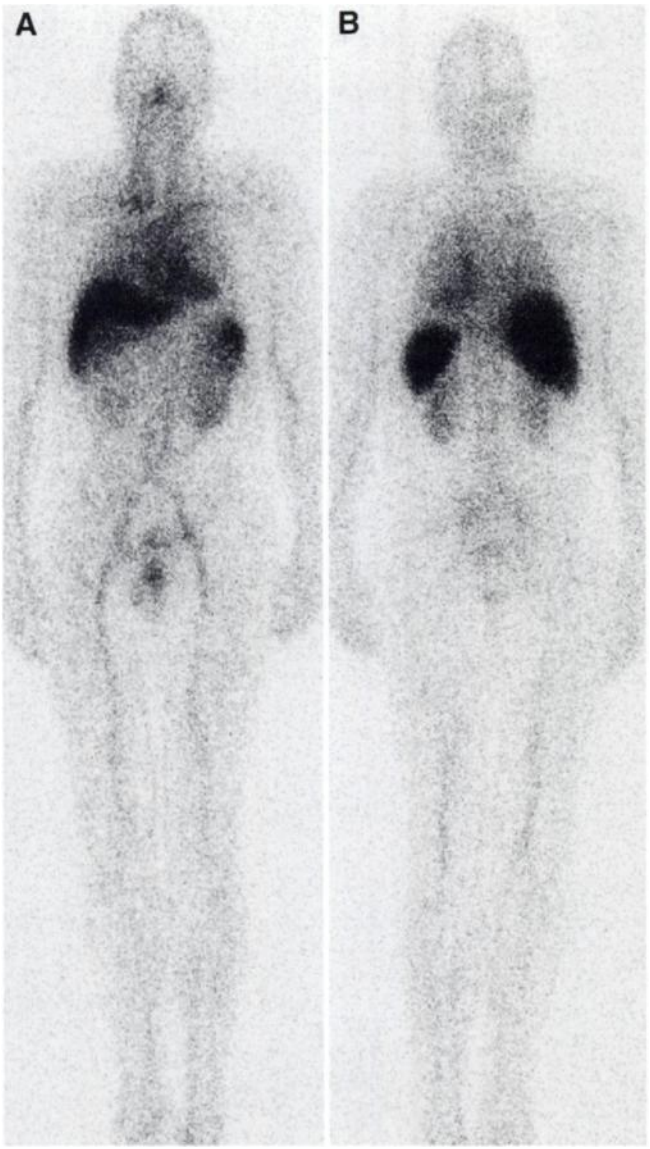

FIGURE 1. Anterior (A) and posterior (B) whole-body ^{99m}Tc-PEG liposome scintigrams show normal distribution of radiolabel 24 h after injection.

DISCUSSION

In recent years, the potential of PEG liposomes to target pathologic lesions has been exploited successfully in the field of drug targeting. As carriers of therapeutic agents, they have been shown to reduce drug-related toxicity and to increase the therapeutic efficacy of a given drug (*14–17,28*). These favorable results have inspired research into the use of radiolabeled PEG liposomes for scintigraphic imaging of infection and inflammation (*18–20*). This article reports on a clinical study with radiolabeled PEG liposomes for evaluation of patients with suspected infectious and inflammatory diseases. In this group of patients with predominantly musculoskeletal pathologies, ^{99m}Tc-PEG liposome scintigraphy showed high sensitivity and specificity. All infectious and inflammatory foci were detected, with only 1 case of endocarditis missed. The visualization of musculoskeletal and abdominal pathology was better than with ^{111}In-IgG scintigraphy. Although ^{111}In-IgG is not considered the gold standard in nuclear medicine for infection imaging, its excellent performance in delineating locomotor infections has been well established (*29*). The more accurate delineation of abdominal inflammation with radiolabeled PEG liposomes compared with radiolabeled IgG is in agreement with our previous findings in experimental colitis (*30,31*). Still, our findings are remarkable, because both liposomes and IgG are thought to accumulate in inflammatory areas according to the same nonspecific mechanism and have similar slow blood clearance (*32*). Although the more optimal physical properties of the ^{99m}Tc label could explain the observed differences, the concordance of ^{99m}Tc-hydrazino nicotinamide (HYNIC) IgG and ^{111}In-IgG in a similar clinical study (*27*) suggests an additional cause. According to Peters and Jamar (*33*), unidirectional transport of macromolecules from plasma to interstitial fluid becomes more predominant with increasing molecular size, because in these situations convective transport rather than diffusion will prevail. Thus, reverse diffusion of the smaller IgG from the interstitial fluid to the bloodstream could result in lower extravascular concentration.

