Imaging Experimental Osteomyelitis Using Radiolabeled Liposomes

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We evaluated radiolabeled liposomes (liposomes labeled both with 99mTc and 111In) for the early detection of osteomyelitis in an experimental model. Methods: Liposomes, containing 5% polyethylene glycol-distearoyl phosphatidylethanolamine with encapsulated glutathione and deferoxamine, were prepared and labeled with 99mTc and 111In by a previously described method. Acute osteomyelitis was induced in male New Zealand rabbits by intramedullary injection of sodium-morphuate and Staphylococcus aureus in the tibial bone marrow. Serial imaging studies, consisting of radiolabeled liposome imaging (2-4 mCi 99mTc and 75-125 μCi 111In), 99mTc-methylene diphosphonate (MDP) (3-5 mCi) and 67Ga-citrate (500 μCi), were performed starting at the third day after injection. Each radionuclide study was separated by at least 2 days. The animals also underwent radiography of the lower extremities. The animals were then killed and the infected tibia was excised for histopathology. Results: For interpreting relative efficacy of individual radiopharmaceuticals, only animals showing positive histopathological findings (n = 9) were considered. Radiographs (Days 12, 13) were conclusive for osteomyelitis in only 3 rabbits. Radiolabeled liposome imaging (Days 4-6) showed positivity in 8 cases and was equivocal in 1. Though the lesion could be delineated as early as 8 hr postinjection in the 99mTc window, the best target-to-nontarget ratio (T/NT) of 1.86 ± 0.19 was obtained at 48 hr in the 111In window. Three-phase 99mTc-MDP scan (Day 7) was positive in only 5 rabbits with 3 hr T/NT of 1.6 ± 0.23. Gallium-67-citrate images (Days 9-11) were positive in 8 cases and equivocal in 1, the mean 48 hr T/NT being 1.74 ± 0.24. These results show liposomes are better than 99mTc-MDP for imaging bone infection. Given the early localization and better quality of the images, radiolabeled liposomes also exhibited advantages over 67Ga-citrate for detection of acute osteomyelitis.

Key Words: osteomyelitis; liposomes; technetium-99m; infection imaging


Though the etiological and pathological basis of acute osteomyelitis is well understood (1), this disease still remains difficult to diagnose and manage (2-7). It is generally believed that the initiation of early antibiotic treatment for osteomyelitis may prevent irreversible changes and progression to chronic disease (5,8). An early and accurate diagnosis is necessary, however, for prompt therapy (9). Three-phase bone imaging using 99mTc-labeled phosphates is recognized as a useful tool for detecting osteomyelitic bone, especially when combined with a 67Ga-citrate scan (7,10). When the osteomyelitic zone overlaps regions of growing bone, a false-negative bone scan may result (11). Also, bone scans become less specific once the bone is violated by an insult, other than infection, that causes increased bone turnover (12). Indium-111-labeled white blood cells are sensitive and specific for osteomyelitis, but they are fraught with several technical disadvantages requiring a tedious labeling process, blood handling and special facilities (13,14). Plain radiographs, on the other hand, are normal during the first few weeks after the onset of infection, and they reflect bone destruction and new bone formation (15,16). Bone biopsy, which is one of the most specific methods, is an invasive procedure and may itself cause osteomyelitis by introducing an infection from surrounding infected soft tissue into the previously sterile bone (17).

Acute hematogenous osteomyelitis initially may behave as a cellulitis of the bone marrow accompanied by ischemia, vasospasm and thrombosis within the marrow space (15). A radiopharmaceutical that is less dependent on bone blood flow and that reflects the presence of bacteria, polymorphonuclear cells and bone marrow inflammation may be effective in the diagnosis of osteomyelitis when the bone scan pattern is not confirmed definitively (10,15). Radiolabeled colloidal particles, which have a tendency to accumulate in bone marrow (18), have shown promise for diagnosing osteomyelitis. In a clinical study, 99mTc-human serum albumin-nanocolloids were found to have the identical specificity and more sensitivity than 111In-oxine white cells for skeletal septic processes (14), while another group reported the same sensitivity, specificity and accuracy for the two methods (13).

