Pharmacokinetics and pharmacodynamics of enrofloxacin and a low dose of amikacin administered via regional intravenous limb perfusion in standing horses

Alberto Parra-Sanchez, MVZ; Joel Lugo, DVM, MS; Dawn M. Boothe, DVM, PhD; Earl M. Gaughan, DVM; R. Reid Hanson, DVM; Sue Duran, RPh, PhD; James K. Belknap, DVM, PhD

Objective—To evaluate the pharmacokinetic-pharmacodynamic parameters of enrofloxacin and a low dose of amikacin administered via regional IV limb perfusion (RILP) in standing horses.

Animals—14 adult horses.

Procedures—Standing horses (7 horses/group) received either enrofloxacin (1.5 mg/kg) or amikacin (250 mg) via RILP (involving tourniquet application) in 1 forelimb. Samples of interstitial fluid (collected via implanted capillary ultrafiltration devices) from the bone marrow (BMIF) of the third metacarpal bone and overlying subcutaneous tissues (STIF), blood, and synovial fluid of the radiocarpal joint were collected prior to (time 0) and at intervals after tourniquet release for determination of drug concentrations. For pharmacokinetic-pharmacodynamic analyses, minimum inhibitory concentrations (MICs) of 16 µg/mL (amikacin) and 0.5 µg/mL (enrofloxacin) were applied.

Results—After RILP with enrofloxacin, 3 horses developed vasculitis. The highest synovial fluid concentrations of enrofloxacin and amikacin were detected at time 0; median values (range) were 13.22 µg/mL (0.254 to 167.9 µg/mL) and 26.2 µg/mL (5.78 to 50.0 µg/mL), respectively. Enrofloxacin concentrations exceeded MIC for approximately 24 hours in STIF and synovial fluid and for 36 hours in BMIF. After perfusion of amikacin, concentrations greater than the MIC were not detected in any samples. Effective therapeutic concentrations of enrofloxacin were attained in all samples.

Conclusions and Clinical Relevance—In horses with orthopedic infections, RILP of enrofloxacin (1.5 mg/kg) should be considered as a treatment option. However, care must be taken during administration. A dose of amikacin > 250 mg is recommended to attain effective tissue concentrations via RILP in standing horses. (*Am J Vet Res* 2006;67:1687–1695)

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From the Department of Clinical Sciences (Parra-Sanchez, Lugo, Gaughan, Hanson, Duran, Belknap) and the Department of Anatomy, Physiology and Pharmacology (Boothe), College of Veterinary Medicine, Auburn University, Auburn, AL 36849. Dr. Belknap's present address is Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210.
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Address correspondence to Dr. Lugo.

Abbreviations

MIC	Minimum inhibitory concentration
RILP	Regional IV limb perfusion
MCIII	Third metacarpal bone
C_{max}	Peak concentration
AUC	Area under the curve

Treatment of septic arthritis and osteomyelitis in horses is difficult, tedious, and expensive. An orthopedic infection can end a horse's athletic career and life.[1] Successful treatment depends on many factors, including virulence of the causative agent; competence of host defense mechanisms; efficacy of a chosen antimicrobial drug; and importantly, tissue concentrations achieved after antimicrobial administration. Thus, resolution of an infection requires delivery of an appropriate antimicrobial to the target tissues in concentrations sufficiently greater than the MIC for the particular organism for an appropriate period of time, as well as adjunct medical and surgical treatments.[2] Systemic administration of antimicrobials can result in therapeutic concentrations of the drugs in affected tissues. However, changes in local blood supply associated with soft tissue sepsis or traumatic injury may negatively impact the efficacy of systemic administration of an antimicrobial because of decreased antimicrobial delivery. The resultant decrease in tissue concentrations of antimicrobials, combined with the isolation of bacteria from host defenses because of the damaged blood supply in infected tissues, can lead to treatment failure and contribute to development of antimicrobial resistance.[2,3]

Regional IV limb perfusion with antimicrobial agents has been described as an effective adjunctive treatment for septic disease conditions in the distal portion of the limbs of horses.[4] This technique delivers high concentrations of antimicrobials to the synovial and osseous structures of the distal portion of a limb. Aminoglycosides are the most common antimicrobials administered via regional limb perfusion because the most common pathogens responsible for orthopedic infections in horses are targeted by those drugs. Furthermore, regional treatment decreases the risk of nephrotoxicosis associated with systemic use of those agents.[5] The dose of amikacin routinely administered by RILP to horses in clinical practice is 500 mg to 2 g of antimicrobial diluted with physiologic saline (0.9%

NaCl) solution to a volume of 20 to 60 mL.[6] A prior investigation[7] revealed that 125 mg of amikacin achieved high antimicrobial concentration in the synovial fluid of the metacarpophalangeal (fetlock) joint following RILP in anesthetized horses. It was of interest to us to determine whether a dose of amikacin lower than that usually used by equine practitioners can be effectively administered to infected tissue sites via RILP in standing horses.

