Comparison of intraosseous and intravenous infusion of technetium Tc 99m pertechnetate in the distal portion of forelimbs in standing horses by use of scintigraphic imaging

Shawn E. Mattson, DVM, DVSc, BSc; Simon G. Pearce, BVSc, PhD; Ludovic P. Boure, DMV, MSc; Howard Dobson, DVM, DVSc; Mark B. Hurtig, DVM, MVSc; William D. Black, DVM, PhD

Objective—To describe and compare the distribution of technetium Tc 99m (99mTc) pertechnetate following intraosseous or IV injection (with or without use of a tourniquet) in the distal portion of the forelimb in standing horses.

Animals—4 horses.

Procedure—Each horse received 4 forelimb treatments in random sequence: intraosseous infusion with tourniquet application (IOT), intraosseous infusion without tourniquet application, IV infusion with tourniquet application (IVT), and IV infusion without tourniquet application. Dynamic nuclear scintigraphic imaging of the third metacarpal bone, proximal and middle phalanges, and distal phalanx was performed from the start of each treatment until 1 hour after infusion was completed. Radionuclide activity was compared within and between treatment groups.

Results—Tourniquet application was necessary to maintain high levels of radionuclide activity in the distal portion of the forelimb after intraosseous or IV infusion with 99mTc pertechnetate; IVT and IOT treatments resulted in similar radionuclide activity in the proximal and middle phalanges and distal phalanx. Of the 4 treatments, there was significantly higher radionuclide activity in the distal aspect of the third metacarpal bone after the IOT treatment.

Conclusions and Clinical Relevance—By use of a tourniquet, radionuclide administration via the intraosseous or IV routes resulted in effective perfusion of the distal portion of the forelimb and similar distribution of the agent in the phalanges of horses. Further studies are required to ascertain whether these findings apply to delivery of therapeutic agents in infected tissues via IOT or IVT. (Am J Vet Res 2005;66:1267–1272)

Techniques for regional perfusion of antimicrobials in limbs involving intraosseous and IV administration routes achieve high concentrations of those agents in synovial fluid and bone, which are effective in treatment of clinical and experimental infections.1-12a Regional perfusion is achieved through application of a tourniquet to the affected limb (proximal to the site of infection), followed by IV or intraosseous injection of an antimicrobial solution at an administration site that is distal to the tourniquet. The tourniquet is believed to prevent dissipation of the drug systemically.11 Injection of the antimicrobial solution under pressure distends the venous vasculature and allows diffusion throughout the tissues distal to the tourniquet.8,11,12

In horses, the route and distribution of perfusate after intraosseous infusion in the third metacarpal bone (MCIII), third metatarsal bone (MTIII), and distal portion of the tibia have been described by use of serial radiography with contrast medium administration.4,5 Following infusion of contrast medium into MCIII, subtraction radiography revealed that the perfusate left the medullary cavity of MCIII through the epiphysyeal veins and entered the adjoining carpal synovial venous system.8 Scheuch et al1 compared intraosseous and IV perfusion of contrast material in the saphenous vein, proximal portion of MTIII, and distal portion of the tibia. There was extensive infiltration of the superficial veins surrounding the tibiotarsal joint following infusion of contrast material into the saphenous vein, proximal portion of MTIII, and distal portion of the tibia. Subjectively, the authors concluded that the density of contrast material in the venous system was greatest after IV infusion; however, the authors also concluded that both techniques were effective in perfusion of the tibiotarsal joint.4

Comparison of antimicrobial concentrations achieved in synovial fluid following intraosseous and IV infusion has been reported.13 In 1 study,14 IV administration of amikacin in the saphenous vein resulted in higher concentrations of amikacin in the synovial fluid of the tibiotarsal joint, compared with that achieved after intraosseous infusion of amikacin in the distal...
portion of the tibia or proximal portion of MTIII, but the antimicrobial concentrations were not significantly different. In another study, IV administration of amikacin in the lateral palmar digital vein resulted in significantly higher amikacin concentration in the synovial fluid of the distal interphalangeal joint, compared with that achieved after intraosseous infusion of amikacin in the distal portion of MCIII; however, similar concentrations of amikacin were achieved in the synovial fluid of the metacarpophalangeal joint and digital flexor tendon sheath.