Liposomes, spontaneously forming lipid vesicles, also have features similar to nano-colloids, which makes them potential imaging agents for osteomyelitis. Earlier, Morgan et al. (19), demonstrated the accumulation of 99mTc-liposomes in deep-seated infections of prosthetic joints. Their studies used liposomes that were rapidly cleared by organs of the reticuloendothelial system. Studies by Goins et al. (20-23), using a newly developed stable 99mTc radiolabeling technique, have renewed interest in infection imaging by radiolabeled liposomes. We have developed a liposome formulation that can be labeled with either 111In, 99mTc or both simultaneously (24). This formulation allows for flexibility in isotope selection, without increasing the lipid dose, depending on the nature of the inflammatory process. In certain clinical settings, it may be advantageous to use liposomes labeled with only 99mTc, while in other situations the use of either 111In alone or both 99mTc and 111In simultaneously may be preferable. One potential situation where both isotopes could be used simultaneously is osteomyelitis since this disease progresses in its own unique way, slower than the more common soft tissue infections.

In our study, we explored the potential of radiolabeled liposomes for imaging experimental osteomyelitis in a rabbit model. We also have compared these radiolabeled liposomes with other standard diagnostic imaging procedures for osteomyelitis.

MATERIALS AND METHODS

The phospholipids, distearoyl phosphatidylcholine (DSPC) and distearoyl phosphatidylethanolamine-polyethylene glycol-5000 (DSPE-PEG) were obtained from Avanti Polar Lipids (Pelham,
Cholesterol was purchased from Calbiochem (La Jolla, CA) and alpha-tocopherol was purchased from Aldrich (Waukegan, IL). Glutathione and deferoxamine were purchased from Sigma (St. Louis, MO). The radiopharmaceuticals, $^{99mTc}$-sodium pertechnetate and $^{111}$In-oxine, were obtained commercially (Medi-Physics Amersham, San Antonio, TX).

### Preparation of Liposomes

Liposomes containing 5% PEG-DSPE of total phospholipid concentration (DSPC:PEG-DSPE:α-tocopherol:cholesterol = 90:80:4:5.3:9 molar ratio) were prepared by the method described earlier with slight modification (25). Briefly, the lipid solution in chloroform was passed through 0.22 μm membrane and evaporated to a dry film in a rotary film evaporator (Brinkmann Instruments, Westbury, NY). After further exposing the lipid film to vacuum for 4–6 hr, the dried lipid film was hydrated with a solution of sucrose (300 mM) in sterile water for injection. The suspension was lyophilized overnight and the dried mixture was again hydrated with a solution of glutathione and deferoxamine (100 mM and 25 mM, respectively) in Dulbecco’s phosphate buffered saline (PBS, pH 6.3) containing 300 mM sucrose. The lipid suspension was extruded sequentially through membranes of 2 μm (8 times), 0.4 μm (8 times) and 0.1 μm (5 times) pore size in an extruder (Lipex Biomembranes, Inc., Vancouver, Canada). The resultant liposomes were centrifuged in a Beckman ultracentrifuge at 45,000 rpm for 45 min to obtain a pellet. Supernatant liquid, containing extravesicular ligands, was discarded. The liposome pellet was washed two times with PBS (pH 6.3). Finally, the liposomes were resuspended in 300 mM sucrose in PBS (pH 6.3) and stored at 4°C till further use. The diameter of the liposomes was determined to be 116 nm with a polydispersity index of 0.02 (Coulter N4-MD particle size analyzer, Hileah, FL). The phospholipid concentration, as determined by the method of Stewart (26), was 39 μmol/ml. The glutathione concentration inside the liposomes was 0.975 μmol/ml and was estimated by using a kit (Bioytech, Cedex, France) and manufacturer’s instructions. The entrapped deferoxamine was extracted from the liposomes and estimated colorimetrically (27) to be 0.308 μmol/ml of liposome suspension.