The nonspecific uptake mechanism of both agents could lead to false-positive results, as illustrated in this study. Uptake of ^{111}In-IgG in pseudoarthrosis has been described previously and is attributed to noninfectious inflammation as a result of irritation from motion at the unstable sites (*29*).

The nonspecific nature of labeled liposomes—in contrast with the current trend in nuclear medicine toward specific receptor binding—is not necessarily disadvantageous. First, uptake at sites of sterile inflammation could very well answer the clinician's question, as illustrated by the patients with polyarthritis and colitis. Second, knowledge of the clinical data and experience with patterns of nonpathologic uptake will help to correctly interpret the scintigraphic results and thus increase specificity. For example, the visualization of the synovial lining in patient 7 could easily be distinguished from the diffuse, intense uptake seen in septic arthritis.

One of the major diagnostic problems in the imaging of infections is the adequate assessment of chronic osteomyelitis. Whether labeled liposomes could be of value remains to be assessed, because chronic osteomyelitis was not evaluated in this study. Still, the adequate visualization of other types of chronic infections and the promising performance of the agent in experimental chronic osteomyelitis (*19*) suggest that ^{99m}Tc-PEG liposomes could be a useful diagnostic tool.

Although the infectious and inflammatory lesions were generally positive at 4 h after injection, visualization was improved at 24 h after injection as a result of increasing focal uptake and decreasing background activity. However, physiologic uptake in liver and spleen could mask abscesses in the upper abdomen. The relatively high background activity of labeled liposomes, resulting from the prolonged

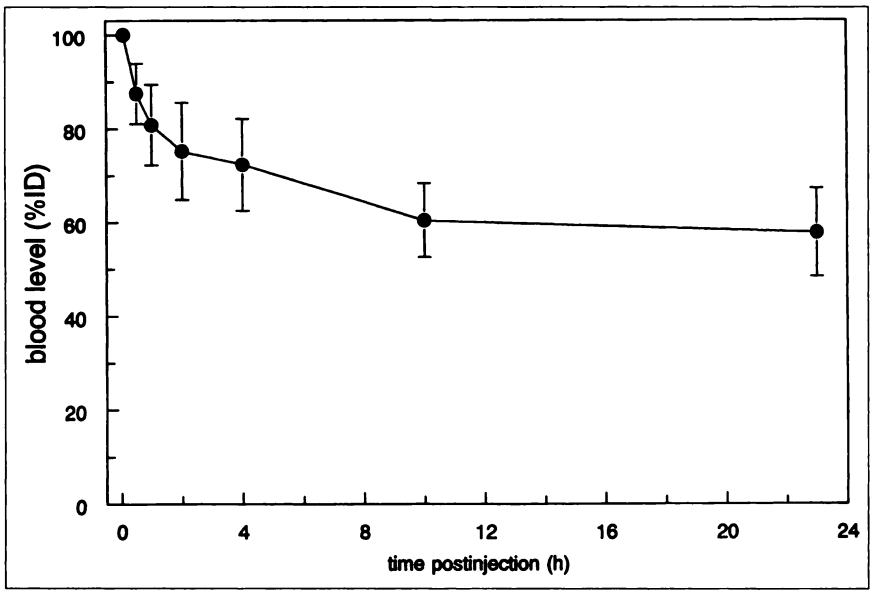

FIGURE 2. Blood clearance of ^{99m}Tc-PEG liposomes in 7 patients. Blood-pool activity measured 5 min after injection was set at 100%. Error bars represent SD.

blood residence time, might hamper adequate delineation of infections in well-perfused tissues, as was apparent in the patient with endocarditis. One of the options to decrease blood-pool activity of liposomes without compromising focal accumulation could be the application of the biotin-avidin system, as we have shown recently (*34*). Whether this approach will lead to improved visualization of infections such as endocarditis and vascular graft infections awaits additional studies.

