

Evaluation of safety and pharmacokinetics of vancomycin after intravenous regional limb perfusion in horses

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Objective—To evaluate clinical variables, regional concentrations, and pharmacokinetics of vancomycin in the synovial fluid of distal forelimb joints of horses after IV regional limb perfusion.

Animals—6 horses.

Procedure—Vancomycin was administered via IV regional limb perfusion to the distal portion of the forelimbs of anesthetized horses. Drug (300 mg of vancomycin hydrochloride in 60 mL of saline [0.9% NaCl] solution) was infused into 1 forelimb, whereas the contralateral limb served as a control and was perfused with 60 mL of saline solution. Solutions were injected into the lateral digital vein after digital exsanguination. Synovial fluid from the metacarpophalangeal (MTCP) and distal interphalangeal (DIP) joints and systemic blood were collected prior to perfusion and 15, 30, 45, 65, and 90 minutes after initiation of the infusion. Synovial fluid from the MTCP joint and blood were also obtained at 4, 8, 12, and 24 hours after infusion. Plasma urea and creatinine concentrations, degree of lameness, and certain clinical variables involving the MTCP joint and infusion site were assessed for 7 days. Results were compared between the vancomycin treatment and control groups.

Results—No complications or significant differences in renal function, lameness, or clinical variables were observed between groups. Vancomycin concentrations exceeded 4 µg/mL in MTCP joints for approximately 20 hours. Higher concentrations were reached in DIP joints than in MTCP joints.

Conclusions and Clinical Relevance—IV regional limb perfusion with 300 mg of vancomycin as a 0.5% solution was safe and may be useful in horses as treatment for distal limb infections. (*Am J Vet Res* 2005;66:2107–2113)

In the decades following the earliest reported isolation¹ of the organism, methicillin-resistant *Staphylococcus aureus* (MRSA) has spread worldwide

and become an important nosocomial pathogen in humans.^{2,3} Infections with MRSA are also reported in veterinary medicine.^{4,5} In horses, MRSA was first isolated in association with infectious metritis and dermatitis.⁶ More recently, MRSA as an agent of nosocomial disease was reported in a veterinary teaching hospital⁷ and in 2 unrelated cases^{8,9} of postsurgical infections in horses.

Concern has been compounded by the fact that MRSA is becoming resistant to an increasing number of antimicrobials, including β-lactam drugs (eg, cephalosporins, cephems, and carbapenems), aminoglycosides, macrolides, clindamycin, tetracyclines, and fluoroquinolones.^{10,11} In 2 recent reports, administration of chloramphenicol⁸ and vancomycin⁹ was required for bacterial eradication in MRSA infections. Vancomycin, a narrow-spectrum bactericidal glycopeptide antimicrobial, is active against staphylococcal organisms, including MRSA. In humans, vancomycin has been associated with nephrotoxicosis, ototoxicosis, neurotoxicosis, and thrombophlebitis in the region of the injection site.^{12,13} The pharmacokinetics of vancomycin after a single dose administered IV have been described.¹⁴ However, parenterally administered antimicrobials alone may fail to successfully resolve orthopedic infections because penetration of antimicrobials into tissues that have become devitalized and ischemic as a result of septic changes is hampered^{15,16} and conditions in local septic environments decrease antimicrobial efficacy, leading to requirements for higher antimicrobial concentrations to kill bacteria.¹⁷

Administration of antimicrobials via IV regional limb perfusion (IVRLP) results in high regional antimicrobial concentrations and low systemic concentrations.^{18–20,a} Consequently, many adverse effects may be avoided, and the high doses of drug that are needed for systemic effects but that make the use of certain antimicrobials economically unfeasible in large animals are unnecessary. The effectiveness of IVRLP in horses in clinical settings has been reported.^{21,22}

We hypothesized that administration of vancomycin via IVRLP would result in high concentrations of the drug in the synovial fluid of distal forelimb joints and avoid systemic signs of toxicosis, supporting a role for use of the drug treatment of infections caused by susceptible organisms. The objectives of the study were to determine local clinical effects, drug concentrations in synovial fluid, and pharmacokinetics of vancomycin after IVRLP.