Although scientific data support the effectiveness of regional administration of aminoglycosides, the increasing incidence of multiple-antimicrobial resistance among bacteria (eg, methicillin-resistant *Staphylococcus aureus*) not only mandates judicious use of antimicrobial drugs, but also mandates validation of novel antimicrobial drug delivery systems such as RILP through administration of antimicrobials with known efficacy against the organisms.[8,9] Other antimicrobial agents have been administered to horses via RILP, including cefotaxime, ampicillin, and vancomycin.[8] However, further investigations are essential to understand the pharmacokinetics of other antimicrobial agents as alternatives for RILP treatments.

Enrofloxacin, a fluoroquinolone approved for use in animals, is characterized by an antimicrobial activity spectrum and distribution pattern desirable for treatment of orthopedic infections in the distal portion of limbs of horses.[10] The pharmacokinetics of enrofloxacin after oral and parenteral administration in horses have been reported.[11,12] However, enrofloxacin has not been studied after RILP because it has been reported to cause vasculitis[13]; furthermore, there is potential for a detrimental effect of high concentrations on chondrocytes or synovial structures. Although adverse articular effects associated with enrofloxacin are known, particularly in young animals,[14] septic arthritis is likely to be associated with more detrimental cartilage damage if not treated early and aggressively.[15]

In pharmacokinetic-pharmacodynamic analyses, the method selected to collect interstitial fluid can influence the concentration of the drug of interest and the estimates of pharmacokinetic parameters.[16] Tissue samples are usually collected after euthanasia or via biopsy procedures or by use of tissue cages in horses that are standing or undergoing surgery. Homogenization of these tissue samples can overestimate lipophilic drug concentrations and underestimate hydrophilic drug concentrations because of the combination of interstitial fluid, intracellular fluid, and blood during the homogenization process.[17,18]

Capillary ultrafiltration is a novel sampling method for collecting low–molecular-weight substances (ie, interstitial fluid) in living biological systems.[19,20] The ultrafiltration device allows the collection of non–protein-bound fluid molecules from the interstitial space that can be directly analyzed without extraction, thus eliminating homogenization-associated problems. Advantages of the capillary ultrafiltration technique for pharmacokinetic studies include minimal invasiveness and preparation and serviceable patency of several weeks' duration that facilitates multiple sample collections.[20] Signs of discomfort are minimal, and animals in which the devices are implanted can move freely throughout a study period. Pharmacokinetic assessments of many drugs have been conducted by use of capillary ultrafiltration probes implanted in the subcutaneous tissues of rats and dogs,[21,22] but to our knowledge, the use of such probes in the limbs of horses has not yet been investigated.

The purpose of the study reported here was to evaluate pharmacokinetic-pharmacodynamic parameters of enrofloxacin and a low dose of amikacin administered via RILP in standing horses. The specific objectives were to collect samples of blood, synovial fluid, and the interstitial fluid of subcutaneous tissue and bone marrow from standing horses for pharmacokinetic evaluation of enrofloxacin and amikacin administered via RILP; investigate pharmacodynamic indices of amikacin and enrofloxacin in these samples by use of MICs that would be applicable to most common equine pathogens (MIC of 16 µg/mL for amikacin and 0.5 µg/mL for enrofloxacin); and assess an ultrafiltration system for collection of interstitial fluid samples from bone marrow and subcutaneous tissues in the area of perfusion for in vivo assessment of drugs in horses.

Materials and Methods

Animals—Fourteen healthy adult horses of various breeds were included in a parallel-design study. The horses weighed 375 to 513 kg (mean ± SD weight, 451.7 ± 44.9 kg), and their age ranged from 2 to 18 years (mean ± SD age, 10.6 ± 5.0 years). Horses were determined to be free of radiocarpal joint disease on the basis of history and findings of clinical and previous radiographic examinations. None of the horses had received antimicrobial treatment within 3 months of the study. Horses were randomly allocated to 1 of 2 groups (7 horses/group); group 1 received enrofloxacin,[a] and group 2 received amikacin.[b] For each horse, the forelimb to be perfused was randomly selected. All procedures were approved by the Auburn University Institutional Animal Care and Use Committee.

Experimental methods—Twenty-four hours prior to RILP, an IV catheter was placed in a jugular vein of each horse for drug administration. Two capillary ultrafiltration probes[c] were positioned in 1 forelimb for in vivo collection of interstitial fluid from the bone marrow of the MCIII and overlying subcutaneous tissue. To obtain serum, blood samples were obtained via venipuncture from either the right or left jugular vein (contralateral to the site of jugular catheter placement). Interstitial fluid samples were collected via the ultrafiltration probes, and synovial fluid was collected from the radiocarpal joint. The first sample collection occurred prior to release of both tourniquets applied during RILP (time 0; approx 25 to 30 minutes after the end of perfusion); subsequent samples were collected at 0.5, 1, 4, 8, 12, 24, and 36 hours after both tourniquets were released. Horses were hospitalized for the duration of the study period. After the end of the experimental protocol (ie, after the final collection of samples), phenylbutazone[d] (4.4 mg/kg, PO, q 24 h) was administered for 4 days. Horses were stall rested for 4 weeks and closely monitored for any evidence of lameness, swelling of the treated limb, or infection. Limbs were bandaged and bandages were changed on alternate days until the surgical staples were removed 14 days after surgery.