To the authors’ knowledge, dynamic comparison of the tissue distribution of drugs after IV and intraosseous administration in the distal portion of limbs and the scientific demonstration that the application of a tourniquet is necessary to achieve regional limb perfusion of the distal portion of limbs in horses have not been reported in the veterinary medical literature. Nuclear scintigraphic imaging can be used to visually and quantitatively assess perfusion of tissues and the distal portion of limbs. Nuclear scintigraphy involving technetium Tc 99m (99mTc) pertechnate has been used in horses to evaluate perfusion of the distal portion of limbs following traumatic injuries. The objectives of the study of this report were to describe and compare the distribution of 99mTc pertechnate after intraosseous or IV injection (with or without a tourniquet) of the distal portion of the forelimb in standing horses. We hypothesized that intraosseous and IV perfusion would result in the same radionuclide counts in regions of the distal portion of the metacarpus, proximal and middle phalanges, and distal phalanx and that a tourniquet is necessary to achieve and maintain high radionuclide counts in the treated limb.

Materials and Methods

Animals—Four mature healthy Standardbreds (3 mares and 1 gelding) from the University of Guelph research herd were used in a randomized crossover study. The ages of the horses ranged from 5 to 15 years (mean age, 9.5 years), and their weights ranged from 457 to 539 kg (mean weight, 497 kg). They were assessed to be healthy and free of lameness on the basis of findings of a clinical examination. The University of Guelph Animal Care Committee approved all of the procedures performed during the study.

Procedures—Each horse was assigned to a random sequence of 4 treatments: intraosseous infusion with a tourniquet (IOT), intraosseous infusion without a tourniquet (ION), IV infusion with a tourniquet (IVT), and IV infusion without a tourniquet (IVN). In the IOT and IVT treatment groups, a 10-cm-wide rubber tourniquet was tightly applied to the proximal aspect of the metacarpus prior to infusion and was removed 30 minutes after the start of the infusion. The tourniquet was applied by the same individual in all experiments (SEM). Both forelimbs of each horse were used in the study; treatments were stratified such that each forelimb had 1 intraosseous treatment and 1 IV treatment only. A washout period of 7 days was used between each of the 4 treatments. Only 1 limb was used at any 1 time.

Horses were treated IV with penicillin G sodium (20,000 U/kg) and phenylbutazone (4.4 mg/kg) 30 minutes prior to the surgical procedure as required by the University of Guelph Animal Care Committee. For each treatment group, horses were sedated with a combination of detomidine (0.01 mg/kg, IV) and butorphanol (0.02 mg/kg, IV) and restrained in the stocks. For the intraosseous treatments, hair on the distal half of the metacarpus was clipped and the skin was aseptically prepared. Three milliliters of 2% mepivacaine solution was infused SC at the junction between the middle and distal thirds of the metacarpus and 1 cm lateral to the common digital extensor tendon. A 1-cm stab incision was made with a No. 10 scalpel blade through the skin, subcutaneous tissues, and periosteum of MCIII. The soft tissue was gently retracted with hemostatic clamps, and a 4-mm-diameter unicortical hole was drilled through the dorsolateral aspect of the cortex of MCIII. The hole was subsequently tapped to a diameter of 5.5 mm, and a custom-made 5.5 × 20-mm cannulated bone screw was inserted by use of sterile piers. The male end of a catheter extension set was attached to a Luer lock adapter that was welded onto the cannulated screw. Five milliliters of 2% mepivacaine solution was infused into the medullary cavity of MCIII through the cannulated screw by use of a 10-ml syringe attached to a catheter extension set. For the IV treatment groups, hair on the palmarolateral aspect of the metacarpophalangeal joint was clipped and the skin was aseptically prepared; a 20-gauge, 1.5-inch catheter was placed in the lateral digital vein.

