Use of scintigraphy for assessment of fracture healing and early diagnosis of osteomyelitis following fracture repair in rabbits

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OBJECTIVE—To evaluate use of technetium Tc 99m disodium hydroxymethylene diphosphonate (99m-Tc-HDP) for assessing fracture healing and 99m-Tc-HDP and technetium Tc 99m ciprofloxacin (99m-Tc-CIPRO) for early diagnosis of osteomyelitis in rabbits.

ANIMALS—32 skeletally mature New Zealand White rabbits.

PROCEDURE—A femoral fracture defect stabilized with bone plates and cortical screws was used. Scintigraphy was performed 4, 8, 12, and 16 weeks after surgery. The 99m-Tc-CIPRO scan was performed 48 hours after the 99m-Tc-HDP scan. The uptake ratio of the experimental limb to the normal limb was calculated by use of multiple regions of interest. Results of radiography performed to determine external callus and lysis grade and percentage defect ossification at 16 weeks were compared with scintigraphy results.

RESULTS—Infected fractures had a higher uptake ratio for 99m-Tc-HDP and 99m-Tc-CIPRO than noninfected fractures. Infected fractures could be differentiated from noninfected fractures late in healing by use of 99m-Tc-HDP. Although 99m-Tc-CIPRO was better than 99m-Tc-HDP for identifying infection, there was a high incidence of false positive and negative results with 99m-Tc-CIPRO. There was an association between 99m-Tc-HDP uptake ratio and callus formation and a good correlation between 99m-Tc-HDP uptake ratio and defect ossification after 4 weeks.

CONCLUSIONS AND CLINICAL RELEVANCE—99m-Tc-HDP and 99m-Tc-CIPRO may be useful for diagnosing osteomyelitis late in fracture healing; however, false positive and false negative results occur. Technetium Tc 99m disodium hydroxymethylene diphosphonate may be useful for evaluating fracture healing. (Am J Vet Res 2003;64:736–745)

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Scintigraphy can be used to detect osteomyelitis several months before osteomyelitis can be detected by radiography. Technetium Tc 99m-labeled phosphonates are commonly used in bone scanning to diagnose osteomyelitis, and for differentiating infected bone from soft tissue. However, because it will accumulate in infected tissue and not be taken up by the bone plate, it will be of no use in evaluating healing of infected nonunion fractures. Technetium Tc 99m-labeled phosphonates are used for assessing bone metabolism because they have an increased uptake in bone with infection and may be useful in evaluating healing of infected nonunions. Advantages of 99m-Tc-phosphonates for detecting infection include high sensitivity, no handling of blood, and minimal technical skills, and laboratory equipment are needed for radionuclide preparation and scanning. To the author's knowledge, there have been no controlled studies evaluating use of 99m-Tc-ciprofloxacin for differentiation of infected and noninfected fractures.

The purpose of the study reported here was to evaluate use of 99m-Tc-HDP for assessing fracture healing and 99m-Tc-HDP and 99m-Tc-CIPRO for early diagnosis of osteomyelitis in rabbits. We hypothesized that there would be a difference in the 99m-Tc-HDP uptake ratio between fractures that healed and fractures that did not heal and that there would be a significant difference in 99m-Tc-HDP and 99m-Tc-CIPRO uptake ratios in infected fractures, compared with noninfected fractures in rabbits.

Materials and Methods

Animal model—Thirty-two skeletally mature female New Zealand White rabbits were used. This study was performed as part of another study evaluating use of adenoviral (Ad) transfer of the bone morphogenetic-2 (BMP-2) gene for enhancing healing of nonunion and infected nonunion fractures in rabbits. Rabbits in the control groups were treated with an adenovector containing the firefly luciferase (LUC) gene, and rabbits in the treatment groups were treated with an adenovector containing the gene for BMP-2. Rabbits were allocated to 4 groups: noninfected Ad-LUC (NON-LUC) control, noninfected Ad-BMP-2 (NON-BMP) treated, infected Ad-LUC (INF-LUC) control, and infected Ad-BMP-2 (INF-BMP) treated.

