Faster perfusate instillation time results in more systemic leakage of amikacin sulfate when performing intravenous regional limb perfusion in horses

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OBJECTIVE
To evaluate if a difference in synovial amikacin concentrations exists in the radiocarpal joint (RCJ) following different durations of instillation of an IV regional limb perfusion (IVRLP) perfusate.

ANIMALS
7 healthy horses.

METHODS
Horses received 2 IVRLPs with 2 g amikacin diluted to 60 mL with 0.9% NaCl via the cephalic vein in a crossover study design with a wash-out period between procedures. Instillation of the perfusate was administered over a 1-minute (technique 1) and 5-minute (technique 5) period. Concentrations of amikacin within the RCJ were measured at time (T) 5, 10, 15, 20, 25, and 30 minutes after instillation of the perfusate. Systemic concentrations of amikacin were measured at T0, 5, 10, 15, 20, 25, 29 minutes, and 1 minute after tourniquet removal (T31). Amikacin concentrations were determined by fluorescence polarization immunoassay.

RESULTS
The median maximum concentration (C\text{MAX}) of amikacin within the RCJ for technique 1 was 338.4 µg/mL (range, 60 to 4,925 µg/mL), while the median C\text{MAX} for technique 5 was higher at 694.8 µg/mL (range, 169.2 to 3,410 µg/mL; \(P = .398\)). There was a higher amikacin blood concentration over time for technique 1 compared to technique 5 (\(P = .004\)).

CLINICAL RELEVANCE
Administration of perfusate at different rates did not significantly affect synovial concentration of amikacin within the RCJ when performing IVRLP. However, increased systemic leakage was noted when the perfusate was administered over 1 minute, which might affect synovial concentrations in a larger group of horses.

Keywords: regional limb perfusion, amikacin, equine, antimicrobials, radiocarpal joint

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Synovial sepsis and wounds to the equine distal limb can have grave consequences for the equine athlete. Wounds on the distal limb often have a higher level of contamination compared to other body sites, and synovial sepsis secondary to these types of wounds is common. Achieving adequate levels of antibiotics in the distal limb is therefore critical, and therapies that can achieve high levels of antibiotics in synovial structures are crucial in treatment of these conditions. IV regional limb perfusions (IVRLPs) result in increased concentrations of antibiotics to the distal limb through administration of an antibiotic distal to a tourniquet. Increased hydrostatic pressure within the venous vasculature will result in diffusion of the antimicrobial to the surrounding tissues, increasing antibiotic levels in tissues and synovial structures. Therefore, IVRLPs have become a common treatment modality to aid in the treatment of synovial infections and distal limb wounds over the last number of years.

IVRLPs are commonly performed with a concentration-dependent antibiotic, most frequently amikacin sulfate, but have also been reported with time-dependent antibiotics. Both historic and recent studies have shown that the most common bacteria isolated from orthopedic infections and septic synovial structures include Streptococcus.
spp., *Staphylococcus* spp., and *Enterobacteriaceae* spp. With a spectrum including gram-negative bacteria as well as *Staphylococcus* spp., amikacin continues to be a good choice that has been proven to be effective against these common isolates. Additionally, performing IVRLP using aminoglycoside antibiotics is advantageous as a higher ratio of maximum drug concentration (Cₘₐₓ) to the minimum inhibitory concentration (MIC) results in an improved bactericidal effect. For susceptible pathogens, the MIC for amikacin is considered to be ≤ 4 μg/mL, while 8 μg/mL is considered intermediate susceptibility, and ≥ 16 μg/mL is considered resistant. For optimal performance of aminoglycosides, a Cₘₐₓ/MIC ratio of between 8:1 and 10:1 is recommended. Therefore, ideal concentrations of amikacin within synovial fluid would be 40 μg/mL for susceptible organisms or > 160 μg/mL for resistant organisms after IVRLP.

Previous studies have demonstrated that these therapeutic levels can be achieved in the radiocarpal joint (RCJ) following IVRLP in the cephalic vein and that these levels may potentially increase in horses with synovitis. Various studies in the literature have looked at different variables that might increase synovial concentrations of amikacin while performing IVRLP, including tourniquet type and number, perfusate volume, and the use of adjunctive medications, etc. However, to the authors’ knowledge, no study has evaluated the effect of perfusate instillation time on Cₘₐₓ and time to maximum concentration (Tₘₐₓ) during IVRLP. Previous studies have administered the amikacin perfusate over times ranging from 1 minute to 5 minutes. The objective of this study is to evaluate the difference in amikacin concentration in the RCJ after different durations of instillation of the perfusate, comparing administration of the perfusate over 1 minute as opposed to 5 minutes. We hypothesize that a slower administration of perfusate over 5 minutes will reduce the amount of systemic leakage of amikacin and increase the Cₘₐₓ of amikacin in the RCJ.