Imaging technique—One hundred microliters of 99mTc pertechnate diluted in sterile saline (0.9% NaCl) solution (administered dose, 0.1 mL/kg) was infused through the intraosseous screw or IV catheter by use of a 10-ml syringe attached to a catheter extension set. The total injection volume ranged from 46 to 54 mL, and time to complete the infusion ranged from 1 to 5 minutes. Radionuclide activity was measured by use of a gamma camera and scintigraphic software in the treated and untreated forelimbs simultaneously over time for each treatment. Radionuclide counts were corrected for time decay of 99mTc pertechnate. Limbs were divided into 3 regions of interest: the distal third of the metacarpus, proximal and middle phalanges, and distal phalanx. Dynamic nuclear scintigraphic imaging of both forelimbs was performed beginning with the start of the infusion of 99mTc pertechnate in one of the limbs until 1 hour after infusion was completed. Thirty-second frames were collected for the duration of each experiment. The scintigraphic software allowed regions of interest to be created for measurement of counts from the defined areas of interest. At the maximum counting rate of 20,000 counts/30 s, dead-time losses were approximately 25% of the total counting rate. At a counting rate of 10,000, dead-time losses were < 5%.

After scintigraphic imaging was completed, treated limbs were bandaged by use of sterile bandage material. A complete physical examination of each horse was performed every 12 hours throughout each experiment (including the 7-day washout period). After each experiment, horses were evaluated for lameness and signs of pain. Horses were monitored for 7 days after the final treatment. Horses that were lame after an experiment were treated with anti-inflammatory medication (phenylbutazone), if required.

Statistical analyses—Radionuclide activity as a percentage of peak radionuclide counts in the treated limb over time was analyzed by use of a 2-factor ANOVA. The 2-factor ANOVA was used to perform comparisons between treatments, between locations, and between treatment-location interactions on mean percentages of peak radionuclide counts (intercept) and slopes (derived from linear regression equations from 30 to 60 minutes). Overall mean percentage of peak radionuclide counts had a significant treatment-location interaction. Therefore, a post hoc Tukey-Kramer t test...
was used to compare treatments within each location and the change in treatments between locations. The ANOVA on the slopes revealed a significant main effect of treatment. The treatment-location interaction and main effect of location were not significant. A post hoc Tukey test was used to compare the overall main effect of treatment for the slope. A value of $P < 0.05$ was considered significant.

**Results**

No major complications developed in any horse, and all horses tolerated the experiments well. Horses were not lame at any time point during the experiment, immediately postoperatively, or during the 7-day washout period.

In all horses, injection of 100 mCi of $^{99m}$Tc pertechnate diluted in sterile saline solution in the distal portion of the forelimb via the intraosseous and IV routes (with or without an tourniquet) resulted in diffusion of the solution in tissues of the distal portion of the forelimb. In the IVT and IOT treatment groups, radionuclide activity was not detected proximal to the level of the tourniquet during tourniquet application (Figures 1 and 2). After tourniquet removal, high levels of radionuclide activity remained in the IOT- and IVT-treated limbs for the duration of that experimental period.

There was no significant difference in the mean percentage of peak radionuclide counts between the IOT and IVT treatment groups at the proximal and middle phalanges ($P = 0.9$) and the distal phalanx ($P = 0.4$; Figure 3). The IOT treatment group had significantly ($P = 0.02$) higher radionuclide counts in the distal portion of the metacarpus, compared with the counts in that region in the IVT treatment group. The ION and IVN treatment groups had significantly ($P < 0.001$) less radionuclide activities in the distal portion of the metacarpus, proximal and middle phalanges, and distal phalanx, compared with activities in those regions in the IOT and IVT treatment groups.

Within the IVT, ION, and IVN treatment groups, there was no significant ($P > 0.2$) difference in mean percentage of peak radionuclide counts between the distal portion of the metacarpus, proximal and middle phalanges, and distal phalanx (Figure 3). Within the IOT treatment group, there was significantly greater peak radionuclide activity in the distal portion of the metacarpus, compared with peak activities in the proximal and middle phalanges ($P = 0.002$) and the distal phalanx ($P < 0.001$). Peak radionuclide counts in the proximal and middle phalanges and the distal phalanx were not significantly ($P = 0.1$) different in the IOT treatment group. Within the IVT treatment group,
there was uniform distribution of radionuclide activity between the distal portion of the metacarpus, proximal and middle phalanges, and distal phalanx.