A nonunion model was created by use of a mid-diaphyseal femoral fracture defect stabilized with a bone plate, cortical screws, and cerclage wire. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC). Briefly, the soft tissues, peristemeum, endostemeum, and bone marrow were removed, and a sclerosing agent (sodium morrhuate) was applied to the ends of the bone to facilitate formation of osteomyelitis and prevent fracture healing as a result of the proliferation associated with infection. Analgesics included morphine, administered epidurally (0.1 mg/kg) and SC (0.5 mg/kg) preoperatively; fentanyl, administered as an IV bolus (0.02 mg/kg), followed by a constant rate infusion (20 µg/kg/hr) during surgery; and flumethrin in meglumine (0.5 mg/kg, SC, 0.1 mg/kg, SC 0.1 mg/kg, SC 0.1 mg/kg, SC 0.1 mg/kg, SC) for 3 days, then as needed. Butorphanol (0.4 mg/kg, SC) was also administered postoperatively, as needed. An IACUC-approved scoring system was used to assess signs of systemic illness and lameness and, subsequently, the need for additional supportive therapy and analgesia twice daily after surgery for the duration of the study. Enrofloxacin (10 mg/kg, SC, once) was administered preoperatively only.

Rabbits in the infected groups were inoculated percutaneously in the fracture defect from the medial aspect of the limb with Staphylococcus aureus (0.5 × 107 colony forming units [cfu] in 0.5 mL saline) 48 hours after surgery. At the same time, Ad-LUC or Ad-BMP-2 was administered percutaneously in the fracture defect. Rabbits were euthanatized 16 weeks after surgery. At necropsy, the fracture was evaluated for gross signs of infection. Quantitative aerobic bacteriologic culture (QAC) of tissues surrounding the fracture site was performed, and a QAC > 103 cfu/mL was used to define infection.

Radiographic evaluation—During general anesthesia, rabbits were evaluated by use of radiography (cranial and lateral views) 16 weeks postoperatively. Results of external callus grade, percentage defect ossification, presence of bridging callus, and lysis grade at 16 weeks (end-point) were compared with scintigraphy data at 4, 8, 12, and 16 weeks. External callus grade, defect ossification, and bridging callus at 16 weeks were used to assess fracture healing, and lysis grade at 16 weeks was used as a measure of osteomyelitis. Radiographs were graded from 0 to +4 for lysis and external callus by a board certified radiologist unaware of experimental group assignment. Bone lysis was graded as 0 (none); 1 (slight) if it was associated with bone adjacent to a single cortical screw or confined to bone immediately adjacent to the defect; 2 (mild) if it was associated with bone adjacent to multiple cortical screws, the defect, or the bone plate; 3 (moderate) if it was associated with bone in between the cortical screws but was not extensive; and 4 (severe) if it was associated with the entire bone. External callus formation was graded as 0 (none); 1 (slight) if there was < 2 cm of thin callus forming on the proximal or distal fragment; 2 (mild) if there was > 2 cm of thin callus extending from the proximal and distal fragment; 3 (moderate) if there was > 2 cm of thick callus extending from the proximal and distal fragment; and 4 (marked) if there was a thick, bridging callus. The percentage defect ossification was subjectively estimated by use of the cranio-caudal and lateromedial radiographic views.