**Methods**

**Animals**

Seven healthy adult horses were chosen at random from the equine research herd at the Center for Equine Health, UC Davis. All selected horses were free of lameness at the walk and had no evidence of carpal disease or vascular abnormalities associated with the cephalic vein. Horses were housed in individual pens throughout the duration of the study and were monitored closely for any joint swelling, lameness, or swelling at the site of perfusion. Horses were turned out to pasture during the wash-out period and after completion of the study. This protocol was approved by the University of California-Davis Institutional Animal Care and Use Committee.

**IV regional limb perfusion**

In February 2023, horses received IVRLP with 2 g amikacin sulfate diluted to 60 mL with sterile saline (0.9% NaCl) under standing sedation, with instillation of the perfusate given over a period of either 1 minute or 5 minutes. For each horse, the choice of forelimb and perfusate instillation time was randomized by use of an online randomization tool (Randomness and Integrity Services Ltd). The second technique was performed on the opposite forelimb in March 2023 after a 3-week washout period.

For every IVRLP, a unilateral median, ulnar, and medial cutaneous antebrachial nerve block was performed with 30 mL of 2% mepivacaine hydrochloride to reduce possible discomfort associated with the tourniquet. Horses were then sedated in standing stocks with IV detomidine hydrochloride (0.01 mg/kg) and butorphanol tartrate (0.01 mg/kg). Additional doses of detomidine hydrochloride (0.005 mg/kg) were administered during the procedure if the patient demonstrated discomfort (weight shifting, lifting the limb, or pawing), and the amount/number of doses was recorded. The cephalic vein of one forelimb was clipped and aseptically prepared. The lateral and dorsal aspects of the carpus were also aseptically prepared.

A wide rubber Esmarch tourniquet was placed approximately 15 cm proximal to the accessory carpal bone, with one roll of gauze placed over the cephalic vein underneath the tourniquet. The tourniquet was wrapped at least 10 turns and was applied by the same investigator (IK) for every perfusion. The regional limb perfusion was then performed in the cephalic vein using a 22-gauge, 2.5-cm catheter over either 1 minute or 5 minutes by the same investigator (LS). The injection of the perfusate was manually timed using a stopwatch with a rate of 1 mL/second for technique 1 and 1 mL/5 seconds for technique 5. After instillation of the perfusate, the catheter was removed, and a bandage was placed over the injection site with gauze and white tape. The tourniquet was left in place and was removed after the 30-minute synovial fluid sample was obtained. The procedure was repeated on the other limb using the alternate instillation time after a washout period of 3 weeks.

During the IVRLP, any movements were graded using a previously described system. Minor movements included a decrease in weight bearing, during which the hoof maintained contact with the ground while major movements were recorded when the entire hoof was lifted off the ground. Minor and major movements were recorded for the 0-to-10-, 10-to-20-, and 20-to-30-minute time periods.

**Sample collection**

A blood sample (2 to 3 mL) was collected into lithium heparin tubes prior to IVRLP and at 5, 10, 15, 20, 29, and 31 minutes (1 minute after tourniquet release) following completion of the perfusion. Synovial fluid (0.5 mL) was collected in lithium heparin tubes by arthrocentesis of the ipsilateral RCJ via the lateral palmar approach at 5, 10, 15, 20, 25, and 30 minutes following completion of the perfusion. If synovial fluid was not obtained from this location, arthrocentesis was performed via the dorsal
was analyzed by a pair concentration in synovial fluid. Normally distributed data were tested for normality with the Shapiro-Wilk test. The presence of an amikacin antibody solution. This is the ability to quantify amikacin concentration within a sample as free amikacin will bind to its antibody and partially inhibit the aggregation reaction. The differences in scattered light and absorbance can be quantified into a concentration-dependent curve, allowing for the measurement of amikacin concentration. Dilution of samples was done as necessary to measure samples that had a concentration outside of the calibrated curve. All analyses were performed by the Biochemical Laboratory at the University of California Davis Veterinary Medical Teaching Hospital.

Statistical analysis

Statistical analysis was performed using commercial statistical software (SPSS Statistics for Windows, version 28.0; IBM Corp). Data was evaluated for normality with the Shapiro-Wilk test. The CMAX and TMAX for each horse were determined by visual inspection of data for the amikacin concentration in synovial fluid. Normally distributed data was analyzed by a pair t test and non-normally distributed data by the Wilcoxon signed-rank test. To determine differences between systemic amikacin concentrations over time, a 2-way repeated measures ANOVA was used. For all analyses, significance was set at values of P < .05.

Results

Horses included in this study were 4 geldings and 3 mares. These horses included 2 Paint Horses, 1 Quarter Horse, 1 Thoroughbred, 1 Westphalian, 1 Oldenburg, and 1 Hanoverian, with a median age of 13 years (range, 5 to 18 years) and a median weight of 560 kg (range, 540 to 660 kg).