In this study, the liposomes were labeled according to the method described by Phillips et al. (*26*). A disadvantage of this method is the relatively complex technique for preparation, requiring postlabeling purification because of the relatively low labeling efficiency (60%–85%). Recently, we described a new method to label liposomes with ^{99m}Tc-pertechnetate using HYNIC conjugated to distearoylphosphatidyl-ethanolamine (*35*). This HYNIC-based method provides ^{99m}Tc-labeled liposomes with high labeling efficiency (>95%) and improved in vitro and in vivo characteristics. Because HYNIC-derivatized PEG liposomes can be made available as a kit for instant ^{99m}Tc labeling with pertechnetate, an additional advantage is the reduction of costs, because extensive laboratory handling for postlabeling purification is no longer necessary and HMPAO kits are no longer necessary as an intermediate for ^{99m}Tc labeling of the PEG liposomes (*35*). Given the advantages of this new labeling method, further clinical studies will be conducted with ^{99m}Tc-HYNIC PEG liposomes.

The acute reaction to liposomal administration observed in 1 patient in this study has been reported in clinical trials with PEG-liposomal doxorubicin (*15,24*). However, we found its occurrence rather surprising because much lower doses were used in our protocol (~0.5 μmol phospholipid/kg body weight) compared with the dose for therapeutic purposes (~10 μmol phospholipid/kg body weight). It has been suggested that activation of complement with subsequent release of vasoactive mediators may have caused the reported side effects (*36*). In this context, enhanced opsonization of the administered liposomes is likely to occur concurrently and could thus explain the rapid blood clearance of the agent in our patient. Because the acute reaction is thought to be provoked by a fast rate of infusion in susceptible patients (*24*), we converted the administration of

TABLE 2
Organ Uptake of ^{99m}Tc-PEG Liposomes (%ID)

Site	Time after injection		
	15 min	4 h	24 h
Liver*	10.5 ± 3.6	9.8 ± 3.4	10.1 ± 3.8
Spleen*	3.4 ± 1.6	4.6 ± 3.0	4.0 ± 2.2
Kidneys*	3.0 ± 1.8	2.8 ± 1.3	3.2 ± 1.7
Testes†	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
Whole body*	100	87.0 ± 4.8	79.8 ± 10.9

*Mean ± SD for 9 patients.
†Mean ± SD for 5 male patients.

TABLE 3
Absorbed Doses in Organs (μGy/MBq)

Site	^{99m}Tc-PEG liposomes
Liver*	11 ± 3
Spleen*	29 ± 14
Kidneys*	15 ± 5
Bone marrow*	4 ± 1
Testes†	13 ± 3
Urinary bladder wall*	16 ± 4

*Mean ± SD for 9 patients.
†Mean ± SD for 5 male patients.