Placement of capillary ultrafiltration devices—Horses were placed in stocks and sedated with detomidine hydrochloride[e] (0.02 mg/kg, IV). A high 4-point nerve block and local infusion of 2% mepivacaine hydrochloride[f]

around the dorsal aspect of MCIII provided regional anesthesia at probe placement sites. Capillary ultrafiltration probes were placed in the medullary cavity of the MCIII and subcutaneous tissue overlying the bone (**Figure 1**). A 2-cm stab incision was made through the skin, subcutaneous tissue, and periosteum on the proximolateral aspect of MCIII, approximately 4 to 5 cm below the carpometacarpal joint. Access to the medullary cavity was achieved by drilling a 4.5-mm bicortical hole (approx 70 mm in length) in a proximolateral to distomedial direction at a 60- to 70-degree angle to the MCIII. A second stab incision was made at the point of exit of the drill bit at the distomedial aspect of the limb. A 70-mm, hollow, stainless steel cannula containing the ultrafiltration probe was introduced into the medullary cavity from the proximal to the distal cortical hole. Once the ultrafiltration probe was inserted into the medullary cavity, the hollow cannula covering was removed through the distal hole, leaving the ultrafiltration probe in place. A second ultrafiltration probe was placed dorsolaterally or dorsomedially in the subcutaneous tissue, approximately 3 cm distal to the first ultrafiltration probe and parallel to MCIII. A 2-cm stab incision was made through the skin and subcutaneous tissue, and the hollow, stainless steel cannula was introduced (proximal to distal direction) into the subcutaneous space. A second incision was made over the distal tip of the cannula, and the ultrafiltration probe was inserted by use of the same technique as described for the medullary cavity probe. The probes were secured with skin staples, and a sterile bandage was applied from the distal aspect of the antebrachium to the proximal metacarpophalangeal joint.

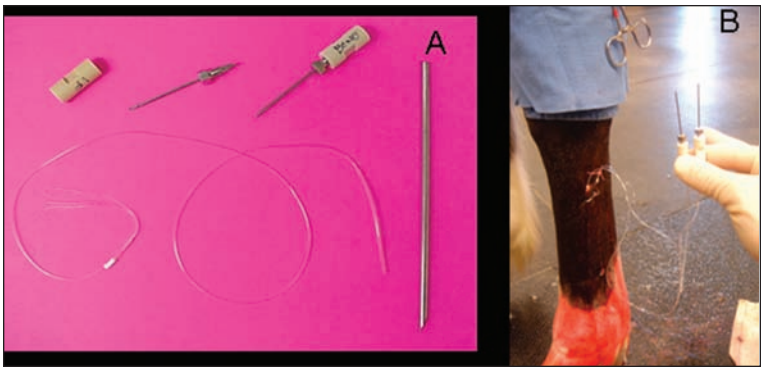

Figure 1—Photographs of components of in vivo capillary ultrafiltration probes used for collection of interstitial fluid samples and their application to the distal portion of the forelimb of a horse. A—Capillary ultrafiltration probe components. In the top row from left to right, the hub connector and needle are shown separately and assembled; the ultrafiltration probe (bottom left) and needle introducer (bottom right) are also displayed. B—Capillary ultrafiltration probes (for collection of interstitial fluid samples) are placed in the medullary cavity of the MCIII and in overlying subcutaneous tissue of the left forelimb of a horse undergoing regional IV perfusion of an antimicrobial agent in that limb. The horse's head is to the left of this image.

Figure 2—Photograph of 2 evacuated tubes attached to their respective ultrafiltration probes. Ultrafiltration probes were placed in the medullary cavity of the MCIII and in the overlying subcutaneous tissue of the left forelimb of a horse undergoing regional IV perfusion of an antimicrobial agent in that limb. The evacuated tubes were used to create the negative pressure necessary to collect interstitial fluid from the bone marrow cavity and subcutaneous tissue space. The horse's head is to the left of this image.

RILP—Horses were restrained in the stock and sedated with detomidine hydrochloride (0.02 mg/kg, IV). An Esmarch bandage[g] tourniquet (width, 5 inches) was placed 25 to 30 cm above the accessory carpal bone, and another tourniquet was placed at the level of the metacarpophalangeal joint. Roll gauze was placed over the cephalic vein before application of the tourniquet to attain better compression of the vascular structures. After aseptic preparation, a 20-gauge, 2-inch catheter[h] was introduced into the cephalic vein and a 7-inch IV extension set[i] was attached to the catheter. Perfusion was performed with either amikacin (250 mg) or enrofloxacin (1.5 mg/kg) diluted with lactated Ringer's solution[j] to a volume of 60 mL. The infusate was slowly administered manually over a period of 5 minutes, and tourniquets remained in place for approximately 30 minutes after perfusion. To limit variability of tourniquet application, the same clinician (AP) placed both tourniquets and perfused all horses in the study. After perfusion of the antimicrobial and removal of the tourniquets, the 20-gauge catheter was removed and a pressure bandage was applied to the venipuncture site.