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Materials and Methods

Animals—Six horses of mixed breeds and sexes, 3 to 18 years of age (mean, 10.5 years), and weighing 345 to 453 kg (mean, 405.8 kg) were used in the study. Horses were considered healthy on the basis of results of physical examination and hematologic and serum biochemical analyses. Radiographs of the distal portion of both forelimbs were obtained, and horses with clinical or radiographic signs of musculoskeletal disease were excluded. Horses were housed in indoor stalls with free access to grass hay and water and also received a ration of oats twice per day. The project was approved by the institutional animal care and use committee.

Experimental design—Each horse received 2 treatments of RLP: 1 control treatment and 1 vancomycin treatment, randomly distributed between the forelimbs. Treatments were performed with the horses anesthetized and were separated by a 2-week washout period. The perfusate was 60 mL of a saline (0.9% NaCl) solution in the control group, and 60 mL of a saline solution containing 300 mg of vancomycin hydrochloride^{b,c} in the vancomycin group. The dose of vancomycin was determined on the basis of results of a pilot study in 3 horses that were to be euthanized, in which each horse received 2 doses of vancomycin (200 and 300 mg) via IVRLP. Each dose was given in 1 of the forelimbs with the horse standing. None of the horses had clinical signs of phlebitis or other adverse effects during an evaluation period of 12 to 18 hours after treatment.

For the present study, horses were sedated with xylazine hydrochloride (1.1 mg/kg, IV) and general anesthesia was induced with ketamine (2.2 mg/kg, IV). Anesthesia was maintained with 2% isoflurane in oxygen at a flow rate of 10 mL/kg/min. Phenylbutazone was administered to all horses on the day of the procedure (4.4 mg/kg, IV, q 12 h), with the first dose administered prior to anesthetic premedication (sedation), and administration was continued for the following 4 days (2.2 mg/kg, IV, q 12 h).

While anesthetized, horses were positioned in lateral recumbency with the limb to be treated uppermost, and the distal portion of the limb (ie, from the carpus to the hoof) was clipped and aseptically prepared. The lateral digital vein was catheterized with a 22-gauge, 2.5-cm over-the-needle catheter inserted at the level of the lateral proximal sesamoid bone and directed distad. A 1.5-cm incision was made along the lateral aspect of the proximal phalanx (P1), and a 2-mm-diameter hole was drilled through the cortex until the medullary cavity was entered.

A 7-cm-wide Esmarch bandage was applied from the hoof proximad to drain blood from the digital vasculature. At the level of the proximal portion of the third metacarpal bone, an 11-cm-wide rubber Esmarch bandage was applied as a tourniquet, and the 7-cm-wide bandage was removed. The metacarpophalangeal (MTCP) joint (fetlock) was entered through the lateral collateral sesamoid ligament with a 23-gauge 25-mm needle attached to a 2-mL syringe. The distal interphalangeal (DIP) joint was entered via dorsomedial or dorsolateral approaches, abaxial to the common digital extensor tendon, with a 20-gauge 40-mm needle attached to a 5-mL syringe. Both needles were secured in place during the 90 minutes of the procedure to minimize trauma to the synovial membrane from repeated punctures. The perfusate was infused at a rate of 2 mL/min by means of an IV infusion pump.^d The infusion lasted 30 minutes; the beginning of the infusion was designated time 0. The tourniquet was released at 45 minutes (ie, 15 minutes after the end of the infusion).

Synovial fluid samples (volume, 0.3 mL) from each joint and blood samples (volume, 2 mL) from the jugular vein were taken prior to infusion and at 15, 30, 45, 65, and 90 minutes after beginning the infusion. After each synovial

fluid sample was collected, a new syringe was attached to the indwelling needle to avoid aspiration of air into the joint. Samples of bone marrow from P1 were taken at the same sampling times for use in a different study.

After collection of samples at 90 minutes, the lateral skin incision over P1 was sutured with nonabsorbable polyamide^e suture material. The distal portion of the limb was bandaged, and horses were allowed to recover from general anesthesia. Samples of blood and synovial fluid from the MTCP joint were collected 4, 8, 12, and 24 hours after beginning the infusion. To facilitate this procedure, horses were sedated with detomidine (0.01 mg/kg, IV). The MTCP joint was entered with a 21-gauge 40-mm needle in the following approaches in the order listed: lateral distopalmar, medial distopalmar, dorsolateral, and dorsomedial.

