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# Different methods of perfusate administration do not have an effect on synovial concentrations of amikacin following intravenous regional limb perfusion

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**Objective**
To determine if different methods of perfusate instillation influence synovial amikacin concentrations in the radiocarpal joint (RCJ) after IV regional limb perfusion (IVRLP).

**Methods**
6 healthy horses received an IVRLP using 2 different methods: (1) 2 g amikacin followed by 52 mL 0.9% NaCl (60 mL total; perfusate-A) and (2) 2 g amikacin diluted to 60 mL with 0.9% NaCl (perfusate-D). For both methods, the perfusion was administered over 5 minutes. Joint fluid from the RCJ was sampled at 10, 15, 20, 25, and 30 minutes after instillation of the perfusate. Systemic concentrations of amikacin were measured prior to IVRLP; at 5, 10, 15, 20, 25, and 29 minutes; and 1 minute after tourniquet removal. Amikacin concentrations were determined by fluorescence polarization immunoassay.

**Results**
Mean ± SD peak synovial concentration in the RCJ was 1,447 ± 1,134 µg/mL with perfusate-D and 1,170 ± 977 µg/mL with perfusate-A. Mean ± SD time to peak concentration was 18 ± 7 minutes with perfusate-D and 20 ± 5 minutes with perfusate-A. There was no difference in peak synovial concentration ($P$ = .684) and time to peak concentration ($P$ = .732) between groups. There was no difference in systemic amikacin concentrations over time between groups ($P$ = .196). All horses included reached the target synovial amikacin concentration of > 160 µg/mL.

**Conclusions**
There was no difference in the systemic or the synovial concentrations of amikacin using different methods of perfusate administration.

**Clinical Relevance**
Different methods of perfusate administration did not affect synovial concentrations of amikacin achieved when performing IVRLP. There is no advantage to administering amikacin first.

**Keywords:** amikacin, synovial, concentrations, perfusate, regional limb

Intravenous regional limb perfusion (IVRLP) has been described as a simple, cost-effective procedure that enables the delivery of a high concentration of antibiotic to an affected region of the distal limb with minimal systemic effects.[1] This technique is commonly used as a therapeutic method for treating traumatic wounds of the limbs of horses, which frequently involve synovial structures that can affect the athletic career, and potentially the life, of the animal.[2–4]

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The antimicrobial most commonly used at our institution when performing IVRLP is amikacin sulfate. Dosages of amikacin routinely administered to horses by IVRLP vary in the literature from 500 mg to 2.5 g, with the diluting volume of 0.9% sodium chloride ranging from 20 to 100 mL.[5–8] When performing IVRLP, the main objective is to achieve a concentration of antimicrobial in the targeted tissue that is higher than the MIC of targeted bacteria. Considering amikacin specifically, an aminoglycoside, the efficacy is maximized with a synovial maximum concentration ($C_{max}$)-to-MIC ratio between 8:1 and 10:1.[9,10] Methods to increase the efficacy of this procedure and maximize synovial levels of amikacin

have been extensively documented in the literature. These include studies[5,7,8,11–15] examining the effect of different tourniquet types and application, and injection characteristics including site of injection, volume of perfusate, previous exsanguination and time taken to administer the perfusate.

Increased hydrostatic pressure within the venous vasculature and the antimicrobial concentration gradient in the distal limb are thought to result in diffusion of the antimicrobial into surrounding tissues, thus delivering therapeutic concentrations of antimicrobials to the affected area.[5,6,16,17] The rise in IV pressure and a concentration gradient creates a depot phenomenon, leading to a slow release of the perfusate back into systemic circulation once the tourniquet is released.[14] To date, the authors are not aware of a study examining different methods of perfusate instillation and their effect on synovial concentrations achieved during IVRLP. Many studies[6,18,19] report performing IVRLP with the antibiotic diluted in varying amounts of 0.9% NaCl. Administration of a high concentration of amikacin followed by the diluent may affect how the antibiotic is distributed within the tissues, thus affecting the synovial concentrations achievable. Further standardization of this technique can help to maximize the synovial concentrations achieved and increase the efficacy of the procedure.

The main objective of this study was to determine if 2 different methods of perfusate instillation when performing IVRLP would affect the synovial concentrations achieved in the radiocarpal joint (RCJ). We hypothesized that administering the antibiotic (amikacin sulfate) first followed by a volume of 0.9% NaCl (52 mL) compared to administering the antibiotic diluted in the same volume of 0.9% NaCl would result in increased systemic leakage of the antibiotic and subsequently lower synovial concentrations ($C_{max}$).