Nuclear scintigraphy—During general anesthesia, rabbits were evaluated by use of scintigraphy at 4, 8, 12, and 16 weeks after surgery. All rabbits received 99m-Tc-HDP (1 mCi/kg, IV), followed by 99m-Tc-CIPRO (1 mCi/kg, IV) 48 hours later. A 1-minute-long pool-phase image was obtained immediately after administration of 99m-Tc-CIPRO and 3-minute-long delayed-phase images were obtained 2 hours after administration of 99m-Tc-HDP and 99m-Tc-CIPRO, by use of a 20 × 14.5-inch rectangular field of view imaging system with a general purpose collimator. A lateral view was obtained for the pool-phase image, and lateral and cranial views were obtained for the delayed-phase image. Additional images were obtained 1 hour later (3 hours after administration) in 4 randomly selected rabbits to determine if there were differences between the 2-hour and 3-hour images for 99m-Tc-CIPRO. These images were analyzed by use of a computer software program. Mean radionuclide uptake was measured for each
region of interest (ROI). The ROI for the experimental limb (numerator; left limb [L]) were defect, whole bone, and proximal fragment. The ROI for the normal bone (denominator) were the same areas of the contralateral femur (right limb [R]) and the first lumbar vertebra. The area of increased radionuclide uptake defined whole bone and proximal fragment ROIs. This area was not consistent among rabbits and time periods. Therefore, the area of radionuclide uptake was measured and expressed as a ratio of experimental-to-normal (L:R) bone. Because the change in area may have altered mean uptake values, the ratio of the maximum and minimum values for whole bone L:R and defect L:R ROI were measured and also expressed as a ratio of L:R bone.

Cranial views on the delayed-phase 99m-Tc-CIPRO images were subjectively evaluated as infected or noninfected by a board certified radiologist (PS) who was unaware of the experimental group assignment. The observer (PS) randomly reevaluated 9 images, and repeatability was determined.

The relationship between the pool- and delayed-phase uptake ratios was investigated further by use of the whole bone L:R ROI by subtracting the pool-phase ratio from the delayed-phase ratio and also by dividing the delayed-phase ratio by the pool-phase ratio.

Statistical analyses—A plot of predicted versus residual values was performed, and if an increase in variance with increase in uptake ratio was observed, a log transformation of the data was performed, and the data were reanalyzed. Data from 99m-Tc-HDP images were analyzed as log values and are given as least squared means (LSM) of the log values, and data from 99m-Tc-CIPRO images are given as LSM of the raw values, because data appeared normal on the plot of predicted versus residual values and were not transformed.

Continuous data were analyzed by use of a mixed-model ANOVA. Class variables were rabbit (1 to 64); time (4, 8, 12, and 16 weeks); treatment (BMP or LUC); infection (infected or noninfected); and lysis grade (0 to 4), defect ossification, or callus grade (0 to 4) at 16 weeks. The random variable was rabbit nested within infection and treatment groups. Data were analyzed by use of 4 models to evaluate the association between uptake ratio (dependent variable) and the fixed effects of time, treatment, infection, and interactions; lysis grade, time, and interactions; defect ossification, time, and interactions; and callus grade at 16 weeks (0 to 4), time, and interactions. The dependent variables for 99m-Tc-HDP and 99m-Tc-CIPRO were defect L:R ratio, whole bone L:R ratio, whole bone:lumbar vertebra ratio, and proximal fragment L:R ratio. Data from cranial and lateral views for each ROI were analyzed separately, and results were reported separately only if there was a difference between the cranial and lateral views. Activities of whole bone L:R ratio on the pool-phase images were also used as dependent variables. For all comparisons, values of P < 0.05 were considered significant.

Pearson’s correlation was used to evaluate the correlation between each dependent variable and callus and lysis grade, as well as the correlation between uptake on the 99m-Tc-HDP and 99m-Tc-CIPRO images. Correlations were rated as none (r² < 0.3), weak (r² = 0.3 to 0.45), moderate (r² = 0.45 to 0.6), or good (r² > 0.6). The probability of the various ROIs in the ability to predict whether a fracture was infected or had bridging callus at 16 weeks was also determined, and the accuracy, true positive (sensitivity), true negative (specificity), positive predictive value, and negative predictive value were calculated.

The coefficient of variation (CV) for 99m-Tc-HDP and 99m-Tc-CIPRO images were determined by measuring radionuclide uptake for 40 randomly selected ROIs 3 times, calculating the mean and SD of the 3 measurements, then dividing the SD by the mean. The CV for the uptake ratio for the 2- and 3-hour 99m-Tc-CIPRO images was calculated similarly.