Sample collection—At each collection time point, samples of the interstitial fluid in the bone marrow of MCIII and overlying subcutaneous tissue, synovial fluid from the radiocarpal joint, and blood (to obtain serum) were collected. Samples of interstitial fluid were collected by replacing the evacuated tubes,[k] which provided continuous suction on the ultrafiltration probes (**Figure 2**). Two milliliters of synovial fluid was collected via aseptic arthrocentesis of the radiocarpal joint. A 20-gauge, 1-inch needle was introduced into the palmar pouch of the radiocarpal joint (1 cm proximal to the accessory carpal bone and 1 cm caudal to the radial epiphysis). Synovial fluid was aspirated with a 3-mL sterile syringe. Whole-blood samples were obtained by venipuncture of a jugular vein. Blood samples were immediately centrifuged, and serum was separated and stored at –80°C until assessed. Limbs were rebandaged after each collection. After

the final collection, the probes were removed and probe placement incisions were closed with surgical staples.

Sample analysis—All samples were stored at –80°C pending assay. Both enrofloxacin and amikacin assays were validated for each sample type. Amikacin was detected by use of fluorescence polarization immunoassays.[l] Enrofloxacin was detected via high-pressure liquid chromatography according to previously described methods.[23] Serum samples were thawed and centrifuged through a 10-kd membrane for 2 hours to remove cellular debris and proteins. The resulting filtrates were analyzed via reverse-phase chromatography,[m] involving a mobile phase comprised of phosphate buffer, methanol, and acetonitrile, followed by fluorescence detection with excitation at 280 nm and emission at 450 nm. The upper and lower limits of detection for enrofloxacin were 30 and 5,000 ng/mL, respectively. By use of control samples spiked with the antimicrobial to spanned concentrations, values were within 15% of the known concentration. For amikacin, the upper and lower limits of detection were 0.8 and 50 ng/mL, respectively. By use of control samples spiked with the antimicrobial to spanned concentrations, values were within 15% of the known concentration.

Data analysis—Drug concentrations (log) in samples-versus-time curves underwent noncompartmental linear regression analysis[n] with the trapezoidal method to determine AUC to infinity. Additional parameters included C_{max} (reported as actual value), time to reach peak concentration, AUC, and disappearance half-life. Two pharmacokinetic-pharmacodynamic indices were calculated for each tissue: the C_{max}:MIC and AUC:MIC ratios. For analyses, MICs of 16 µg/mL and 0.5 µg/mL were used for amikacin and enrofloxacin, respectively.[17] Descriptive statistics were determined for pharmacokinetic parameters and pharmacodynamic-pharmacokinetic indices for each sample type and drug. Data were expressed as mean ± SD (normally distributed data) or as median and range (nonnormally distributed data). Comparisons of indices between drugs were not made because doses were not comparable. Thus, comparisons of C_{max}:MIC and AUC:MIC ratios were limited within treatment groups among sample types by use of an ANOVA on ranks or a 1-way ANOVA test and respective multiple comparison test (Tukey test for ANOVA on ranks or Holm-Sidak test for 1-way ANOVA) to identify significant differences. A significant difference was set at a value of $P \leq 0.05$. Computer software was used for data analysis.[o]

Results

Lameness was not detectable at the walk in any of the horses following drilling of the MCIII and implantation of the ultrafiltration device. No complications developed as a result of placement and maintenance of the ultrafiltration probes or during RILP with amikacin. Signs of inflammation (transient perivascular edema and mild cellulitis) were evident at the site of infusion in 3 horses that received enrofloxacin perfusion; clinical signs were detected approximately 6 to 8 hours after perfusion and resolved without treatment within 48 hours. Surgical incisions healed well in all horses. At 4 weeks following the experimental protocol, horses were in good health and not lame, and eventually all of the horses returned to the large animal teaching herd.

Collection of interstitial fluid with the ultrafiltration probe was successful at all time points in 12 of the 14 horses. In 1 horse, the permeable area of the ultrafiltration probe decreased, and in another horse, constant slippage of the limb bandage obstructed both probes at the level of the nonpermeable portion; as a result, at some time points, insufficient fluid was collected from these horses for antimicrobial detection. For purposes of analysis, these samples were considered as uncollected or missing samples. At each time point, the mean volume of interstitial fluid collected with the ultrafiltration probes from the bone marrow of MCIII and from the overlying subcuta-

Table 1—Mean ± SD volumes of interstitial fluid collected by use of ultrafiltration probes placed in the bone marrow of the MCIII and overlying subcutaneous tissue of 1 forelimb in each of 7 horses before (time 0) and at intervals after release of tourniquets applied during RILP of that limb with 250 mg of amikacin.

	Interstitial fluid volume (µL)	
Time (h)	Subcutaneous tissue	Bone marrow
0	61.4 ± 20.3	114.2 ± 62.6
0.5	87.1 ± 93.9	57.1 ± 18.8
1	178.5 ± 99.4	257.0 ± 123.9
4	314.2 ± 157.3	335.7 ± 165.1
8	314.2 ± 121.2	335.6 ± 165.1
12	928.3 ± 345.3	814.2 ± 409.9
24	800.0 ± 288.6	635.7 ± 430.8
36	928.5 ± 411.1	771.4 ± 394.6

Table 2—Pharmacokinetic values (median [range]) determined in samples of interstitial fluid from the bone marrow of the MCIII and overlying subcutaneous tissue, synovial fluid from the radiocarpal joint, and serum obtained from 7 horses administered amikacin (250 mg) and 7 horses administered enrofloxacin (1.5 mg/kg) via regional IV perfusion of 1 forelimb.