### Radiolabeling of Liposomes

Liposomes (4 ml) were mixed with 2 ml $^{99mTc}$-HMPAO prepared by reconstituting the HMPAO kit (Ceretec, Amersham International, Amersham, UK) with 25–30 mCi sodium pertechnetate in 5 ml normal saline. Reconstituted kits were checked for contamination using a three-step, paper chromatography system outlined in the package insert. In all cases, the kits used for labeling liposomes contained > 80% lipophilic $^{99mTc}$-HMPAO. After 30 min of incubation at room temperature, the liposomes were mixed further with 1 ml $^{111}$In-oxine (300–400 μCi). After an additional 30 min incubation at room temperature, the liposomes were passed through PD-10 columns (Pharmacia Biotech, Uppsala, Sweden) to separate any radioactivity not associated with the liposomes.

### Animal Model

Experimental osteomyelitis was induced in New Zealand white rabbits (2–3 kg) by the method of Norden (29). The rabbits were anesthetized by intramuscular injection of ketamine-xylazin mixture (50 mg/kg and 10 mg/kg, respectively). On a shaven and disinfected hind leg, an 18-gauge hypodermic needle was inserted percutaneously through the lateral metaphysis of the tibia into the medullary cavity. Through the needle, 0.1 ml of 5% sodium mormhatue (American Regent Lab, Inc., Shirley, NY) was injected followed 5 min later by 0.1 ml of bacterial suspension in saline. To ensure complete injection, 0.1 ml saline also was flushed through the needle. The needle was removed carefully, the site of injection disinfected again and the rabbits were returned to their cages.

### Organism

Staphylococcus aureus (ATCC 19095) was grown on trypticase soy broth. The concentration of the bacterial cells was estimated by comparison with McFarland® tubes prepared in house and adjusted to $9 \times 10^8$ cells per ml saline (29).

### Scintigraphic Studies

Since the degree of infection and inflammation varied from one rabbit to another, serial scintigraphic studies were performed on all the rabbits instead of randomizing them into separate groups. The animals were subjected to scintigraphic studies consisting of radiolabeled liposomes, $^{99mTc}$-MDP and $^{67}$Ga-colate. All radionuclide studies were prepared by at least 2 days and were done on rabbits under ketamine-xylazin anesthesia. Liposome imaging always was performed first on Day 4 after infection induction; it was followed by $^{99mTc}$-MDP study on Day 7 and $^{67}$Ga-colate injection on Day 9. This order was chosen by considering the respective decay half-lives of the radionuclides, which precluded using $^{67}$Ga first. Anterior images of the lower extremities were acquired in 128 × 128 matrix under Picker Gamma camera interfaced to a Pinnacle computer workstation (Medasys, Ann Arbor, MI). In a few cases, lateral images were also acquired. The collimator was changed from a low-energy, all-purpose (LEAP) to a medium-energy collimator according to the isotope being imaged.

### Radiolabeled Liposome Scanning

Liposomes labeled with 2–4 mCi $^{99mTc}$ and 75–125 μCi $^{111}$In were injected intravenously through the marginal ear vein. Images were obtained at 4–6 hr (under LEAP collimator) and 24 hr and 48 hr (under medium-energy collimator) postinjection. To assess the relative contribution of $^{111}$In in $^{99mTc}$ window, phantom studies were done. Total number of counts of both 3 mCi $^{99mTc}$ and 100 μCi $^{111}$In together were obtained in 140 keV (± 20%) and 173 keV (± 20%) windows under the gamma camera. The counting was repeated with the same amount of activity of each isotope taken individually. The magnitude of mutual contribution was estimated by the ratio (A-B)/A × 100 where:

- A = Counts by two radionuclides together in one window under one collimator and;
- B = Actual counts by one radionuclide in the same window under the same collimator.