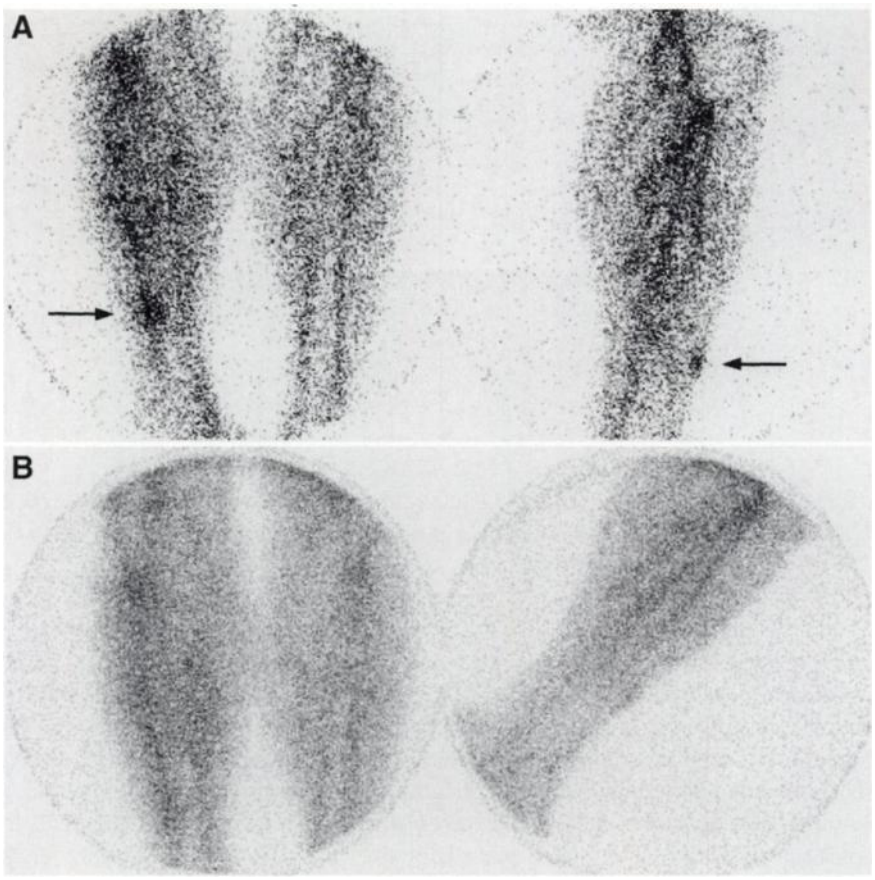

FIGURE 3. True-positive ^{99m}Tc-PEG liposome scintigram and false-negative ^{111}In-IgG scintigram in patient 1 (40-y-old man) with painful swelling and redness at level of old tibial fracture. (A) ^{99m}Tc-PEG liposome scintigram, lateral view, 24 h after injection. Increased uptake in lower leg consistent with soft-tissue infection (arrow). (B) ^{111}In-IgG scintigram, 24 h after injection. Absence of abnormal accumulation in lower leg.

the radiopharmaceutical from a bolus injection to a slow infusion. Furthermore, the new HYNIC-labeling method allows the preparation of liposomes with a higher specific activity and thus the administration of a lower lipid dose.

Comparing the biodistribution data for ^{99m}Tc-PEG liposomes with those reported for ^{111}In-IgG (*37*), the uptakes of activity in the liver and kidney were relatively low (9.9% versus 19.2% and 3.2% versus 7.2%, respectively). The relatively low liver uptake illustrates the MPS-evading properties of the PEG liposomes. The uptake in the spleen was somewhat higher, and the testicular uptake was similar to that of ^{111}In-IgG (*37*). The effective dose for ^{99m}Tc-PEG liposomes was 7.6 μSv/MBq. This is in the same range as that of other ^{99m}Tc-labeled agents used for infection imaging, such as ^{99m}Tc-labeled WBCs (11 μSv/MBq) and ^{99m}Tc-labeled diphosphonate (6.0 μSv/MBq) (*38*). Therefore, from a radiation safety point of view, this radiopharmaceutical can be administered safely.

CONCLUSION

This clinical evaluation of ^{99m}Tc-PEG liposomes indicates that this new agent offers a safe and effective scintigraphic

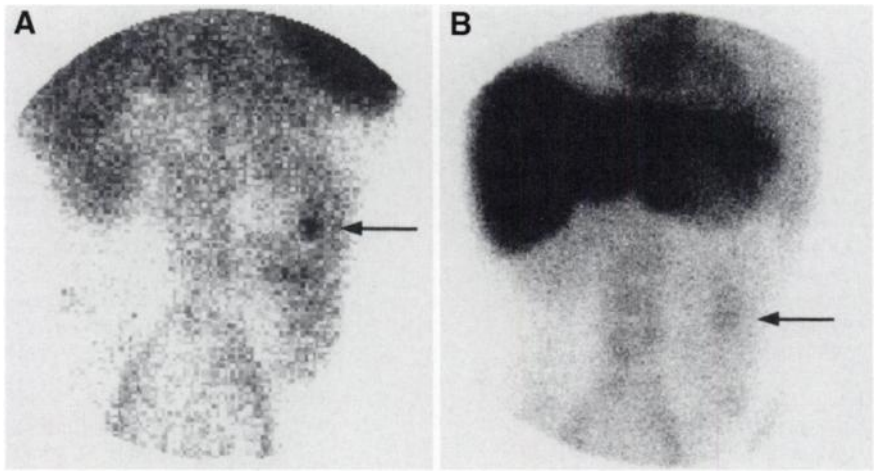

FIGURE 4. Abdominal planar views, 24 h after injection of ^{99m}Tc-PEG liposomes (A) and ^{111}In-IgG (B) in patient 2 (20-y-old man) with Crohn's disease. Although both scans show diffuse accumulation in distal colon, liposomes scintigram more accurately depicts extent and severity of colitis. Arrow points to hot spot midway in colon, consistent with focal ulceration as confirmed by endoscopic and histologic examination.