Variable	Treatment	Bone marrow	Subcutaneous tissue	Synovial fluid	Serum
AUC (µg•h/mL)	Amikacin	62.96 (28.30–419.50)	108.74 (28.36–176.99)	66.01 (12.77–214.82)	10.70 (1.70–943.85)
	Enrofloxacin	25.37 (5.43–262.81)	70.76 (5.26–469.50)	71.49 (7.60–126.70)	6.63 (3.14–45.80)
C_{max} (µg/mL)	Amikacin	6.63 (2.81–11.18)	19.41 (4.1–29.4)	26.27 (5.7–50.0)	1.95 (1.19–3.57)
	Enrofloxacin	1.09 (0.27–67.90)	3.99 (0.44–198.00)	13.22 (0.25–167.9)	0.70 (0.58–8.70)
$T_{½}$ (h)	Amikacin	5.41 (2.15–67.07)	2.01 (1.64–15.40)	2.04 (1.06–10.59)	4.47 (0.77–269.64)
	Enrofloxacin	27.76 (9.80–37.17)	12.04 (4.22–463.45)	13.24 (7.08–90.00)	6.76 (5.12–16.99)
T_{max} (h)	Amikacin	4 (1–4)	4 (1–4)	0.25 (0–1)	0.25 (0–4)
	Enrofloxacin	1 (0–4)	1 (1–4)	0 (0–4)	0 (0–0.5)

$T_{½}$ = Disappearance half-life of the antimicrobial. T_{max} = Time to reach peak antimicrobial concentration.

Table 3—Pharmacokinetic-pharmacodynamic indices (median [range]) determined from samples of interstitial fluid from the bone marrow of the MCIII and overlying subcutaneous tissue, synovial fluid from the radiocarpal joint, and serum obtained from 7 horses administered amikacin (250 mg) and 7 horses administered enrofloxacin (1.5 mg/kg) via regional IV perfusion of 1 forelimb.

Variable	Treatment	Bone marrow	Subcutaneous tissue	Synovial fluid	Serum
AUC:MIC ratio	Amikacin	3.94 (1.77–26.22)	6.80 (1.77–11.06)	4.13 (0.79–13.43)	0.67 (0.11–58.99)
	Enrofloxacin	50.7 (10.8–525.5)	141.5 (10.5–939.0)	142.9 (15.2–253.4)	13.2 (6.2–91.5)
C_{max}:MIC ratio	Amikacin	0.41 (0.17–0.70)	1.23 (0.26–1.84)	1.64 (0.36–3.10)	0.12 (0.07–0.22)
	Enrofloxacin	2.19 (0.54–135.80)	7.96 (0.98–396.13)	26.5 (0.50–335.95)	1.40 (1.16–17.40)

For analyses, MICs of 16 µg/mL and 0.5 µg/mL were used for amikacin and enrofloxacin, respectively.

neous tissues did not differ in horses perfused with amikacin (**Table 1**).

Pharmacokinetics—The highest concentration after RILP of enrofloxacin was detected in synovial fluid (median concentration, 13.22 µg/mL; range, 0.254 to 167.9 µg/mL), followed (in decreasing order) by the concentration in interstitial fluid from the subcutaneous tissue (median concentration, 3.99 µg/mL; range, 0.44 to 197.6 µg/mL) and the concentration in interstitial fluid from the bone marrow (median concentration, 1.09 µg/mL; range, 0.27 to 67.9 µg/mL). The median value of C_{max} in serum was 0.70 µg/mL (range, 0.58 to 8.7 µg/mL; **Table 2**). For amikacin, the highest C_{max} value was also detected in synovial fluid (median value, 26.2 µg/mL; range, 5.78 to 50.0 µg/mL), followed (in decreasing order) by the value in interstitial fluid from the subcutaneous tissue (median C_{max}, 19.4 µg/mL; range, 4.1 to 29.4 µg/mL) and the value in interstitial fluid from the bone marrow (median C_{max}, 6.6 µg/mL; range, 2.8 to 11.1 µg/mL). The median C_{max} value for amikacin in serum was 1.9 µg/mL (range, 1.1 to 3.5 µg/mL). The times to reach C_{max} were highly variable among sample types (Table 2).

Pharmacokinetic-pharmacodynamic indices—Results of in vitro studies of concentration-dependent antimicrobials suggest that antibacterial efficacy is more likely to be maximized if the C_{max}:MIC ratio is ≥ 10 or the AUC:MIC ratio is ≥ 125. On the basis of those targeted indices, the required C_{max}:MIC ratio for enrofloxacin was achieved in all samples from 3 of 6 horses; the required AUC:MIC ratio was achieved in synovial fluid and interstitial fluid of the subcutaneous tissue in all 6 horses and in the bone marrow interstitial fluid in 4 of 6 horses, but was not achieved in serum in any of the 6 horses. For amikacin, the target C_{max}:MIC and AUC:MIC ratios were not achieved in any of the 7 horses or samples (**Table 3**). There were no significant differences in C_{max}:MIC or AUC:MIC ratios among sample types in horses perfused with either enrofloxacin or amikacin.