At an initial ratio of 3 mCi to 100 μCi ($^{99mTc}$/111In), the contribution of $^{111}$In in total counts acquired by the camera peaked at 140 keV (± 20%); it was 7.3% after 6 hr. Contribution of $^{99mTc}$ in $^{111}$In images was negligible under MEC collimator at 173 keV (± 20%).

### Technetium-99m-MDP and Gallium-67 Imaging

Approximately 3–5 mCi of $^{99mTc}$-MDP was injected intravenously through the marginal ear vein while 3-sec flow images were obtained. A 1-min blood-pool image was acquired at 5–6 min postinjection followed 3 hr later by a delayed 3–5 min acquisition. Approximately 500 μCi $^{67}$Ga-colate was injected intravenously and images were acquired at 24 hr and 48 hr postinjection.

### Radiograph

A radiograph (ELEMA-SCHÖNANDER, Stockholm, Sweden) of the lower extremities of the rabbits was the last study performed on Days 12–13. The rabbits were under ketamine-xylazin anesthesia during the procedure. The radiographs were interpreted by an independent bone radiologist who was kept blind about the study.
Table 1

<table>
<thead>
<tr>
<th></th>
<th>Radiolabeled liposomes (Day 4)</th>
<th>$^{99m}$Tc-MDP (Day 7)</th>
<th>$^{67}$Ga-citrate (Day 9)</th>
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<tbody>
<tr>
<td></td>
<td>6 hr</td>
<td>48 hr</td>
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<tr>
<td>Qualitative</td>
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<td>Positive</td>
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<td>Negative</td>
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<td>Total</td>
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<tr>
<td>T/NT ratio</td>
<td>1.36 (0.16)</td>
<td>1.86 (0.19)</td>
<td>1.60 (0.23)</td>
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T/NT = target-to-nontarget.

Animal Kill

After the last study, the rabbits were killed and their tibia and femur were isolated. The soft tissue was cleared carefully off the bone. The tibia was cut distally, leaving approximately 5 cm of the bone. Marrow was aspirated aseptically by inserting an 18-gauge needle through the cut end of the bone and suctioning by syringe. Before marrow isolation, the bones were cleaned thoroughly with an alcohol swab. The isolated marrow was inoculated in 5 ml trypticase soy broth in a sterile tube and cultured on shaker water bath set at 37°C. The cultures were observed for 5 days and sent for identification of the microorganism. After marrow isolation, the bones were fixed in 10% neutral formalin for at least 1 wk before decalcification and further processing. Longitudinal sections, two at the metaphyseal region and one at the diaphyseal region, were processed for histopathology.

Scan Interpretation and Final Diagnosis

All scans were analyzed quantitatively as well as qualitatively. Regions of interest (ROIs) were drawn outlining the accumulation of radioactivity in the infected tibia. A scan was interpreted as positive if focal hyperactivity was detected in this ROI compared to the identical ROI of same pixel size on the contralateral tibia. For the qualitative analysis, all of the nuclear scans were interpreted by six nuclear medicine physicians who were kept blind about the procedures and interventions. The images were rated as either positive for osteomyelitis, negative for osteomyelitis or equivocal when they could not be interpreted definitively. Final diagnosis was made on the basis of the interpretation of histopathological findings of the tibia isolated surgically. Signatures of past infections, such as lymphocytic infiltration and fibrosis, were taken as indications of positive infections.