Results

Animal model—Twenty rabbits completed the 16-week study (NON-LUC, [n = 6], NON-BMP-2 [5], INF-LUC [6], INF-BMP [3]). Twelve rabbits were euthanatized for humane reasons before completion of the study. Three rabbits were euthanatized after week 4, and data from these rabbits before euthanasia were included. Reasons for euthanasia included sepsis, surgical failure, and lumbar vertebra fracture. At necropsy, all rabbits in the infected group and none of the rabbits in the noninfected group had gross signs (accumulation of purulent material and abscess formation) of infection, and S aureus was identified on QAC of tissues.

99m-Tc-HDP delayed-phase—Median CV for 99m-Tc-HDP images was 3.5% (range, 1 to 10.4%). There was no association between treatment and uptake ratio. There was a significant association between infection and uptake ratio on both views and all ROIs at all time periods except 4 weeks. There was a significant association between time and uptake ratio on both views and all ROIs. The change in uptake ratio with time was different for infected and noninfected fractures (ie, there was a significant interaction between the fixed effects of

Figure 1—Delayed-phase technetium Tc 99m disodium hydroxymethylene diphosphonate (99m-Tc-HDP) uptake (least square means ± SEM log [ln] of uptake ratios) in whole bone (expressed as a ratio between left and right femurs [L:R; panel A] and as a ratio between whole bone and a lumbar vertebra [panel B]) in infected and noninfected rabbits 4, 8, 12, and 16 weeks after surgery. *Significant (P < 0.05) differences between infected and noninfected groups and among different time periods.
infection and time; Fig 1). Whole bone L:R area ratio increased in rabbits with infected fractures and decreased in rabbits with noninfected fractures with time. Rabbits with infected fractures had a significantly higher whole bone L:R area ratio than rabbits with noninfected fractures at 12 and 16 weeks only.

There was a good correlation \((r^2 = 0.7)\) between lysis grade and uptake ratio of whole bone L:R and whole bone-to-lumbar vertebra ROI at 8, 12, and 16 weeks. The association between lysis grade and uptake ratio of the defect and whole bone L:R ROI was different at different times, and the change in uptake ratio with time was different for different lysis grades (ie, there was a significant interaction between the fixed effects of lysis grade and time; Fig 2). Use of 99m-Tc-HDP for determining the probability of a fracture being infected was determined (Table 1).

Overall, there was moderate correlation between percentage defect ossification and uptake ratio of the defect L:R. There was no correlation at 4 weeks \((r^2 = 0.1)\), and the correlation peaked at 8 and 12 weeks \((r^2 = 0.6)\) then slightly decreased at 16 weeks \((r^2 = 0.5)\). There was no correlation between percentage defect ossification and uptake ratio for whole bone L:R or whole bone:lumbar ROI. There was no correlation between uptake ratio for any ROI and external callus grade. However, the association between external callus grade and uptake ratio of the defect L:R ROI was different at different times, and the change in uptake ratio with time was different for different external callus grades (ie, there was a significant interaction between the fixed effects of external callus grade and time; Fig 3). There was no association between external callus grade and uptake ratio for whole bone L:R or whole bone:lumbar ROI. The uptake ratio of the defect L:R ROI on the lateral view at 4 weeks was useful for determining the probability of fractures developing a bridging callus with a sensitivity of 57%, specificity of 88%, and overall accuracy of 78%. A radionuclide uptake ratio of 2.9 was the minimum value calculated to differentiate fractures that had bridging callus at 16 weeks from those that did not develop bridging callus.