Samples were identified by use of the acronym of the location sampled (ie, MTCP, DIP, or blood) and the sampling time (15, 30, 45, 65, and 90 minutes and 4, 8, 12, and 24 hours after the start of the infusion). For instance, the designation MTCP-30 referred to synovial fluid obtained from the MTCP joint 30 minutes from the time the infusion was begun. Samples taken prior to the beginning of infusion were labelled 00 (ie, MTCP-00). The sampling interval was defined as the time period between 2 consecutive sampling times (eg, MTCP-00 and MTCP-15).

Clinical signs after IVRLP—Horses were evaluated prior to the procedure and during the ensuing 7 days by the same investigator who was unaware of treatment group assignments. Clinical, lameness, and neurologic examinations were conducted daily. Renal function variables (eg, blood urea and plasma creatinine) were measured prior to perfusion and on days 1, 2, 3, 5, and 7 after perfusion. Thereafter, clinical observations regarding the perfused limb were made and scored on the basis of a numeric scale. These variables included lameness (0 = no lameness; 1 = no lameness at a walk but lameness at a trot with head movements; 2 = gait altered at a walk without head movements, but head movements present at a trot; 3 = obvious lameness at a walk, and head movements present at a trot; and 4 = non-weight-bearing), MTCP joint effusion (0 = no effusion observed or palpated, 1 = mild effusion palpable in the lateral joint pouch, 2 = moderate effusion in the lateral pouch, and 3 = severe or prominent effusion in both the lateral and dorsal joint pouches), palpable heat in the MTCP joint region (0 = negative, 1 = mild, 2 = moderate, and 3 = severe), sensitivity of the MTCP joint to digital pressure (0 = no sensitivity [horse does not move the limb in response to mild palpation or firm pressure], 1 = mild [horse moves limb when firm pressure is applied], 2 = moderate [horse moves limb when superficial pressure is applied], and 3 = severe [horse abruptly lifts the limb when lightly touched]), and signs of inflammation, discharge, or sensitivity to palpation at the infusion site on the lateral digital vein (range, 0 = negative to 3 = severe).

Sample processing and vancomycin assay—All blood and synovial fluid samples were collected into heparinized tubes.¹ Blood samples were centrifuged at 1,207 × g for 5 minutes, and plasma and synovial fluid were stored at -20°C until assayed. Determination of vancomycin concentrations was performed from 5 to 7 days after collection by use of a fluorescence polarization immunoassay.⁸

To validate the assay of vancomycin in equine samples, regression analysis had been performed previously on a series of equine plasma and synovial fluid samples from healthy horses that contained known concentrations of vancomycin (range, 1 to 100 µg/mL). This range was selected because all experimental samples were diluted to a final concentration ≤ 100 µg/mL. For plasma samples, the regression coefficient was 0.991 with a slope of 1.17 and an intercept of 0.26. For

synovial fluid, the regression coefficient was 0.994 with a slope of 1.235 and an intercept of 0.796. The inter- and intra-assay reproducibility was < 6% (coefficient of variation).

Pharmacokinetic analyses—After synovial fluid and plasma concentrations of vancomycin were measured, the disposition of vancomycin was analyzed via noncompartmental pharmacokinetic analysis by use of a computer program.^b Variables determined included the **elimination rate constant** (β [minutes⁻¹ calculated as the slope of the elimination phase of the concentration-time curve]), **terminal half-life** ($t_{1/2}$ β [minutes]), **area under the concentration-time curve** (AUC, [$\mu\text{g}\cdot\text{min}/\text{mL}$]), and mean residence time ($\mu\text{g}\cdot\text{min}/\text{mL}$) of vancomycin. For MTCP and blood, variables were calculated for the period of 24 hours ($t = 24$). For DIP, variables were calculated for the 90 minutes studied in that joint ($t = 90$). Variables in MTCP up to 90 minutes were also calculated for comparison with DIP values.