The 2-factor ANOVA on the slopes derived from linear regression equations revealed a significant ($P < 0.05$) main effect of treatment. The treatment-location interaction and main effect of location were not significant ($P > 0.05$) for each treatment group. The mean slopes of the IOT and IVT treatment groups were not significantly ($P = 0.5$) different in the distal portion of the metacarpus, proximal and middle phalanges, and distal phalanx (Figure 6). The mean slopes of the ION and IVN treatment groups were not significantly ($P = 0.9$) different in the distal portion of the metacarpus, proximal and middle phalanges, and distal phalanx.
Discussion

In the present study, diffusion characteristics of $^{99m}$Tc pertechnate diluted in sterile saline solution injected via intraosseous and IV routes in the distal portion of the forelimbs of horses could be evaluated dynamically by use of nuclear scintigraphy. Previously, regional limb perfusion techniques in horses have been evaluated at specific time points by use of serial radiography with contrast medium administration.1,8 Although such radiographic techniques can be used to monitor diffusion of a perfusate after intraosseous or IV administration, they do not provide a means with which to objectively compare different perfusion techniques. In our study, dynamic nuclear scintigraphy allowed (in a noninvasive manner) visual evaluation and quantification of regional tissue perfusion following intraosseous or IV infusion. It should be noted that dynamic nuclear scintigraphy involving $^{99m}$Tc pertechnate does not compare radionuclide activity to concentrations of a therapeutic agent that can be achieved via regional limb perfusion techniques.

Concentrations of antimicrobials in synovial structures after regional limb perfusion of such agents vary considerably.1,4 This variability has been attributed to the volume of perfusate used, loss of perfusate, and variations in tourniquet pressure. In the horses used in our study, we attempted to minimize the variability of radionuclide activity associated with these factors by administration of a predetermined perfusate volume per kilogram of body weight (ie, 0.1 mL/kg), placement of a custom-made cannulated screw to minimize leakage from the metacarpal administration site, and application of the 10-cm-wide rubber tourniquet by the same individual each time. The volume of perfusate used by other investigators has ranged from 20 to 60 mL/horse.1-10,a The volume of perfusate used in the present study is comparable to the volume used clinically by the authors to treat soft tissue, bone, and synovial infections of the distal portion of the limb in horses.

Finsterbusch et al12 evaluated perfusion of radiolabeled albumin (an endovascular tracer) in healthy and infected limbs in rabbits. Those investigators injected radiolabeled albumin into the medullary cavity of the femur of rabbits following application of an Esmarch bandage and tourniquet. Counts of radiolabeled albumin were measured at the distal metaphysis of the femur and at the ear (representative of systemic levels of activity). Counts at the distal metaphysis of the femur were high after perfusion and decreased rapidly after removal of the tourniquet, compared with counts recorded at the ear, which increased slowly until the tourniquet was removed.12 In our study, application of a tourniquet for as long as 30 minutes was essential for the maintenance of high radionuclide counts in the distal portion of the forelimb in horses, regardless of which route of administration of $^{99m}$Tc pertechnate was used. The intraosseous and IV techniques without the use of a tourniquet resulted in rapid redistribution of $^{99m}$Tc pertechnate and low radionuclide counts in all 3 regions of interest. Use of a tourniquet prevented accumulation of detectable levels of $^{99m}$Tc pertechnate activity in the limb proximal to the tourniquet. Unlike findings of the study by Finsterbusch et al,12 use of the tourniquet resulted in maintenance of high radionuclide activity in the distal portion of the limb of horses after the tourniquet was removed for the duration of the experiment for both the intraosseous and IV techniques. This was likely because $^{99m}$Tc pertechnate diffuses into the extracellular space, unlike radiolabeled albumin.13 Results of our study indicate that solutions that are administered via intraosseous or IV routes to achieve regional limb perfusion remain localized after tourniquet removal. The use of a tourniquet probably forces the perfusate out of the vascular compartment, and diffusion occurs along a drug concentration gradient distal to the tourniquet.11 Therefore, once the tourniquet is removed, redistribution of the solution occurs gradually. Data from further studies to evaluate the duration of tourniquet application that is necessary to maintain high radionuclide counts (ie, achieve effective regional limb perfusion) in the distal portion of the limb after administration of radiopharmaceuticals would be valuable.