Synovial amikacin sulfate concentrations are presented (Table 1). With the infusion given over 5 minutes (technique 5), all horses reached the target minimum synovial concentration of 160 µg/mL needed to treat organisms with an MIC of ≤ 16 µg/mL. When the infusion was given over 1 minute (technique 1), only 5 of 7 horses reached the target minimum synovial concentration of 160 µg/mL.

The median synovial CMAX for technique 1 was 338.4 µg/mL (range, 60 to 4,925 µg/mL), while the median synovial CMAX for technique 5 was higher at 694.8 µg/mL (range, 169.2 to 3,410 µg/mL), although not statistically significant (P = .398).

The mean synovial (± SD) TMAX for technique 1 was 20 ± 7 minutes, with the mean synovial TMAX for technique 5 being 19 ± 9 minutes. In technique 1, 4 of the 5 horses that reached a CMAX of over 160 µg/mL had done so by 15 minutes, with the remaining horse reaching a CMAX of over 160 µg/mL by 30 minutes. In technique 5, 6 of the horses reached a CMAX of over 160 µg/mL by 20 minutes, with the remaining horse reaching a CMAX of over 160 µg/mL by 30 minutes. There was no significant difference in synovial TMAX between technique 1 and technique 5 (P = .783).

The systemic concentration of amikacin over time is presented (Figure 1). There was a higher amikacin concentration of the perfusate being infused over a period of 1 minute after IV regional limb perfusion using the cephalic vein with the perfusate being infused over a period of 1 minute (technique 1) or 5 minutes (technique 5).

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**Table 1**—Median (range) concentrations of amikacin (µg/mL) in synovial fluid samples from the radiocarpal joints of 7 healthy horses 5 (T5), 10 (T10), 15 (T15), 20 (T20), 25 (T25), and 30 (T30) minutes after IV regional limb perfusion using the cephalic vein with the perfusate being infused over a period of 1 minute (technique 1) or 5 minutes (technique 5).

<table>
<thead>
<tr>
<th>Time</th>
<th>Technique 1 synovial concentration of amikacin sulfate (µg/mL)</th>
<th>Technique 5 synovial concentration of amikacin sulfate (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T5</td>
<td>16 (0–1634.4)</td>
<td>44.4 (2–1684.8)</td>
</tr>
<tr>
<td>T10</td>
<td>154.8 (27.4–4925)</td>
<td>252 (20.7–1425.6)</td>
</tr>
<tr>
<td>T15</td>
<td>226.8 (16–4925)</td>
<td>277.2 (81.4–3140)</td>
</tr>
<tr>
<td>T20</td>
<td>291.6 (27.5–4925)</td>
<td>435.6 (93.6–2881)</td>
</tr>
<tr>
<td>T25</td>
<td>200.4 (58.8–4925)</td>
<td>608.4 (115.2–1051.2)</td>
</tr>
<tr>
<td>T30</td>
<td>237.6 (17.7–4925)</td>
<td>457.2 (147.6–1425.6)</td>
</tr>
</tbody>
</table>

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**Figure 1**—Systemic amikacin concentrations over time from horses undergoing regional limb perfusion with 1- or 5-minute infusion time. Blood samples were collected before infusion and at different time points before removal of the tourniquet. Black line represents concentrations from technique 1. Gray line represents concentrations from technique 5. Data is presented as a Log-10 scale. There was a significant interaction (P = .004) between time and group.
blood concentration over time in the 1-minute infusion time group compared to the 5-minute infusion time group ($P = .004$).

No difference was noted between minor and major movement events between groups ($P = .932$ and $P = .588$, respectively).

**Discussion**

Contrary to our hypothesis, no difference in synovial concentrations was apparent in this study comparing instillation of the perfusate over 1 minute or 5 minutes during IVRLP; however, there was a significant difference noted in the systemic levels over time. The median $C_{\text{MAX}}$ for both techniques was greater than 160 µg/mL, which is ideal for resistant organisms with an MIC $\geq 16$ µg/mL. However, it is noteworthy that 2 of 7 horses did not reach a concentration of 160 µg/mL using technique 1, while all 7 horses reached 160 µg/mL with technique 5.