Statistical analysis—Statistical analyses were performed by use of a computer program.¹ Clinical observations were analyzed with a Kolmogorov-Smirnov test for nonparametric data for comparison between the vancomycin and control groups. Vancomycin concentrations were analyzed with a repeated-measures ANOVA test with time as the unique factor to study the changes in vancomycin concentration over time at each location. Synovial fluid concentrations of vancomycin in the DIP and MTCP joints up to 90 minutes were analyzed with a repeated-measures ANOVA test with 2 factors (time and articulation). A Student *t* test was used for comparison of vancomycin concentrations in the MTCP and DIP joints at each sampling time. Pharmacokinetic variables that were determined for synovial fluid from both joints until 90 minutes were compared with a Kolmogorov-Smirnov test. For all statistical methods, values of $P < 0.05$ were considered significant.

Results

None of the horses developed complications during the experimental procedure. Results of clinical and neurologic examinations in the 7 days after the experimental procedure were unremarkable. Concentrations of urea and creatinine remained within reference ranges (≤ 50 mg/mL and ≤ 2 mg/dL, respectively). Minimal evidence of lameness (maximal mean \pm SD values of 0.33 ± 0.408 and 0.5 ± 0.447 for the vancomycin and control groups respectively) and mild to moderate MTCP joint effusion (maximal mean \pm SD values of 1.58 ± 0.801 and 1.50 ± 1.095 for the vancomycin and control groups, respectively) were observed. Signs of heat on palpation were mild (maximal mean \pm SD values of 1.08 ± 0.801 and 1.17 ± 1.125 for vancomycin and control groups, respectively), and sensitivity to palpation (maximal mean \pm SD values of 0.42 ± 0.801 and 0.50 ± 1.225 for vancomycin and control groups, respectively) was mild. No evidence of inflammation, discharge, or sensitivity was observed at the site of injection in any of the horses. The surgical wounds on the lateral aspect of P1 healed normally in horses of both groups. The Kolmogorov-Smirnov test results indicated no significant differences in clinical observations between groups.

Vancomycin was not detected in blood samples collected before perfusion, and there were no detectable concentrations until the tourniquet was released. The **maximum plasma concentration** (C_{max}) of vancomycin was reached at 65 minutes (Table 1). In

both joints, tourniquet release was associated with a change in the slope of the vancomycin concentration-versus-time curves (Figure 1), reaching synovial fluid C_{max} at 45 minutes. Time was a significant factor in the overall change in vancomycin concentrations in plasma and fluid from the MTCP and DIP joints. Time was

Table 1—Vancomycin concentrations ($\mu\text{g}/\text{mL}$) measured in synovial fluid of joints of the distal portion of the forelimb and peripheral blood of 6 horses in which vancomycin was administered as an IV regional limb perfusion.

Sample	Mean \pm SD	95% confidence interval	Range
MTCP-00	0.000 \pm 0.000	0.000–0.000	0.000–0.000
MTCP-15	7.545 \pm 9.903	0.000–16.966	1.620–27.640
MTCP-30	24.585 \pm 16.574	4.478–44.692	8.940–50.210
MTCP-45	80.179 \pm 37.802	44.406–115.952	45.935–145.240
MTCP-65	63.125 \pm 41.152	27.316–98.934	23.010–136.170
MTCP-90	48.167 \pm 33.159	21.546–74.788	15.840–107.960
MTCP-4h	17.105 \pm 8.351	5.157–29.053	8.740–28.660
MTCP-8h	9.765 \pm 5.133	1.053–18.477	4.220–16.340
MTCP-12h	6.102 \pm 3.206	2.998–9.205	2.950–10.480
MTCP-24h	2.528 \pm 1.741	1.192–3.865	0.650–5.770
DIP-00	0.000 \pm 0.000	0.000–0.000	0.000–0.000
DIP-15	39.220 \pm 32.207	15.466–62.974	8.500–89.410
DIP-30	72.276 \pm 39.357	44.232–100.320	18.500–125.560
DIP-45	98.735 \pm 46.434	62.045–135.425	23.280–157.560
DIP-65	90.590 \pm 41.645	59.572–121.608	26.270–143.230
DIP-90	73.878 \pm 39.505	45.664–102.093	20.880–119.300
B-00	0.000 \pm 0.000	0.000–0.000	0.000–0.000
B-15	0.000 \pm 0.000	0.000–0.000	0.000–0.000
B-30	0.000 \pm 0.000	0.000–0.000	0.000–0.000
B-45	0.000 \pm 0.000	0.000–0.000	0.000–0.000
B-65	1.910 \pm 1.169	1.039–2.781	0.370–3.740
B-90	0.868 \pm 0.524	0.371–1.366	0.040–1.560
B-4h	0.373 \pm 0.344	0.009–0.738	0.000–0.830
B-8h	0.103 \pm 0.119	0.000–0.296	0.000–0.270
B-12h	0.018 \pm 0.045	0.000–0.047	0.000–0.110
B-24h	0.000 \pm 0.000	0.000–0.000	0.000–0.000