RESULTS

The rabbits survived the course of our investigation without any apparent distress. To relieve pain, they were given nalbuphane 1 mg/kg by subcutaneous injection. The only undesired effect we observed was infection in the soft tissue adjacent to the injection site in 3 rabbits. Of the 12 rabbits used in the main study, only 10 underwent a complete set of investigations including radiolabeled liposome imaging, $^{99m}$Tc-MDP scanning, $^{67}$Ga-citrate scanning, radiography, bone marrow culture and histopathology. The remaining 2 rabbits were subjected only to liposome imaging and $^{99m}$Tc-MDP scan. These 2 rabbits showed positive accumulation of radiolabeled liposomes in the infected tibia, whereas the bone scan turned out to be negative. All the results presented in the next section refer to the 10 rabbits who underwent a complete set of investigations. A comprehensive view of the results is in Table 1.

Nine of the 10 rabbits were found to have histopathological signs of active (n = 8) or resolving (n = 1) bone infection. While the resolved infection was evident by the presence of lymphocytes, few neutrophils and fibrosis, the active bone infection was characterized by new bone formation, periosseous elevation, cortical thinning, supuration and the presence of granulocytic infiltrate. A representative light-micrograph of an active infection is shown in Figure 1. Among these, 3 rabbits demonstrated infection extension in the distal tibia, whereas the rest of the rabbits had infection localized in the metaphyseal tibia. The microbial culture of the bone marrow aspirates gave vague results. Since the marrow was drawn from the distal end of the tibia, it gave culture-positivity for coagulase positive Staphylococcus aureus in only 4 cases. It was thought prudent not to aspirate all bone marrow from the metaphyseal region, as it might have violated the histopathological observations. One culture showed the presence of Gram negative rods, while another showed growth of coagulase negative Staphylococcus aureus. All histopathologically-positive rabbits (n = 9) were selected for the calculation of mean target-to-nontarget (T/NT) ratio and the comparison of one technique with the other. Figure 2 shows cumulative data from the radionuclide studies in these 9 rabbits.

A representative radiolabeled liposome scan is shown in Figure 3. Radiolabeled liposome imaging was positive in 8 rabbits and equivocal in 1 rabbit by qualitative interpretations. The rabbit with an equivocal radiolabeled liposomes image was positive in $^{67}$Ga-citrate scan. The reason for this was the high accumulation of radioactivity in the infected soft tissue, which made it difficult to visualize actual tibial accumulation. In a histopathologically-negative rabbit, all the investigations gave negative findings. In rabbits with adjacent soft tissue infection

FIGURE 1. Light microscopic view (450 X) of a tibia infected with Staphylococcus aureus showing typical features of acute osteomyelitis. Very large number of neutrophils with abscess formation in intramedullary space. Necrotic bone can also be seen. Lesion is characterized by fibroplasia in an attempt to circumcise the infection.

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or inflammation, liposome uptake was higher in the infected soft tissue, but it did not overlap the tibial infection site. The soft tissue involvement was toward the femur, which may be because of upward stretching of the soft tissue mass in the imaging position. This interpretation was confirmed by one or more lateral views of the lower extremities.

Though the radiolabeled liposomes yielded the best T/NT ratios at 48 hr, in 3 rabbits the infected tibia accumulated enough radioactivity in the 4–6 hr image to delineate it from the normal tibia. The other animals became positive on 24-hr scans. The average T/NT ratio at 4–6 hr postinjection was 1.36 ± 0.16, which increased to 1.61 ± 0.18 at 24 hr postinjection and 1.86 ± 0.19 at 48 hr postinjection. Considering the uniqueness of the site and the pathophysiology of osteomyelitic infection, T/NT ratios of such a magnitude may be regarded as good. The liposomes also have a tendency to accumulate in bone marrow, therefore, a considerable amount of activity was found in normal bone harboring red marrow. The utility of using two radionuclides within the liposomes was evident from the observation that 5 rabbits who were either equivocal or negative for bone infection at 4–6 hr yielded positive scans at 24 and 48 hr. Figure 4 shows one such rabbit. The other 3 rabbits were imaged positive even at 4–6 hr and the contrast increased with time.