Table 1—Performance of delayed-phase technetium Tc 99m disodium hydroxymethylene diphosphonate uptake ratios for various regions of interest (ROI) for detecting infection in fractures in rabbits 4, 8, 12, and 16 weeks after surgery

<table>
<thead>
<tr>
<th>ROI</th>
<th>Week</th>
<th>Ratio*</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
<th>Accuracy (%)</th>
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Defect L:R = Radionuclide uptake in defect ROI expressed as a ratio of the left (L, experimental) femur to the right (R, normal) femur. Proximal fragment L:R = Radionuclide uptake in proximal fragment ROI expressed as a ratio of L:R. Whole bone L:R = Radionuclide uptake in the whole bone ROI expressed as a ratio of L:R. Whole bone:lumbar vertebra = Radionuclide uptake of whole bone ROI of the left limb expressed as a ratio of the uptake in a normal lumbar vertebra. 

*Ratio values differ significantly \((P < 0.05)\) from those of noninfected fractures. 

Infected fractures have a radionuclide uptake ratio \(>\) this calculated ratio, and noninfected fractures have a radionuclide uptake ratio \(<\) this calculated ratio.
99m-Tc-CIPRO delayed-phase—Median CV for the 99m-Tc-CIPRO images was 1.4% (range, 0 to 6.4%). Overall, there was no association between treatment and uptake ratio, except on the cranial view for rabbits in the BMP-2 treated group that had a significantly higher uptake ratio at 16 weeks on the defect L:R ROI, and at 4 and 16 weeks on the whole bone and proximal fragment L:R ROI (ie, there was a significant interaction between the fixed effects of treatment and time). Overall, there was a significant association between infection and uptake ratio, and the change in uptake ratio with time was different for infected and noninfected fractures on both views and all ROIs (ie, there was a significant interaction between the fixed effects of infection and time; Fig 4). There was no association between infection and uptake ratio at 4 weeks with the defect L:R ROI; however, with whole bone or proximal fragment L:R ROI, there was a significant association between infection and uptake ratio at 4 weeks. Use of 99m-Tc-CIPRO for determining the probability of a fracture being infected was determined (Table 2).

Results of subjective analysis of cranial views of 99m-Tc-CIPRO images revealed low specificity and high sensitivity for detecting infection with 99m-Tc-CIPRO (Fig 5). Sensitivity was 94% (33/35 images), and specificity was 73% (35/48 images), with an overall accuracy of 82% (68/83 images). Repeatability for results of subjective analysis of cranial views of 99m-Tc-CIPRO images was 89% (8/9 images).

Rabbits with infected fractures had a greater uptake ratio of whole bone L:R area at all time periods on the lateral view, compared with rabbits with noninfected fractures. The uptake ratio of whole bone L:R ROI area decreased with time on both views in rabbits with infected and noninfected fractures. There was no difference between rabbits with infected and noninfected fractures in the maximum or minimum values at 4 weeks in the defect ROI. However, by use of the whole bone ROI, rabbits with infected fractures had a higher maximum value at all time periods.

There was a significant association between lysis grade and uptake ratio of 99m-Tc-CIPRO (Fig 6). There was no correlation between uptake ratio for whole bone L:R ROI and lysis grade at 4 weeks, but the correlation increased with time and was good after 4 weeks. The correlation was highest for the whole bone L:R ROI and for the lateral view ($r^2 = 0.7$).

Overall, for all treatment groups and both views,
there was a correlation between the 99m-Tc-HDP and 99m-Tc-CIPRO images for the uptake ratio of the defect and whole bone ROI. The correlation was weak at 4 weeks and increased with time.

Four rabbits were rescanned 3 hours after administration of Tc-99m-CIPRO to determine if there was a difference between the 2- and 3-hour images. Subjectively, the images appeared identical, and this was supported by the uptake ratios of the defect L:R and whole bone L:R. The CV for the difference in uptake ratio between the 2- and 3-hour scan was <7% and within the CV range for the 99m-Tc-CIPRO images.