MTCP = Metacarpophalangeal joint. DIP = Distal interphalangeal joint. B = Blood. 00, 15, 30, 45, 65, 90, 4h, 8h, 12h, and 24h = 0, 15, 30, 45, 65, and 90 minutes and 4, 8, 12, and 24 hours, respectively, after beginning regional limb perfusion.

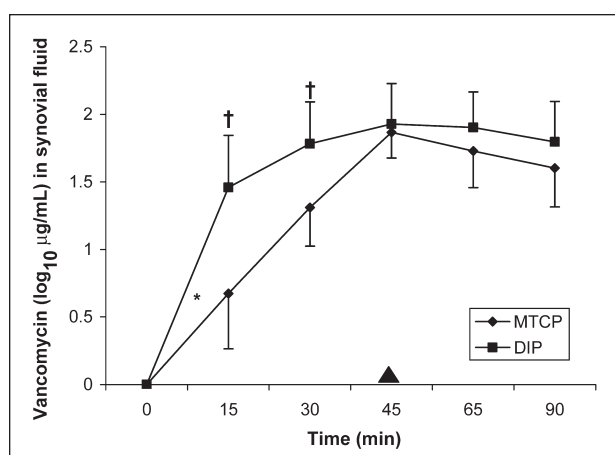


Figure 1—Changes in log₁₀ vancomycin concentration over time in the metacarpophalangeal (MTCP) and distal interphalangeal (DIP) joints up to 90 minutes after initiating IV regional limb perfusion in 6 horses. *Significant ($P \leq 0.05$) difference between both joints in synovial fluid vancomycin concentration during the interval from 0 to 15 minutes. †Significant ($P \leq 0.05$) differences in synovial concentrations of vancomycin at sampling times of 15 and 30 minutes. ▲Time of tourniquet release.

Table 2—Pharmacokinetic variables for vancomycin after IV regional limb perfusion in the same horses as Table 1.

Variable	24 hours		90 minutes		P value
	Blood	MTCP	MTCP	DIP	
$\beta \times 10^{-3}$ (min ⁻¹)	2.8 ± 0.141	1.78 ± 0.72	NA	NA	NA
t _{1/2} β (min)	246.17 ± 8.726	423.18 ± 117.43	NA	NA	NA
AUC _t (μg•min/mL)	225.352 ± 52.921	17,038.04 ± 7,988.76	3,907.47 ± 2,074.47	6,362.06 ± 3,239.13	0.441
MRT _t (min)	163.398 ± 75.159	357.54 ± 74.67	56.135 ± 3.865	53.09 ± 1.22	0.139

Results of Kolmogorov-Smirnov tests (P values) of comparison of variables up to 90 minutes between the MTCP and DIP joints are given.
 β = Elimination rate constant. t_{1/2} = Terminal half-life. AUC_t = Area under the concentration-time curve. MRT = Mean residence time. t = Last sampling time included in the calculation of the variables. NA = Not applicable.
 See Table 1 for remainder of key.

also a significant factor for the sampling intervals from 45 minutes to 8 hours in plasma and for all sampling intervals in both synovial joints, except for the first interval in MTCP (MTCP-00 to MTCP-15; $P = 0.121$). The changes in synovial concentrations up to 90 minutes in both joints were not significantly different between joints overall ($P = 0.191$) but were significantly different during the first interval (from 0 to 15 minutes; $P = 0.044$). The mean ± SD concentration of vancomycin in MTCP joint synovial fluid (37.26 ± 26.73 μg/mL) was lower than that in the DIP joint (62.45 ± 26.73 μg/mL), but the difference was not significant ($P = 0.134$). Vancomycin concentrations in fluid from the DIP joint were higher than those in the MTCP joint at all sampling times, but differences were significant only at 15 minutes ($P = 0.044$) and 30 minutes ($P = 0.021$).