Discussion

The activities of fluoroquinolones and aminoglycosides against microorganisms are regarded as concentration dependent.[10] Thus, as the extent to which the antimicrobial concentration attained in a given tissue exceeds the MIC increases, the rate of bacterial killing increases.[24] Therefore, RILP is an excellent method for administration of concentration-dependent antimicrobials in horses because high drug concentrations can be achieved in the distal portion of treated limbs.[7] Recent scientific investigations[5,8] have revealed high concentrations of gentamicin and amikacin in bone and synovial fluid of anesthetized horses for as long as 24 hours after RILP. Other investigators have also described the pharmacokinetics of time-dependent antimicrobials such as vancomycin and ceftiofur after RILP in anesthetized horses.[7,25] In field and hospital conditions, equine practitioners routinely use chemical restraint to perform RILP in standing horses. The procedure in standing horses appears to be well tolerated and is easy to perform because no specialized facilities or equipment is required. In the present study, administration of 1.5 mg of enrofloxacin/kg via RILP in standing horses resulted in apparently therapeutic antimicrobial concentrations for approximately 24 hours within subcutaneous tissue and synovial fluid and for 36 hours in bone marrow. In marked contradiction, a low dose of amikacin (250 mg) administered via RILP did not result in apparently effective therapeutic antimicrobial concentrations in any of the analyzed samples (interstitial fluid, synovial fluid, or serum) collected from horses.

The pharmacokinetic parameters (C_{max} and AUC) provide guidance concerning achievable concentrations in living tissues; high values of C_{max} and AUC are important for the efficacy of concentration-dependent antimicrobial agents. Conversely, pharmacodynamic indices for antimicrobial agents describe the interaction between the drug and the microorganisms (ie, in vitro activity). Pharmacodynamic predictors that evaluate the efficacy of concentration-dependent antimicrobials include the ratio of C_{max} to MIC and the AUC:MIC ratio. The maximum efficacy of these concentration-dependent antimicrobials appears to be achieved at a C_{max}:MIC ratio ≥ 10 or an AUC:MIC ratio ≥ 125. The AUC:MIC ratio is the most accurate predictor of bacteriologic and clinical response to fluoroquinolone administration.[26] Exceeding these targeted indices not only enhances bacterial killing, but also reduces the risk of development of microbial resistance. In the present study, the potential efficacy of the antimicrobial agents (as indicated by the C_{max}:MIC and AUC:MIC ratios) varied. Our data indicated that a dose of 1.5 mg of enrofloxacin/kg resulted in sufficient interstitial fluid, synovial fluid, and serum concentrations to achieve C_{max}:MIC and AUC:MIC ratios that were sufficiently high to target most of the susceptible bacteria associated with orthopedic infections in horses.[27] By apply-

ing an MIC value of 0.5 µg/mL, enrofloxacin administered at a dose of 1.5 mg/kg via RILP resulted in pharmacodynamic values well in excess of the cited indicator of successful treatment (C_{max}:MIC ratio) in 3 of 6 horses. In treated limbs, this dose also exceeded target AUC:MIC ratios for synovial fluid and interstitial fluid of the subcutaneous tissue in all 6 horses and the AUC:MIC ratio for interstitial fluid of the bone marrow in 4 of 6 horses.[28] In contrast, 250 mg of amikacin did not result in C_{max}:MIC nor AUC:MIC ratios that were potentially effective for treatment of organisms with an MIC of 16 µg/mL in interstitial fluid, synovial fluid, or serum.

Although apparently effective concentrations of enrofloxacin were more predictably attained in the different samples, the interstitial fluid, serum, and synovial fluid concentrations of either antimicrobial investigated were highly variable. To our knowledge, no information is available regarding the MICs for equine pathogens causing orthopedic infections in horses that can be used for pharmacokinetic-pharmacodynamic indices. Therefore, we selected targets on the basis of susceptible MIC values published by the Clinical and Laboratory Standards Institute because of the high possibility that these results would be relevant for any isolate considered susceptible to amikacin or enrofloxacin.[29] Although an MIC of 16 µg/mL is known to be satisfactory for amikacin, there are no official MIC standards for enrofloxacin to apply to pathogens that affect horses. An MIC of 0.5 µg/mL is sufficient for the most important pathogens, including coliforms, enterobacters, and *Staphylococcus* spp.[29] On the basis of the pharmacodynamic findings in our study, enrofloxacin (1.5 mg/kg) administered via RILP in standing horses may be a good alternative for the treatment of orthopedic infections in horses. In contrast, a comparatively higher dose of amikacin (500 mg to 2 g) for RILP administration in standing horses is recommended.