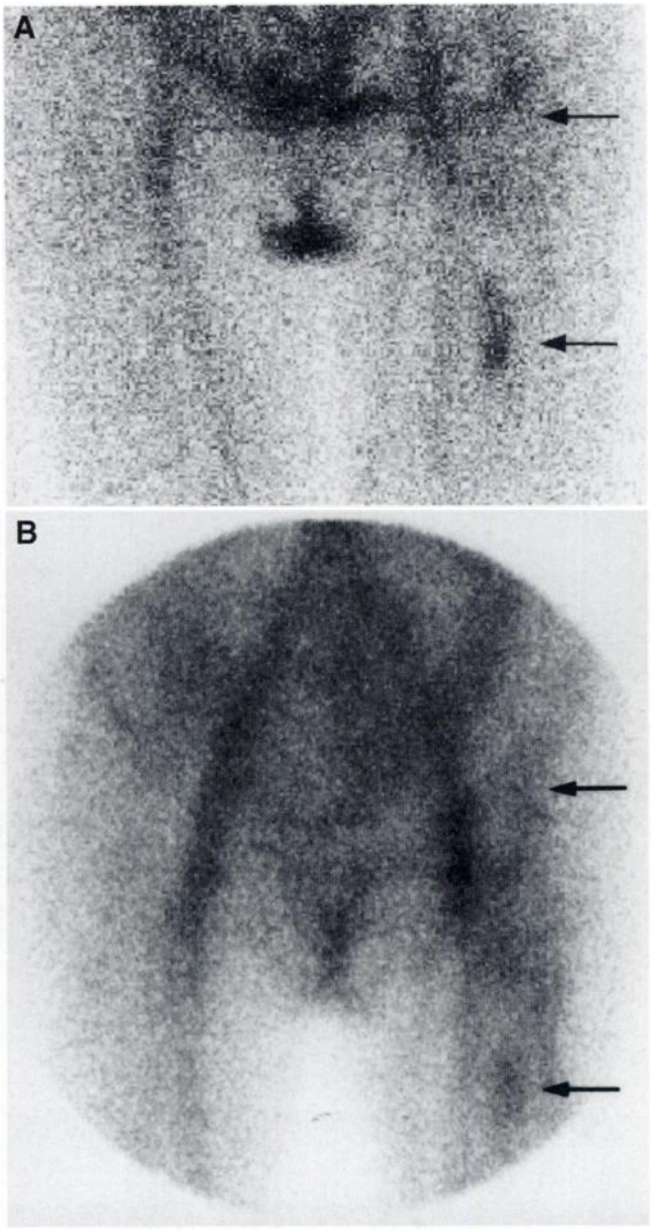

FIGURE 5. Concordantly positive ^{99m}Tc-PEG liposome scintigram (A) and ^{111}In-IgG scintigram (B) in patient 13 (66-y-old woman) with fever, elevated erythrocyte sedimentation rate, and painful left hip prosthesis. Both scans show increased uptake around prosthesis and femoral shaft (arrows). Cultures revealed growth of *S. aureus*. Patient responded favorably to initiation of antibiotic treatment.

method to target focal infection and inflammation. Its performance is at least as good as that of ^{111}In-IgG scintigraphy. The sensitivity and specificity of ^{99m}Tc-PEG liposomes for imaging infection or inflammation (or both) appeared to be in the range needed for routine clinical practice. The agent is easily prepared and has the physical and logistic advantages of a ^{99m}Tc label. These studies show that ^{99m}Tc-PEG liposomes is a promising radiopharmaceutical for the scintigraphic evaluation of infection and inflammation.

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