Pharmacokinetic variables at the different locations were summarized (Table 2). Values for β and t_{1/2} β could not be calculated for plasma or the DIP joint because of low concentrations and the finalization of the study at 90 minutes after beginning the perfusion, respectively. No significant differences were observed when variables were compared between joints up to 90 minutes.

Discussion

Vancomycin was administered via IVRLP in horses with no adverse effects and synovial concentrations of vancomycin were greater than the **minimum inhibitory concentration (MIC)**^{23,24} for MRSA for approximately 20 hours in the MTCP joint. No signs of vancomycin toxicosis were observed. Although the safe range of vancomycin concentrations in equine plasma is not known, values for C_{max} in this study (range, 1.47 to 3.74 μg/mL) were significantly lower than concentrations that have been associated with nephrotoxicosis (30 μg/mL) and ototoxicosis (60 μg/mL) in humans.^{25,26} Neurologic signs of toxicosis caused by vancomycin are rare,¹² and vancomycin was administered into the intraventricular space in the CNS of humans with no adverse effects in a recent report.²⁷ The plasma concentrations achieved in our study were substantially lower than those reported (C_{max}, approx 40 μg/mL) in another study¹⁴ of systemic administration in horses. Those authors reported no adverse effects, but signs of toxicosis are more likely to occur after repeated administration, which is usually necessary with orthopedic infections.²⁸ In such instances,

use of the IVRLP technique could help diminish the risk of systemic toxicosis.

Measurement of additional variables such as creatinine clearance, urine albumin concentration, urine lactate dehydrogenase activity, urine α -N-acetyl-D-glucosaminidase activity, and urine maleate dehydrogenase activity as well as histologic study of renal tissue from horses that received vancomycin would have provided more extensive assessment of changes associated with nephrotoxicosis.²⁹⁻³¹ However, results of studies³¹⁻³⁴ in human patients and experiments with animal models indicate that measurement of blood urea and serum creatinine over a period of 5 days accurately detects changes associated with nephrotoxicosis.

Concurrent administration of phenylbutazone, a known nephrotoxin, could confound making conclusions regarding vancomycin-induced nephrotoxicosis. Phenylbutazone was administered to the horses of our study because horses with orthopedic infections are typically treated with nonsteroidal anti-inflammatory drugs and antimicrobials. It was also administered for humane concerns and to remain in accordance with animal care committee requirements for the study protocol. The fact that no clinical signs of nephrotoxicosis were observed in the study horses, which were receiving both drugs, supports the protocol's safety for clinical use.

Because of the drug's irritant properties, the safety of intra-articular and regional administration of vancomycin has been questioned.^{14,35} However, no signs of phlebitis or thrombophlebitis after IV administration were evident in the horses. No comments can be made, however, regarding the safety of intra-articular injection of vancomycin. We administered a solution in which the concentration of vancomycin was 5 mg/mL, a concentration that is biocompatible with endothelial cell function.³⁶ Adverse reactions have been related to infusion rate, and not exceeding a rate of 10 mL/min purportedly minimizes the likelihood of phlebitis.³⁷ Other local or regional clinical variables (eg, lameness in the perfused limb) and those related to the fetlock joint (eg, effusion, heat, and sensitivity to palpation) evaluated in our study were minimal and clinically unimportant. Although the concurrent administration of phenylbutazone may have influenced the development or detection of vancomycin-induced inflammation and phlebitis, there were no significant differences in values between horses in the vancomycin and control groups. If vancomycin-induced toxic effects were

similar in severity to those described in humans,^{38,39} differences between values in the vancomycin and control groups would have been expected. However, further studies would be necessary to assess the safety of repeated IVRLP treatments with vancomycin.

The high variability of vancomycin concentrations detected in synovial fluid samples was comparable to results of other studies^{20,40-43} in which administration of other antimicrobials via IVRLP to horses and cattle was investigated. Inadequate tourniquet application, variation in pressure while applying the Esmarch bandage, differences in the ratio between antimicrobial dose and the distal limb vascular volume, differences in medullary volumes of the involved bones, and atypical vascularization patterns have been indicated as possible causes for such variation.^{20,41-44} To minimize individual variation in our study, the Esmarch bandage and tourniquet were applied by the same investigator (LMR) in each horse.