**Pool-phase**—There was no difference in the radionuclide activity of whole bone L:R ROI on the pool-phase between rabbits with infected and noninfected fractures at 4 weeks. Activity in rabbits with noninfected fractures decreased with time but did not change with time in rabbits with infected fractures, and the difference between infected and noninfected rabbits was significant after 4 weeks. Therefore, rabbits

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**Table 2—Performance of delayed-phase technetium Tc 99m ciprofloxacin uptake ratios for various ROI for detecting infection in fractures in rabbits 4, 8, 12, and 16 weeks after surgery**

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*Ratio values differ significantly (P < 0.05) from those of noninfected fractures.

See Table 1 for key.
with noninfected fractures had a higher blood flow to the experimental limb or an increase in capillary permeability at 4 weeks, compared with that at 8, 12, and 16 weeks.

When the relationship between the pool- and delayed-phase uptake ratio was investigated further by use of the whole bone L:R ROI by subtracting the pool-phase ratio from the delayed-phase ratio and by dividing the delayed-phase ratio by the pool-phase ratio, the difference between rabbits with infected and noninfected fractures was not significant at 4 weeks. The ratio of uptake on delayed-to-pool-phase for rabbits with noninfected fractures was > 1, and the difference in uptake between delayed and pool-phase for rabbits with noninfected fractures was > 0.

Qualitatively, there was reduced activity in the defect region, compared with the proximal and distal fragment (Fig 7), and reduced activity associated with areas of abscess formation on the pool-phase image (Fig 8).

**Discussion**

Results of this study indicate that 99m-Tc-HDP may be useful for differentiating infected from noninfected fractures late in healing. Technetium Tc 99m-labeled ciprofloxacin was better than 99m-Tc-HDP at identifying infection; however, the accuracy was lower than expected, particularly at 4 weeks. The lack of association between infection and uptake ratio on the 99m-Tc-HDP images at 4 weeks may be explained by an increase in bone metabolism associated with fracture healing in infected and noninfected fractures and a lack of bone formation in infected fractures early in fracture healing. In another study, serum bone marker concentrations were measured, and there was a decrease in the concentration of markers of bone formation in rabbits with infected fractures and with an increase in lysis grade at 4 weeks.

There was an increase in 99m-Tc-HDP activity on the pool-phase images in infected and noninfected fractures early in healing, which may be attributed to an increase in blood flow or capillary permeability, presuming that blood volume and extracellular fluid volume were constant. Therefore, although an increase in bone metabolism associated with fracture healing may have been the major cause of an increase in uptake ratio in the delayed-phase images, an increase in blood flow and capillary permeability may also have contributed in both infected and noninfected fractures. Although it was not significant, there was also a decrease in 99m-Tc-HDP uptake ratio with an increase in lysis grade at 4 weeks.

Other studies have found that 99m-Tc-HDP is beneficial for diagnosing late infections that develop several months after surgery. Technetium Tc 99m phosphonates could be used to diagnose infection following total hip arthroplasty 6 to 8 months after surgery; however, in 1 study, surgical trauma and thermal necrosis of the bone resulted in an increase in uptake ratio in patients without infections after arthroplasty before 6 to 8 months. However, 99m-Tc-HDP could be used to identify patients with infection after arthroplasty before radiographic signs develop. Most authors agree that no uptake of 99m-Tc-HDP can be used to rule out infection. As in our study, patients with infections...
after arthroplasty had an increase, and patients without infections had a decrease in uptake ratio with time.\textsuperscript{11,16} Differentiation of soft tissue from bone infection is also significant in evaluation of fractures postoperatively. Use of the delayed-phase 99m-Tc-HDP images to differentiate cellulitis from osteomyelitis has been reported.\textsuperscript{3,4,18} Although further evaluation of the use of 99m-Tc-HDP to differentiate soft tissue infection from osteomyelitis after fracture repair is needed, this could not be evaluated in our study, because all rabbits with infected fractures had soft tissue infection determined by gross necropsy findings, results of QAC, and osteomyelitis determined by bone lysis on radiographic examination.\textsuperscript{19}