Enrofloxacin was selected for investigation in the present study because its antibacterial properties are ideal for use against most common pathogens involved in orthopedic infections in horses.[30] However, high concentrations of enrofloxacin, as might be obtained with local administration (intra-articular route or via RILP), are of concern because of associated adverse effects on chondrocytes and tendon metabolism in vitro.[14,15] The chondrotoxic effects are characterized by suppression of proteoglycan synthesis, proteoglycan degradation, and chondrocyte necrosis.[15] In our study, the mean ± SD C_{max} of enrofloxacin in synovial fluid was 54.37 ± 69.07 µg/mL (median, 13.22 µg/mL; range, 0.254 to 167.9 µg/mL). On the basis of findings of previous in vitro studies, synovial fluid concentration following RILP would not be expected to be high enough to cause chondrocyte damage (ie, ≤ 1,000 µg/mL).[14] The effects of sustained concentrations of antimicrobial agents, which can be achieved with multiple RILP treatments, still require further investigation. Until proven otherwise, we do not recommend administration of enrofloxacin via RILP in young horses (≤ 2 years old) because these horses appear to be more susceptible to chondrotoxic effects of quinolones.[31]

The enrofloxacin concentration achieved in synovial fluid following RILP was approximately 5 to 10 times as great as that reported after systemic administration of 5.0 mg of enrofloxacin/kg in horses.[12] In the interstitial fluid samples collected from the bone marrow and subcutaneous tissue, concentrations of enrofloxacin were also high, and compared with the synovial fluid concentration, remained high for a longer period (36 hours vs 24 hours). The high lipid solubility of the drug most likely resulted in excellent distribution and preferential accumulation in these tissues. In horses with orthopedic infections, these properties are crucial to maintaining high concentrations of antimicrobials for extended periods while minimizing dosing intervals during the treatment of infected soft tissues or osseous structures. In addition to increased bacterial killing and prolonged postantimicrobial effect, longer dosing intervals can reduce the risk for development of adaptive resistance by microorganisms.[32] Considering the strong postantimicrobial effect of enrofloxacin and the results of the present study, which indicated that concentrations of enrofloxacin were greater than the MIC for 36 hours in the interstitial fluid of bone marrow and for 24 hours in the interstitial fluid of subcutaneous tissue and synovial fluid, we recommend administration of enrofloxacin via RILP no less frequently than every 36 hours. However, because antimicrobial activity at infected sites can be diminished, pharmacokinetic-pharmacodynamic studies under septic conditions require further investigation.[33]

The perivascular edema and vasculitis of the distal portion of the limb that developed after perfusion with enrofloxacin in 3 horses were most likely attributable to extravasation of the drug solution during RILP, which caused an inflammatory response in the surrounding tissue. Irritation is most likely caused by the vehicle used to suspend the drug or the pH adjustment required for stability of the product. In a pilot study to assess different doses and formulations for use in the present study, the preparation of enrofloxacin for use in small animals (50 mg/dL) or doses > 2.0 mg/kg caused severe cellulitis in the perfused limb of 3 horses. In our clinical experience, there is always some degree of solution extravasation after RILP in standing horses because high hydrostatic pressure results in solution leakage at the venipuncture site. To limit extravasation, we recommend the use of an IV catheter for perfusion, positioning the infusion site 6 to 10 cm distal to the proximal tourniquet, dilution of the perfusate with physiologic saline solution to a volume of 60 mL, slow administration of the perfusate during a 5-minute period, and removal of catheters after tourniquets are released. By following these steps, we have administered enrofloxacin via RILP to 12 horses with infections of the distal portion of limbs (ie, septic arthritis) with no adverse effects.

Amikacin has been considered the antimicrobial of choice for local and regional administration because it is effective against a wide range of microorganisms implicated in septic conditions. The administration of 150 mg to 1 g of amikacin by RILP and regional intraosseous limb perfusion have been reported to result in high antimicrobial concentrations in synovial

fluid and bone in anesthetized horses.[7,34] In the present study, a smaller dose of amikacin (250 mg) was used to determine whether a dose lower than that usually administered by practitioners (typically 500 mg to 2 g) would provide effective antimicrobial concentrations in interstitial fluid, synovial fluid, and serum. Amikacin tissue concentrations in the horses of this study were 20% to 50% less than those previously reported.[7,34,35] In addition, pharmacodynamic data (AUC:MIC and C_{max}:MIC ratios) suggested that administration of 250 mg of amikacin did not provide adequate concentrations for effective therapy. The most likely reason for the difference between the findings of our RILP study (ie, attainment of inadequate amikacin concentrations) and those reported previously is different methodologies. As indicated, in most of the other studies, a higher dose of amikacin was used. Furthermore, we performed RILP in standing horses rather than in anesthetized and laterally recumbent horses. Systemic blood pressure in anesthetized horses is decreased as a result of decreased cardiac output and peripheral vascular resistance because of the influence of anesthetic agents and position.[36,37] In addition, compared with recumbent horses, systemic blood pressures in the distal portion of limbs of standing horses are likely greater because of the weight of the blood in the veins and changes in peripheral vascular resistances as a result of motion.[38] Perhaps the high systemic blood pressure in standing horses (compared with values in anesthetized horses) influences leakage of the perfusate into the systemic circulation and leads to lower antimicrobial concentrations in the perfused region.

The high variability of concentrations in interstitial fluid, synovial fluid, and serum for both antimicrobials investigated in the present study was comparable with results of previous studies[5,7,34,35] involving RILP with amikacin and other antimicrobials in horses. This variability may be attributable to differences in tourniquet placement (including location [distal limb region vs antebrachium] and pressure application [ie, individual variances between antebrachial muscles]), differences in medullary volume of involved bones, and leakage of the perfusate during perfusion.[34] Other investigators[8] have speculated that discrepancies in body weight and antimicrobial dose were causes of high C_{max} variability associated with RILP. However, in our investigation, similar C_{max} variability was identified for both antimicrobials, horses perfused with amikacin received the same dose of 250 mg, and horses perfused with enrofloxacin received a dose that was based on body weight. Even with tourniquet placement and RILP procedures performed by the same investigator (AP), discrepancies likely occurred among horses with regard to tourniquet positions and pressures.