In the present study, use of the IOT and IVT techniques resulted in the same radionuclide activity in the distal portion of the metacarpus, proximal and middle phalanges, and distal phalanx. This finding is probably attributable to the fact that by use of either technique, the injected drug gains access to the vascular compartment and diffuses throughout the tissues of the distal portion of the limb, depending on the volume of perfusate and the drug concentration gradient. Further research to compare regional perfusion characteristics of drugs administered via intraosseous and IV routes in infected limbs would be necessary to confirm the findings of our study and ascertain whether both techniques may be used equally effectively in horses with infections of the distal portion of limbs.

Compared with findings in the IVT treatment group, there was a higher radionuclide count in the distal portion of the metacarpus in the IOT treatment group. Although not significant, radionuclide counts in the distal portion of the metacarpus also appeared to be higher in the ION treatment group, compared with those in the IVN treatment group, for the duration of the experiment. This could be explained by the fact that the $^{99m}$Tc pertechnate solution was directly injected at this site and diffuses primarily as a result of a drug concentration gradient. Within the IVT treatment group, radionuclide activity was more evenly distributed throughout the distal portion of the limb; this is most likely related to the fact that after IV administration, the agent diffuses initially within the vascular compartment; subsequently, the concentration gradient becomes increasingly important as the drug diffuses out of the vascular compartment to the tissue compartment.

The experiment of the present study was designed as a randomized crossover study with a washout period of 7 days. Consequently, some of the IVT and IVN treatments were performed following the IOT and ION treatments. It is possible that there was leakage of perfusate through the hole in the distal portion of the metacarpus when an IV or IVN treatment was performed following an ION or IOT treatment; however, this was not evident grossly during the infusions or in the scintigraphic images.
Because the present study was designed to evaluate perfusion and clearance of radionuclide activity in the distal portion of the forelimb of horses, \( ^{99m} \)Tc pertechnate was used. Differentiation of radionuclide activity between soft tissue and bone was not possible in our study because the radionuclide used was not bound to a phosphonate compound. The ability to differentiate between radionuclide activity in bone and soft tissue of the distal portion of the horses’ limbs would have provided useful information but would have required the use of a radionuclide that was bound to a phosphonate compound.

A major limitation of the study was the relatively short period during which radionuclide counts were measured after infusions. Ideally, radionuclide activity in the distal portion of the limb would have been measured dynamically for the first hour of the study, and subsequently, static images could have been acquired during the following 24 hours. Such an assessment may have revealed differences between the IV and intraosseous infusion techniques that were not detected in our study.

Results of the present study have suggested that the intraosseous and IV techniques used to achieve regional limb perfusion are likely to be similarly effective in delivering antimicrobial agents to distal portions of infected limbs in horses, particularly when concentration-dependent antimicrobials, such as aminoglycosides, are used. The high level of radionuclide activity that was maintained in the distal portion of limbs treated with \( ^{99m} \)Tc pertechnate via the intraosseous and IV techniques after removal of the tourniquet (and for the duration of the experiment) implies that the maintenance of localized high concentrations of drugs can be achieved by use of either technique. This is likely to be particularly important when administering antimicrobials that act in a time-dependent fashion. However, because of potential differences in diffusion characteristics of drugs in infused tissue, additional studies in horses would be necessary before a conclusion can be reached that IOT and IVT infusions of antimicrobials in the distal portion of the limbs would be similarly effective in achieving regional perfusion of those drugs in the distal portion of limbs with soft tissue, bone, or synovial infections.

References

b. Technicare Omega 500, Solon, Ohio.
c. Mirage, Segami Corp, Columbia, Ohio.