## Methods

### *Animals*

Six healthy horses from the equine research herd at the University of California-Davis Center for Equine Health were chosen randomly. Physical examinations were performed to rule out abnormalities of the cephalic veins, distal limb vasculature, and carpal joints. A lameness examination was performed to confirm that there was no lameness associated with either forelimb. Horses were housed in stalls and monitored for the duration of the study and for 48 hours following completion of the procedure for localized swelling at the site of perfusion, swelling of the RCJ, and lameness associated with the treated limb. This protocol was approved by the University of California-Davis IACUC (#23718).

### *Procedures*

This was a randomized, crossover experiment with similar methodology to those previously reported.[7,8,20] Each horse underwent IVRLP with 2 g of amikacin administered via 2 methods: (1) 2 g amikacin administered followed by 52 mL 0.9% NaCl (60 mL total; perfusate-A) and (2) 2 g amikacin diluted to 60 mL with 0.9% NaCl (perfusate-D). The choice of forelimb and method was randomized for each horse (www.randomization.com). The second method was performed on the opposite forelimb following a minimum 2-week washout period.

For each part of the experiment, unilateral median, ulnar, and medial cutaneous antebrachial nerve blocks were performed using aseptic technique with 10 mL of 2% mepivacaine hydrochloride per site to reduce possible discomfort associated with the tourniquet.[21] Horses were restrained in standing stocks and sedated with IV detomidine hydrochloride (0.01 mg/kg [0.005 mg/lb]) prior to the start of each experiment. Additional sedation was administered during the procedure if the horse demonstrated discomfort (shifting weight, lifting the limb, or pawing). The cephalic vein of 1 forelimb was clipped and aseptically prepared. A wide rubber tourniquet (12-cm wide; Esmarch Bandage; Skylar Instruments) was placed at the level of the proximal antebrachium, approximately 10 cm proximal to the accessory carpal bone, and wrapped around the limb 10 full circumferential turns as tightly as possible by the same investigator (IK). An IV catheter (22 gauge, 2.5 cm) was placed in the cephalic vein below the tourniquet at the level of the chestnut, and the perfusate was infused using either method over 5 minutes. The injection of the perfusate was manually timed using a stopwatch. After the instillation of the perfusate was complete, the catheter was removed, and a pressure bandage was placed over the injection site. The tourniquet was removed after the 30-minute sample was obtained. The procedure was repeated on the other limb using the alternate method of perfusate administration after a washout period of at least 2 weeks.

### *Sample collection*

A venous blood sample was collected in lithium heparin tubes by jugular venipuncture prior to IVRLP; at 5, 10, 15, 20, and 25 minutes; 1 minute before tourniquet release; and 1 minute following tourniquet release. Synovial fluid (0.5 mL) was aseptically collected in lithium heparin tubes by arthrocentesis of the RCJ from the lateral palmar approach[22] at 10, 15, 20, 25, and 30 minutes following completion of the perfusion. Topical diclofenac ointment was applied to the cephalic vein,[23] and a light bandage consisting of soft roll gauze secured with an elastic adhesive bandage was applied to the carpal region after the 30-minute sample was taken. A full-limb bandage consisting of 2 cotton rolls secured with 1 layer of brown gauze and 1 layer of elastic wrap secured with an elastic adhesive on the proximal and distal ends was applied to the metacarpal and carpal regions and maintained for 24 hours. Horses received 1 dose of flunixin meglumine (1.1 mg/kg, IV) after the last sample was taken. Horses were assessed every 24 hours for the first 48 hours after IVRLP for signs of lameness at the walk, effusion or swelling associated with the RCJ sampling sites, or signs of inflammation at the perfusion instillation site of the cephalic vein.

*Sample processing*

Samples were centrifuged at 1,700 X *g* for 5 minutes immediately after collection. The supernatant was collected and stored at −80 °C when all sampling was completed. Amikacin sulfate concentrations (micrograms per milliliter) were determined by fluorescence polarization immunoassay as previously validated (Roche Diagnostics GmBH).[8,15,20] Testing was performed at the Clinical Diagnostic Laboratories of the Veterinary Medical Teaching Center at the University of California-Davis.

*Target concentrations*

Target concentrations for synovial amikacin were based on previous research and the Clinical and Laboratory Standards Institute Subcommittee on Veterinary Antimicrobial Susceptibility Testing's reported MIC for common orthopedic pathogens.[24] The Clinical and Laboratory Standards Institute reports the MIC for susceptible bacteria as ≤ 4 µg/mL, 8 µg/mL for bacteria with intermediate susceptibility, and ≥ 16 µg/mL for resistant bacteria. To maximize the efficacy of amikacin, an aminoglycoside antibiotic, a concentration 8 to 10 times the MIC is considered therapeutic.[9,10,16] To ensure appropriate antimicrobial levels to treat bacteria with an MIC of 16 µg/mL or less, ≥ 160 µg/mL was chosen as the target concentration.