Absorption of the perfusate into the systemic circulation during the RILP procedure occurred in all horses in the present study, and serum antimicrobial concentrations were detected prior to release of the tourniquets in 11 of 12 horses. This apparent failure of the tourniquets may have happened for several reasons, including inadequate pressure application. In our study, the intent was to imitate clinical situations in which an elastic tourniquet would likely be used, and consequently, a pneumatic tourniquet was not used. Thus, application of pressure via each tourniquet was not monitored. Also, there was no exsanguination of the perfused region. The increased venous blood volume in the region as a result of administration of the perfusate may have caused a reduction in tourniquet efficacy.[39] Differences between performing the RILP procedure in standing and anesthetized horses may also affect physiologic effects of tourniquet application. Ideally, vascular occlusion occurs when external pressure is higher than arterial systolic pressure. However, tourniquet application to the distal portion of the limbs of anesthetized horses at a pressure of 600 mm Hg does not completely occlude blood flow.[39] This might be of greater influence in standing horses. Continued arterial blood flow with a greater degree of venous obstruction can cause engorgement of the vessels distal to the tourniquet and contribute to development of pain, which can induce restlessness and motion of the limb; this might then result in variations of tourniquet pressure, compared with pressures achieved in totally immobilized limbs during anesthesia and lateral recumbency. Another important factor to consider is the aforementioned difference in cardiovascular variables between the anesthetized and awake horses. These findings have not been previously reported and are very important concepts because equine practitioners more often perform RILP in standing horses using techniques similar to that used in our study.

In the present study, capillary ultrafiltration probes were successfully used for collection of fluid samples in standing horses. These devices allowed us to successfully evaluate the in vivo pharmacokinetic and pharmacodynamic data for the 2 antimicrobials in interstitial fluid, synovial fluid, and serum after RILP. Overall, the collection device was well tolerated, easy to place and maintain, and effective for the duration of the collection period. The volume of fluid samples collected by the probes did not differ significantly; in almost all collections (ie, in the absence of minor technical difficulties), the volume obtained was adequate for direct analysis. The volume of sample collected was representative of the interval between collections rather than representative of a specific measurement time point. Therefore, higher volumes were collected when more time elapsed between collections. Ultrafiltration probes have been used to collect interstitial fluid in different species including rats, dogs, mice, cats, and humans for analysis of drugs and interstitial tissue concentrations of electrolytes, proteins, and metabolites for as long as 30 days.[20,40] The main advantages of the use of such ultrafiltration probes in horses include continuous fluid collection, little or no manipulation of a sample prior to analysis, the ability to use the catheter 24 hours after implantation, and free movement of animals in a stall following implantation. The only disadvantage of these devices is that they are friable and care must be taken during use. In 2 horses, shrinkage of the capillary portion of the probe and obstruction of the nonpermeable portion of the probe resulted in collection of insufficient volumes of samples for

analysis. This technical error can be avoided by correct placement of the evacuated tubes and careful bandage application over the device.

Our data suggest that administration of 1.5 mg of enrofloxacin/kg via RILP should be considered as a treatment option in horses with orthopedic infections. However, care must be taken to avoid extravasation of the drug, which can result in serious complications. Further studies are required to determine the efficacy of enrofloxacin (1.5 mg/kg) administered via RILP to treat infectious conditions of the distal portion of the limbs of horses. With regard to administration of amikacin via RILP in standing horses, a dose greater than 250 mg is recommended when a cephalic vein is used for perfusion to achieve effective therapeutic concentrations of antimicrobial at the carpus and MCIII. The findings of the present study also emphasize the importance of careful placement of tourniquets for performing RILP in standing horses. In addition, to our knowledge, this is the first study in which the effectiveness of capillary ultrafiltration probes for in vivo collection of interstitial fluid in standing horses has been established.

a. Baytril 100, Bayer Animal Health, Shawnee Mission, Kan.
b. Amikacin, Sicor Pharmaceuticals Inc, Irvine, Calif.
c. Bioanalytical Systems Inc, Baltimore, Md.
d. Schering-Plough Animal Health, Union, NJ.
e. Dormosedan, Pfizer Animal Health, New York, NY.
f. Carbocaine-V, Abbott Laboratories, North Chicago, Ill.
g. Vet Surgical Resources Inc, Darlington, Md.
h. Terumo Medical Co, Elkton, Md.
i. Hospira Inc, Lake Forest, Ill.
j. Abbott Laboratories, North Chicago, Ill.
k. Vacutainer serum, 7.0 mL, BD Franklin Lakes, NJ.
l. Abbott TDX Laboratories, Abbott Park, Ill.
m. Luna C8 column, Phenomenex Inc, Torrance, Calif.
n. WINONlin, Pharsight Inc, Apex, NC.
o. Sigma Stat, Systat Software Inc, Richmond, Calif.

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