Leakage of perfusate into the systemic circulation across the tourniquet is unlikely to have occurred because no vancomycin was detected in any samples collected before the tourniquet was released. Pneumatic tourniquets may permit better control of applied pressure, and successful results have been reported with the use of rubber tourniquets.¹⁸⁻²⁰ The effectiveness of a tourniquet depends on the width of the cuff, rate of infusion of fluids, cuff pressure, and removal of blood prior to placement of the tourniquet cuff.⁴⁴ The tourniquet should be at least 20% wider than the diameter of the limb or structure to which it is applied,⁴⁴ and on that basis, we calculated that a width of 11 cm would be appropriate for use on the proximal portion of the equine metacarpus. By evacuating blood from the extremity and infusing the perfusate solution at a slow rate (ie, 2 mL/min), the likelihood of reaching the maximal venous pressure (resulting in leakage into systemic circulation⁴⁴) was minimized.

Peak concentrations of vancomycin in systemic plasma were achieved at 65 minutes, a logical finding in that it corresponds with the first sampling time after tourniquet removal. The fact that synovial fluid concentrations of the drug peaked at 45 minutes in both joints indicates that the vancomycin concentration was higher in the synovial membrane capillaries than in synovial fluid from 30 to 45 minutes, and as a result, the antimicrobial continued to diffuse into the synovial cavity, reducing the concentration gradient.⁴⁵ It is logical to suppose that local intravascular vancomycin concentration was maximal at the end of infusion and then decreased as the drug diffused into the different structures while the tourniquet was maintained, as has been described for ceftiofur.¹⁹ Therefore, instead of releasing the tourniquet at the time the infusion is completed,^{41,46,47} we recommend that the tourniquet be maintained for a period of time after the infusion is finished. Factors such as drug physicochemical characteristics⁴⁸ will affect the rate of diffusion into the tissue and the length of time that the tourniquet should be maintained. Consequently, this interval will differ among pharmaceuticals, as has been observed with cephalosporins and amikacin.^{18,20,42} It cannot be concluded

from our results whether 45 minutes of tourniquet duration was long enough to yield maximum synovial fluid concentrations of vancomycin or whether maintenance of the tourniquet for a longer period would have improved those values.

It has been recommended that infusion of antimicrobials be performed from a site distal to the septic focus because the distribution of drug follows the venous system.^{41,45} However, according to our results and those of other studies,^{18,20,43} structures distal to the point of infusion are adequately perfused with the technique used in our study. In fact, our results indicate that vancomycin initially diffused more quickly and to a larger degree into the DIP joint than into the MTCP joint, a finding also reported with amikacin.²⁰

Antimicrobials diffuse from blood into tissues in accordance with Fick's principle.⁴⁹ Histologic properties of the synovial membrane and the antimicrobial gradient may be considered equal for the DIP and MTCP joints. Therefore, it is possible that the observed differences were related to the ratio between synovial fluid volume and synovial membrane surface area in each joint. It should also be considered that the tip of the catheter was situated 2.5 cm distal to the site of entry, and because the perfusate was infused in a distad direction under pressure, the IVRLP technique used may have favored perfusion of distal structures over more proximally located structures. Nonetheless, our results are not conclusive. Further studies are needed to describe the elimination phase of vancomycin in the DIP joint and investigate whether there were other differences in the distribution of the drug over time.