*Statistical analyses*

Data were analyzed for normality by the Shapiro-Wilk test using commercial statistical software (SPSS Statistics for Windows, version 19.0; IBM Corp). The $C_{max}$ and time to obtain maximum synovial concentration ($T_{max}$) for each horse was determined for both methods of perfusate administration by visual inspection of the median data for the amikacin concentration in synovial fluid. Data were analyzed by a paired *t* test. Differences in systemic amikacin concentrations over time between groups were analyzed by a 2-way repeated-measures ANOVA. For all analyses, significance was set at $P < .05$.

## Results

Six geldings were used in this study, of which there were 4 warmbloods, 1 Thoroughbred, and 1 Quarter Horse. Age ranged from 4 to 21 years (median, 10.5 years). Weight ranged from 568 to 715 kg (median, 618 kg).

No systemic or local complications were observed following IVRLP in any of the horses. Mean ± SD systemic and synovial amikacin sulfate concentrations at the various sampling points were compiled **(Tables 1 and 2)**. The mean ± SD $C_{max}$ of amikacin in the RCJ was 1,447 ± 1,134 µg/mL with perfusate-D and 1,170 ± 977 µg/mL with perfusate-A. The mean ± SD $T_{max}$ was 18 ± 7 minutes with perfusate-D and 20 ± 5 minutes with perfusate-A **(Table 3)**. There were no significant differences in $C_{max}$ ($P = .684$) and $T_{max}$ ($P = .732$) between groups. All horses in both groups reached the target peak concentration of ≥ 160 µg/mL.

There were no differences in systemic concentrations over time between groups ($P = .196$).

**Table 1**—Mean ± SD synovial concentrations of amikacin sulfate (µg/mL) in the radiocarpal joint (RCJ) following IV regional limb perfusion (IVRLP) of 6 healthy horses using the cephalic vein at different time points following administration of the perfusate using 2 different methods: (1) 2 g amikacin followed by 52 mL 0.9% NaCl (60 mL total; perfusate-A) and (2) 2 g amikacin diluted to 60 mL with 0.9% NaCl (perfusate-D).

| | Synovial amikacin concentration (µg/mL) | |
|---|---|---|
| **Time (min)** | **Perfusate-A** | **Perfusate-D** |
| 10 | 154 ± 85 | 555 ± 684 |
| 15 | 446 ± 323 | 823 ± 517 |
| 20 | 1,170 ± 977 | 1,447 ± 1,134 |
| 25 | 950 ± 755 | 766 ± 286 |
| 30 | 1,024 ± 651 | 856 ± 513 |

**Table 2**—Mean ± SD plasma concentrations of amikacin sulfate (µg/mL) following IVRLP of 6 healthy horses using the cephalic vein at different time points following administration of the perfusate using 2 different methods: (1) 2 g amikacin followed by 52 mL 0.9% NaCl (60 mL total; perfusate-A) and (2) 2 g amikacin diluted to 60 mL with 0.9% NaCl (perfusate-D).

| | Plasma amikacin concentration (µg/mL) | |
|---|---|---|
| **Time (min)** | **Perfusate-A** | **Perfusate-D** |
| 0 | 0 | 0 |
| 5 | 1.0 ± 0.9 | 4.5 ± 5.2 |
| 10 | 1.0 ± 0.9 | 4.2 ± 4.2 |
| 15 | 0.9 ± 0.7 | 3.8 ± 3.6 |
| 20 | 0.9 ± 0.7 | 3.9 ± 2.9 |
| 25 | 0.8 ± 0.6 | 4.0 ± 2.6 |
| 29 | 0.8 ± 0.5 | 4.0 ± 2.7 |
| 31 | 10.9 ± 8.5 | 10.9 ± 5.0 |

**Table 3**—Peak synovial concentrations in the RCJ and time to peak synovial concentration ($T_{max}$) for horses after an IV regional limb perfusion with 2 g of amikacin in the cephalic vein using 2 different methods of administration: (1) 2 g amikacin followed by 52 mL 0.9% NaCl (60 mL total; perfusate-A) and (2) 2 g amikacin diluted to 60 mL with 0.9% NaCl (perfusate-D).