The lack of specificity early in fracture healing with 99m-Tc-CIPRO is difficult to explain. Ciprofloxacin should bind specifically to DNA gyrase of live bacteria. Technetium and ciprofloxacin have low protein binding; therefore, the blood pool-phase should be rapidly cleared,\textsuperscript{46} resulting in a high uptake ratio in infected fractures only. In our study, specificity and sensitivity at 4 weeks was lower than the reported specificity of > 90% and sensitivity of 70 to 84% for diagnosing various types of infection with 99m-Tc-CIPRO,\textsuperscript{14,15} but there were limitations with these studies. First, infection was not well defined.\textsuperscript{3,46} In 1 study,\textsuperscript{39} humans with no growth on bacteriologic culture were considered infected if they were treated for infection by the attending physician; however, criteria used by the attending physician were not described. Second, specificity and sensitivity for diagnosing infection in patients with osteomyelitis at a fracture site could not be determined from these studies, because all patients with osteomyelitis were included in 1 group. However, in 1 study,\textsuperscript{39} false-positive results in patients with fractures were reported. Third, specificity and sensitivity are dependent on the number of animals with or without disease, and the high specificity may be attributable to higher numbers of animals with infection.

False-positive results obtained early in our study may have been caused by several factors. Surgery and the use of a sclerosing agent caused inflammation. Infection and inflammation caused vasodilation, increase in vascular permeability, expansion of the extracellular space, and an increase in leukocyte accumulation, all of which may cause an increase in non-specific accumulation of 99m-Tc-CIPRO. The radionuclide activity in the pool-phase was the same in infected and noninfected fractures at 4 weeks. Therefore, an increase in blood flow to the femur or increase in capillary permeability may have contributed to the false-positive results in the noninfected fractures. In a similar study,\textsuperscript{12} an increase in uptake with an increase in blood flow with other Tc-labeled pharmaceuticals has been reported. Thrall et al\textsuperscript{10} reported that increase in uptake of 99m-Tc-HDP extended beyond the lesion, and the cause was thought to be an increase in blood flow to the region. Other authors have emphasized the importance of bone blood flow in determining uptake patterns for Tc-labeled agents\textsuperscript{15} and that images with radionuclide uptake indicate distribution of the radio-label, rather than the pharmaceutical to which it is attached.\textsuperscript{24} However, when the pool-phase uptake ratio for whole bone L-R ROI was subtracted from the delayed-phase uptake ratio for 99m-Tc-CIPRO images, rabbits with noninfected fractures had an uptake ratio > 0, and there were no significant differences between rabbits with infected and noninfected fractures at 4 weeks. This finding suggests that there was also a decrease in elimination of the 99m-Tc-CIPRO from the fracture site.

The decrease in elimination of 99m-Tc-CIPRO may have been caused by reduced venous and lymphatic drainage associated with inflammation and accumulation of 99m-Tc-CIPRO in edema fluid, or the 99m-Tc-CIPRO may have bound to something other than bacteria. By use of microradiography, studies\textsuperscript{16} evaluating radiolabeled immunoglobulin G, which binds specifically to inflammatory cells, found nonspecific uptake through increased vascular permeability and accumulation in edema fluid, rather than specific uptake associated with inflammatory cells. Ciprofloxacin concentrates in bone and phagocytic cells;\textsuperscript{45} therefore, it may also accumulate in sterile inflammation of bone that develops with a fracture. Although accumulation of unassociated Tc may have also been the cause of the increase in uptake in noninfected fractures, this is unlikely, because Tc is covalently bound to the pharmaceutical and has a low dissociation rate.\textsuperscript{4}

Rabbits with infected fractures had a higher maximum uptake by use of the whole bone ROI and a higher uptake in the proximal fragment, compared with rabbits with noninfected fractures at all time periods. The pattern of uptake was different in rabbits with infected and noninfected fractures. Infected fractures had mixed areas of increased and decreased uptake, whereas noninfected fractures had a more uniform uptake pattern. Therefore, if the area of the highest uptake, such as the proximal fragment, is used (rather than a defined region, such as the whole bone or defect), then 99m-Tc-CIPRO may be accurate for early diagnosis of infection. The lack of significance in the whole bone ROI at 4 weeks on the lateral view could be attributed to a greater area ROI, which resulted in inclusion of regions with a lower uptake. At 4 weeks, there was no difference in the maximum and minimum uptake values between infected and noninfected fractures in the defect ROI. This suggests that the lack of significant difference in the defect region was real and most likely attributed to reduced blood flow.