The 99mTc-MDP bone scans were positive in only 5 of the 9 rabbits when imaged on Day 7 after infection induction. Among the remaining 4 rabbits, 1 was equivocal and the other 3 were negative. Quantitatively, the mean T/NT ratios at 5 min and 3 hr postinjection were 1.32 ± 0.15 and 1.6 ± 0.23, respectively; however, we found that for a 99mTc-MDP image to be qualitatively positive, the T/NT ratio must at least 1.4. Below this ratio, the abnormal accumulation in the infected tibia could not be discerned from that in the normal tibia by visualization of the scan. In fact, the site of infection in this rabbit model overlaps with the metabolically active regions of bone, which makes it difficult to differentiate the normal bone from abnormal bone unless major changes in osteoblastic activity have taken place. In the perfusion phase of the three-phase bone scan, there was a very subtle flow increase in the infected bone of all rabbits, but in the subsequent 3-hr images only 5 of the 9 rabbits showed an increased accumulation of tracer. In 2 rabbits, the 3-hr T/NT ratios were of the order of 2.66 and 2.07. Histopathology of these rabbits revealed severe bone infection extending into the diaphysis of the tibia. Figure 5 shows images of a rabbit that were positive for bone infection by all investigations including culture.

The third nuclear medicine modality tested for detection of osteomyelitis was 67Ga-citrate imaging. The average T/NT ratios in the 24-hr and 48-hr images were 1.62 ± 0.19 and 1.74 ± 0.24, respectively. The difference between these ratios, as a function of time, was not statistically significant; however, a decrease in background activity made lesion visualization better at 48 hr than at 24 hr. The scans gave a positive impression in 8 of the 9 rabbits, whereas in 1 rabbit the findings were equivocal (Fig. 6). The 99mTc-MDP scan of the equivocal rabbit was negative, but a radiolabeled liposome scan depicted a positive accumulation in infected tibia of this rabbit. Histopathologically, the tibia had signs of early infection. In the rest of the cases, 67Ga-citrate accumulated in concordance with radiolabeled liposomes, the maximum T/NT ratio for 67Ga-citrate being 2.74.

Radiography, which is known to be the least sensitive among the routine diagnostic procedures for osteomyelitis, was performed last. Of the 9 histopathologically-positive rabbits, only 3 showed positive signs of osteomyelitis in the infected tibia. Periosteal elevation and new bone formation were the marked

![FIGURE 2. Dot diagram showing comparative efficacy of radiolabeled liposomes (RL), 99mTc-MDP and 67Ga citrate as factors of T/NT ratio.](image1)

![FIGURE 3. Radiolabeled liposome images of rabbit with right tibial infection. Increased accumulation of radioactivity is seen in right tibia at all time points.](image2)

![FIGURE 4. Radiolabeled liposome scan of rabbit with right tibial infection that was found negative in an early (6-hr) 99mTc image, but became positive in delayed 111In images at 24 and 48 hr.](image3)
observations. Abnormal lucency in the metaphyseal region also was observed in a few cases. Obviously, the radiolabeled liposomes were more sensitive than the radiography.

**DISCUSSION**

Recent breakthroughs in liposome technology and improved radiolabeling methods have led to the successful use of radiolabeled liposomes in imaging infectious sites in rats (20–22). Our study demonstrated that liposomes also are very useful for imaging osteomyelitis. During the early imaging phase (6–24 hr), the 99mTc label provides useful information with greatly increased photon flux, while later imaging (beyond 24 hr) with 111In fills in critical details. Of the 8 unequivocally positive radiolabeled liposomes ‘imaging cases, during the early imaging period from 4–6 hr, 3 rabbits were found to be positive, 2 were equivocal, while 3 yielded negative scans. By 24–48 hr, the scans became clearer leading to definite diagnosis in these 8 rabbits.