| | $T_{max}$ (min) | | Amikacin (µg/mL) | |
|---|---|---|---|---|
| | **Perfusate-A** | **Perfusate-D** | **Perfusate-A** | **Perfusate-D** |
| Horse A | 20 | 15 | 2,815 | 940 |
| Horse B | 20 | 20 | 1,922 | 1,202 |
| Horse C | 20 | 20 | 410 | 3,686 |
| Horse D | 15 | 30 | 626 | 608 |
| Horse E | 30 | 10 | 1,577 | 1,908 |
| Horse F | 15 | 15 | 922 | 1,764 |

## Discussion

Contrary to our hypothesis, this study did not find a difference in synovial amikacin concentrations in the RCJ achieved during IVRLP by different methods of perfusate administration. Although the mean synovial $C_{max}$ for horses undergoing IVRLP using perfusate-D was greater compared with perfusate-A (1,447 ± 1,134 µg/mL vs 1,170 ± 977 µg/mL, respectively), the difference was not statistically significant ($P = .684$). It is possible that our statistical power is

low due to a small sample size and a large SD, leading to a type II error.

All horses using either perfusate-A or perfusate-D reached the target minimum synovial concentration ( ≥ 160 µg/mL) necessary to treat infections caused by organisms with an MIC of 16 µg/mL.[25] Similar to previous studies,[1,6,8,12,17,18,26,27] the present study demonstrated a wide variability in synovial amikacin sulfate concentrations achieved using IVRLP. This variation should be considered a limitation of this procedure in a clinical setting, since synovial levels achieved clinically are not routinely measured. This variability is likely due to multiple factors, including differences in how individual patients metabolize the drug, variations in tourniquet placement, or movement during the procedure resulting in systemic leakage. Additionally, the use of a set dose of 2 g likely resulted in differences in drug dosing and levels achieved between horses of different weights and should be considered a limitation.

For both methods in the current study, the instillation time for both perfusates was performed over 5 minutes. One study[8] previously examined the effect of different perfusate instillation times (1 minute vs 5 minutes) on the synovial concentrations achieved in the RCJ and reported that no significant difference in synovial concentrations was apparent. It was noted, however, as part of that study that systemic levels of amikacin recorded 5 minutes after completion of the procedure were higher in the 1-minute group, suggesting more systemic leakage of amikacin when the perfusate was administered over a shorter time period. The peak synovial levels achieved in the current study are higher than previous studies with similar methodology looking at concentration within the RCJ (1,331.4 µg/mL [range, 246.4 to 4,620.8 µg/mL],[7] 338.4 µg/mL [range, 60 to 4,925 µg/mL],[8] and 1,153 µg/mL [range, 588 to 1,950 µg/mL][18]). The length of time used to administer the perfusate in these studies ranged from 1 to 3 minutes. Based on these results and those of a previous study,[8] it would seem prudent to deliver the perfusate slowly over 5 minutes when performing IVRLP to maximize peak synovial concentrations.

One advantage of using perfusate-A in a clinical setting is that the antibiotic is instilled first. If, for example, the horse moves or there is a handling error and the perfusion has to be discontinued, the horse has received the complete dose of antibiotic. With reduced total volume, however, synovial levels may not reach adequate concentrations without a sufficient volume of diluent to follow the antibiotic. While some studies[5] have shown that perfusate volumes as low as 20 mL can achieve adequate synovial levels of certain antibiotics, another study[6] has documented that synovial levels of amikacin in the metacarpophalangeal joint were significantly higher using higher perfusate volumes (100 mL vs 60 mL or 30 mL).

The mean $T_{max}$ for each group (18 ± 7 minutes vs 20 ± 5 minutes) was not significantly different and suggests that maintaining the tourniquet for 20 minutes following completion of the IVRLP is appropriate, which aligns with other studies.[7,8,13,18]

There are several limitations to this study. The main one being a small study population and large differences in amikacin concentrations. A greater number of horses would have increased the statistical power of our findings. This study also only examined the effect of different instillation methods using 1 set volume of 60 mL. Ideally, a third group looking at just administration of the antibiotic (ie, 8 mL total volume) could have been examined to ascertain the effect of instilling just the antimicrobial with no diluent. The decision to use a wide rubber as the tourniquet was based on its clinical practicality; however, in future studies, using a pneumatic tourniquet could be considered for more control within the protocol. The use of clinically healthy horses is another limitation for this study as it has been shown previously that the pharmacokinetics and pharmacodynamics of amikacin may change in inflamed joints.[17] Complete pharmacokinetic analysis was also not performed in this study as no samples were obtained after the 30-minute timepoint.

In conclusion, there was no difference in the systemic or the synovial concentrations of amikacin achieved using different methods of perfusate administration, with either method achieving adequate synovial amikacin concentrations in all horses.


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