Reducing systemic absorption is desirable to limit the development of possible resistance to amikacin where subtherapeutic doses may be released. In the current study, there was a significant difference in the systemic concentration of amikacin between groups. The systemic concentration was higher at all timepoints using technique 1 ($P = .004$), suggesting more systemic leakage in these horses. While movement has been previously suggested as a potential source of leakage resulting from tourniquet failure,$^{36}$ there was no significant difference in movement between groups. We hypothesize that the quicker instillation of the perfusate in technique 1 had resulted in increased venous hydrostatic pressures as compared to technique 5, leading to tourniquet failure and increased systemic leakage of amikacin. The systemic amikacin concentration over time was higher using technique 1 compared to concentrations achieved when the perfusate was instilled over 5 minutes (technique 5), which supports this hypothesis. The reduced systemic absorption of horses in technique 5 will be valuable in practice to limit drug resistance and the potential development of unwanted systemic adverse effects, thus improving our ability to use amikacin in other necessary situations.

In this study, all horses were treated with 2 g of amikacin despite a moderate range in weight of the patients. While this is similar to how IVRLPs are performed in clinical practice, this resulted in a dose range of 3.0 to 3.7 mg/kg. As all of our patients in technique 5 achieved a $C_{\text{MAX}}$ of $> 160$ µg/mL, reducing the dosage of amikacin used in IVRLPs may be feasible to achieve adequate synovial concentrations while further reducing systemic amikacin concentrations.

The large variation in amikacin concentration across groups also aligns with previous studies,$^{27,31,37,38}$ looking at amikacin sulfate concentrations in the RCJ after IVRLP. This variability can likely be attributed to a variety of factors, including relative amikacin dosage, patient drug metabolism, and the possibility of tourniquet leakage. Variation in amikacin concentration continues to be a potential downfall of IVRLP in clinical patients, where amikacin concentration within the target structure is not routinely measured. Despite trying to control for breed, sex, weight, tourniquet placement, and perfusate instillation, this study and others have been unable to document consistent amikacin concentrations within the target synovial structure. This should remain a consideration when using this modality in a clinical patient as there is likely patient variation in amikacin concentrations. While most studies are performed in normal horses and not those with clinical manifestations of disease, a study inducing synovitis in the RCJ prior to IVRLP saw an increased $C_{\text{MAX}}$ of intra-articular amikacin compared to normal joints, indicating that levels in clinical patients may be higher than expected.$^{24}$

The range for concentration of amikacin in the study was quite large, with a high peak concentration of 4,925 µg/mL in 1 horse using technique 1 and 3,140 µg/mL in 1 horse using technique 5. Two horses using technique 1 and 4 horses using technique 5 reached synovial amikacin concentrations greater than 800 µg/mL, which has been shown in previous in vitro research to be cytotoxic to synoviocytes.$^{39}$ An additional horse in each group was also above the concentration of 310 µg/mL, which has been shown to have cytotoxic effects on chondrocytes.$^{39}$ This shows that while synovial concentrations greater than 160 µg/mL were routinely achieved for best effect against resistant bacterial organisms, excess concentrations are also routinely achieved during IVRLP that may have negative effects on the synovial structures. However, it is important to note that this study$^{39}$ was performed in vitro, and the degree of penetration of amikacin into the articular cartilage in vivo is currently not known. More research is necessary to determine if IVRLP with amikacin would have a detrimental effect on articular cartilage in vivo and if the possibility of using lower doses in the future may ameliorate this risk. Additionally, one must also consider that when treating a potentially septic synovial structure, the risk associated with cartilage damage due to the presence of bacteria likely outweighs a potential risk of cartilage damage due to the ensuing treatment.

The limitations of this study include a small sample size in combination with large standard deviations in the data. These large differences in amikacin concentration resulted in low power, limiting the ability to prove difference in synovial concentration between groups and resulting in the possibility of a type II error (failure to detect a significant difference). Additionally, instillation of perfusate was performed manually, while a syringe pump could have been utilized for more consistent antibiotic infusion as has been previously reported.$^{40}$ Repeat arthrocentesis throughout the IVRLP also frequently resulted in blood contamination, which may alter the results of the amikacin concentration in subsequent synovial fluid samples. The authors are unaware of previous studies examining the effects of repeated arthrocentesis and hemorrhage on synovial amikacin concentrations. A further limitation of this study is that...
complete pharmacokinetics were unable to be calculated as amikacin concentrations were not measured past the 30-minute timepoint. Lastly, this study only looked at one perfusate volume of 60 mL total volume. Previous studies\textsuperscript{5,6,41} have used perfusate volumes ranging from 10 mL to 250 mL, with higher volumes being documented to result in higher synovial concentrations. Obviously, varying perfusate volumes will result in an alteration in the rate that the perfusate is administered, and this should be taken in to consideration if different volumes are used.

In conclusion, this study found no difference between instillation of perfusate time and the concentration of amikacin within the RCJ after IVRLP. However, significantly more systemic leakage was observed when the perfusate was administered rapidly over 1 minute. In clinical practice, instillation of perfusate at a slower rate than 60 mL over 1 minute (1 mL/s) seems prudent to reduce the amount of systemic leakage, with an aim to maximize synovial concentrations.

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Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

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References