Radiolabeled liposomes performed well in our study with 8 positive cases and 1 equivocal case where the bone infection was overwhelmed by the tremendous accumulation in the adjacent soft-tissue infection. The exact mechanism of liposome accumulation in inflammatory sites is not known. The liposomes probably extravasate through the leaky capillaries at the inflammatory sites; however, the role of bacteria and phagocytic leukocytes present at these sites cannot be ignored (21,30). Although the physiological basis of liposome accumulation in infection does not appear to be specific to the presence of infection, its usefulness in imaging inflammation and infection cannot be ruled out because none of the other imaging methods is infection specific (14).

Gallium-67-citrate has been shown to localize in bone infection earlier than 99mTc-MDP (10,31,32). We also found 67Ga-citrate to be a sensitive indicator of bone infection. Eight of 9 rabbits in our study showed definite indication of its accumulation in the infected tibia. Only 1 was equivocal where the T/NT ratio was found to be only 1.19. Since 67Ga-citrate scanning was done 5 days after the liposome scan, we expected it to perform equally well, if not better than the liposomes, assuming that infection consolidated its presence by Day 9. On the basis of this assumption, we believe that if the liposome study had been performed on Day 9, it would have yielded better images than those obtained on Day 4. Despite its widespread use, 67Ga-citrate imaging has several serious drawbacks. These include its slow clearance from background, accumulation in tumor and bowel excretion that interfere in detection of vertebral and pelvic osteomyelitis (13). It is also taken up by reactive bone, which may increase the possibilities of false-positive results (33).

Uptake of 99mTc-MDP in bone depends on two factors – blood flow and osteoblastic activity (34). Despite its well-established importance in diagnosing acute osteomyelitis, it has been found unreliable in neonates and children (11,35). Also, it may not be possible to differentiate its accumulation due to bone repair secondary to trauma or surgery from that due to bone infection. A simultaneous 111In white cell scan or 67Ga-citrate scan adds more specificity to the conventional three-phase bone scan (10); however, these additional procedures increase the total cost of investigation. In our study, 99mTc-MDP was positive in 5 of 9 rabbits with osteomyelitis. The slight increase in blood perfusion in phase I/II of the imaging in a few rabbits with a negative 3-hr image indicates that hyperemia and increase in blood flow precede the elevation in bone metabolism. In very early disease when the intramedullary pressure of the infected bone is high, the 99mTc-MDP scan may, in fact, show a photopenic focus. It
is only after revascularization and manifestation of the inflammatory reaction that an increased uptake of the bone-seeking radiopharmaceutical occurs (34).

In a previous rabbit study, it was found that radiography was not indicative of osteomyelitis until Days 14–21 after onset of infection (36), and it was normal in histologically-confirmed early infected bone (31). Our results confirm these earlier investigations. Only 3 of 9 rabbits in our study were found to demonstrate conclusive signs of acute osteomyelitis. Other investigators also have found radiography lacking the sensitivity and specificity of the scintigraphic procedures (37). The delay in radiographic changes is due to the slow rate of anatomical changes, lytic lesions and areas of increased density, which can be visualized by radiography (5,17).

CONCLUSION

In our study, radiolabeled liposomes were found more sensitive than 99mTc-MDP and radiography, and they were comparable to 67Ga-citrate. Nevertheless, liposomes have obvious advantages over 67Ga such as the absence of bowel disposition of radioactivity, ideal energy characteristics and lack of uptake by reactive bone. A major limitation of our study was that we were unable to compare the performance of 99mTc-liposomes with 111In-leukocytes in rabbits because of the difficulties associated with the separation of rabbit leukocytes. Another drawback was the time lag between the various investigations after induction of osteomyelitis. From our study, we conclude that radiolabeled liposomes are of great use in diagnosing infectious sites, which accumulate the injected radiopharmaceuticals slowly over a period of 1–2 days. Since the liposomes were labeled with 111In, as well as 99mTc, it was easier during our investigation to predict the accumulation of liposomes in early images and to confirm them in the late images.

REFERENCES