Because vancomycin is a time-dependent antimicrobial (ie, antimicrobial for which drug concentration must be maintained above the MIC for efficacy), bactericidal activity is determined mainly by the surrogate variable $T > MIC$, where $T = \text{time}$.⁵⁰ Isolates of *S aureus* with MIC values $\leq 4 \mu\text{g/mL}$ are considered to be susceptible to vancomycin.^{23,24} Three recent equine MRSA isolates at the University of Guelph had MIC values $\leq 4 \mu\text{g/mL}$.¹ The concentration of vancomycin in the DIP joint remained $> 4 \mu\text{g/mL}$ during the 90 minutes of study and were maintained at that concentration for approximately 20 hours in the MTCP joint. Vancomycin exerts a postantimicrobial effect against staphylococcal species,^{50,51} indicating that antibacterial activity may be maintained for nearly 24 hours after administration of 300 mg of vancomycin via IVRLP. Synovial fluid concentrations of vancomycin after IVRLP were substantially higher than those measured after systemic administration.¹⁴ To maintain synovial fluid concentrations $\geq 2 \mu\text{g/mL}$ (the trough concentration targeted by those investigators), systemic administration of vancomycin (4.3 to 7.5 mg/kg, IV, q 8 h) was necessary, whereas a single dose of 300 mg of vancomycin given via IVRLP resulted in the desired concentration (MTCP-24h, 2.52 $\mu\text{g/mL}$). The mean $t_{1/2}$ of vancomycin in synovial fluid was > 7 hours, a value that was higher than $t_{1/2}$ β values described in synovial fluid (1.2 \pm 0.42 hours) and blood (3.69 \pm 0.81 hours).¹⁴ Although the synovial fluid concentrations of vancomycin in the DIP joint were higher than those in the MTCP joint during the 90 minutes of study and

were significantly different at 15 and 30 minutes, no conclusions can be drawn regarding a possible difference in antimicrobial activity between the joints. Further studies would be needed to clarify this possibility. Up to 90 minutes, however, pharmacokinetic variables were not significantly different between the joints.

Despite the time-dependent nature of vancomycin, increasing the C_{max} has been correlated with greater bacterial killing⁵² and AUC_t/MIC has been associated with therapeutic efficacy and outcome in a mouse model.⁵³ Intravenous RLP provides much higher synovial C_{max}/MIC (> 20) and AUC_t/MIC (AUC , $17,038.04 \pm 7,988.76 \mu\text{g}\cdot\text{min}/\text{mL}$) values than does systemic administration ($C_{max}/MIC < 10$; AUC , $2,100 \pm 354 \mu\text{g}\cdot\text{min}/\text{mL}$).¹⁴ Consequently, administration of vancomycin via IVRLP may result in higher bactericidal activity and, potentially, a higher bacteriologic response rate than parenteral administration. Further studies on bactericidal efficacy and repeatability of vancomycin RLP need to be done.

The concentrations of vancomycin attained in synovial fluid with IVRLP may also be helpful in the management of infectious arthritis caused by *S aureus* strains with an intermediate level of susceptibility, in which eradication of the bacteria is an important concern because of the high MIC values (ie, 8 to 16 $\mu\text{g}/\text{mL}$).⁵⁴ The administration of vancomycin by means of IVRLP would avoid exposing organisms to suboptimal concentrations of the antimicrobial, the most important risk factor for emergence of resistant strains.⁵⁵ However, the synovial fluid concentrations and pharmacokinetics of vancomycin described in this study may be different under septic conditions^{46,48,56} in which vascular drug permeability may be greatly altered.^{15,16} In septic tissue, acidic and anaerobic environments reduce the activity of certain antimicrobials, including vancomycin.¹⁷ Antimicrobial efficacy could also be affected by increased protein concentrations in a septic site because the concentration of free drug (ie, non-protein-bound) is important to the success of treatment.⁵⁷ Further studies in horses with infectious arthritis would be necessary to ascertain these differences.

As recommended by AVMA guidelines^k for the judicious use of antimicrobials, administration of vancomycin should be reserved for infections for which results of bacterial culture and susceptibility testing have determined that there are no other antimicrobial alternatives. Vancomycin has been considered a last-resort drug in human medicine and should be a last-resort drug in veterinary medicine as well, given that emergence of new resistant bacterial strains is an ongoing and serious concern.

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- c. Vancomycin, Xechem International Inc, New Brunswick, NJ.
- d. Perfusor fm B/Braun, Barcelona, Spain.
- e. Nylon Unifilar, Lab Aragó SA, Barcelona, Spain.
- f. Tapval, JP Selecta SA, Barcelona, Spain.

- g. AxSYM vancomycin II, Abbot Científica SA, Madrid, Spain.
- h. Pcnnonlin, version 4.0, Statistical Consultants Inc, Lexington, Ky.
- i. SPSS, version 10.0, SPSS Inc, Chicago, Ill.
- j. Dr. M. Archaumbaut, University of Guelph, ON, Canada: Personal communication, 2004.
- k. AVMA. Guidelines for the judicious use of antimicrobials. Available at: www.avma.org. Accessed July 13, 2004.

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