The delayed-phase scan was performed 2 hours after administration of 99m-Tc-CIPRO. Increasing the time from administration to scanning may have caused an increase in uptake ratio in infected fractures and a decrease in noninfected fractures as a result of clearing of nonspecifically bound 99m-Tc-CIPRO. Scanning was repeated 3 hours after administration of 99m-Tc-CIPRO, and there was no difference between the 2- and 3-hour images. Technetium has a short half-life (6 hours) and rapid urinary excretion; therefore, if scanning is delayed 24 to 72 hours to allow for adequate clearance of nonspecifically bound 99m-Tc-CIPRO, the radionuclide would have decayed. In another study\textsuperscript{20} evaluating radiolabeled antimicrobials, tetracycline labeled with \textsuperscript{125}I, rather than Tc, was used to
scan for abscesses, because the longer half-life of $^{111}$I would permit imaging 72 hours after administration, when the lesion-to-background ratio is higher. Therefore, labeling ciprofloxacin with a radionuclide with a longer half-life would allow scanning to be performed at a later time when the soft tissue and blood are cleared of unbound ciprofloxacin.

Rabbits were inoculated with S. aureus in the fracture defect. There was no difference in 99mTc-CIPRO uptake ratio between infected and noninfected fractures at 4 weeks by use of the defect ROI. This was thought to be associated with reduced blood flow to the defect region as indicated on the pool-phase image, resulting in false-negative results for infection. In our model, the soft tissue, periosteum, endosteum, and bone marrow were removed from the bone during surgery, and a sclerosing agent was applied to the defect to prevent healing; therefore, reduced blood flow to the defect was expected. Additionally, there was abscess formation associated with the defect region in all rabbits with infected fractures, which may also have caused a decrease in uptake of 99mTc-CIPRO. Ciprofloxacin penetrates abscesses well; however, abscesses contain dead bacteria, and because 99mTc-CIPRO does not bind to dead bacteria, there is a diffuse increase in uptake around (but not in) the abscess.30

In our study, 99mTc-HDP uptake was correlated with callus formation and defect ossification. Fractures that developed a grade 1 external callus (nonunion) had almost no radiographic signs of defect ossification and a consistently low uptake of 99mTc-HDP. Fractures that developed a grade 2 and 3 callus had radiographic signs of proliferation and external callus formation without bridging callus, and uptake of 99mTc-HDP increased throughout the study in these rabbits. Our results were similar to results in another study31 in that fractures that healed or developed a bridging callus reached a peak in 99mTc-HDP uptake at 8 to 12 weeks, and then uptake declined, whereas 99mTc-HDP uptake in fractures that had delayed union or nonunion remained increased. Serum markers of bone formation followed a similar pattern.

The association between 99mTc-CIPRO uptake ratio and the interaction between treatment group and time is also difficult to explain. The 3 rabbits that were euthanized after week 4 were in the infected BMP treated group; therefore, the change over time may have been a result of the loss of these rabbits. However, when these rabbits were excluded from the analysis, the results were still significant. There was no 3-way interaction between infection, treatment, and time; hence, these findings were not a result of an interaction with infection. Therefore, this was most likely a result of an increase in blood flow in the infected and noninfected BMP treated rabbits. Only a lateral view was obtained for the pool-phase image, and although BMP treated rabbits had a slight increase in activity, it was not important; consequently, this assumption could not be confirmed.

References


