Evaluation of safety and pharmacokinetics of vancomycin after intraosseous regional limb perfusion and comparison of results with those obtained after intravenous regional limb perfusion in horses

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Objective—To evaluate the clinical effects and pharmacokinetics of vancomycin in plasma and synovial fluid after intraosseous regional limb perfusion (IORLP) in horses and to compare results with those obtained after IV regional limb perfusion (IVRLP).

Animals—6 horses.

Procedures—1 forelimb of each horse received vancomycin hydrochloride (300 mg in 60 mL of saline [0.9% NaCl] solution) via IORLP; the contralateral limb received 60 mL of saline solution (control). Solutions were injected into the medullary cavity of the distal portion of the third metacarpal bone. Synovial fluid from the metacarpophalangeal (MTCP) and distal interphalangeal (DIP) joints and blood were collected prior to perfusion and 15, 30, 45, 65, and 90 minutes after beginning IORLP, and synovial fluid from the MTCP joint only and blood were collected 4, 8, 12, and 24 hours after beginning IORLP. Plasma urea and creatinine concentrations and clinical appearance of the MTCP joint region and infusion sites were determined daily for 7 days. Results were compared with those of a separate IVRLP study.

Results—Clinical complications were not observed after IORLP. Mean vancomycin concentration in the MTCP joint was 4 μg/mL for 24 hours after IORLP. Compared with IVRLP, higher vancomycin concentrations were detected in the DIP joint after IVRLP. Compared with IORLP, higher vancomycin concentrations were detected in the MTCP joint for a longer duration after IORLP.

Conclusions and Clinical Relevance—IORLP with 300 mg of vancomycin in a 0.5% solution was safe and may be clinically useful in horses. Intravenous and intraosseous routes may be better indicated for infectious processes in the DIP and MTCP joints, respectively. (Am J Vet Res 2006;67:1701–1707)

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>IVRLP</td>
<td>IV regional limb perfusion</td>
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<tr>
<td>MRSA</td>
<td>Methicillin-resistant Staphylococcus aureus</td>
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<tr>
<td>IORLP</td>
<td>Intraosseous regional limb perfusion</td>
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<tr>
<td>MTCP</td>
<td>Metacarpophalangeal</td>
</tr>
<tr>
<td>DIP</td>
<td>Distal interphalangeal</td>
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<tr>
<td>β</td>
<td>Elimination rate constant</td>
</tr>
<tr>
<td>t1/2 β</td>
<td>Terminal half-life</td>
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<tr>
<td>AUC</td>
<td>Area under the concentration-versus-time curve</td>
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<tr>
<td>MRT</td>
<td>Mean residence time</td>
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<td>Cmax</td>
<td>Maximum concentration</td>
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<td>MIC</td>
<td>Minimum inhibitory concentration</td>
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Administration of vancomycin by IVRLP to healthy horses has been described.1 Vancomycin, a bactericidal glycopeptide highly active against staphylococci organisms such as MRSA, has been reported for control of orthopedic infections in horses.2 High vancomycin concentrations in regional synovial joints were described following IVRLP,3 which could be applied clinically.

Intravenous regional limb perfusion and IORLP provide high regional antimicrobial concentrations while maintaining low systemic concentrations, thus reducing the possibility of systemic adverse effects.4,5 Use of regional limb perfusion can also counteract inadequate drug distribution into infected tissues after systemic administration of antimicrobials.6 The clinical effectiveness of regional limb perfusion with other antimicrobials has been reported in horses.7,8

We hypothesized that administration of vancomycin via IORLP would be a safe clinical procedure and would result in high drug concentrations in synovial fluid of joints in the distal portion of the forelimb. This could be applied for the treatment of infections by organisms that are susceptible to vancomycin, while preventing vancomycin-associated toxicosis. We also hypothesised that vancomycin concentrations and synovial fluid pharmacokinetics in distal joints as well as clinical effects would be similar after IVRLP and IORLP.

The purposes of the study reported here were to evaluate the clinical effects and pharmacokinetics of vancomycin in plasma and synovial fluid after IORLP in horses and to compare results with those obtained in a separate study after IVRLP.
Materials and Methods
Horses—Six horses of mixed breeds and sexes, 4 to 20 years old (mean, 11.2 years) and weighing from 380 to 484 kg (mean, 432.5 kg) were used in the study. The horses were owned by the Universidad Complutense de Madrid. Horses were considered to be 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 daily. The study was approved by the institutional animal care and use committee. Characteristics of horses and methodology used during IVRPL have been described elsewhere.1

Experimental design—Each horse received 2 treatments of regional limb perfusion: 1 control treatment and 1 vancomycin treatment, randomly distributed between the forelimbs. Treatments were performed during general anesthesia and separated by a 2-week washout period. For the control treatment, the perfusate was 60 mL of saline (0.9% NaCl) solution; for the vancomycin treatment, the perfusate was 60 mL of saline solution containing 300 mg of vancomycin hydrochloride.1 The dose of vancomycin was determined as previously described.1 The perfusate was injected into the medullary cavity of the distal portion of the third metacarpal bone.

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 (+4 mg/kg, IV; q 12 h) was administered to all horses on the day of the experimental procedure, with the first dose administered prior to anesthetic premedication (sedation), and administration of phenylbutazone (2.2 mg/kg, IV; q 12 h) was continued for 4 days.

While anesthetized, horses were positioned in lateral recumbency with the limb to be perfused uppermost and the distal portion of the limb (ie, from the carpus to the hoof) was clipped and aseptically prepared. A 2-cm skin incision was made along the lateral aspect of the distal portion of the third metacarpal bone. The periosteum was incised and elevated, and a 3.2-mm-diameter hole was drilled into the medullary cavity of the third metacarpal bone. Threads were created by use of a 4.5-mm tap, and a custom-made cannulated infusion screw was inserted. The infusion screw was manufactured by use of a 20-mm-long and 4.5-mm-diameter cortical bone screw, which was cannulated (1-mm diameter) and welded to a luer-lock head. A 1.5-cm-long incision was made on the lateral aspect of the proximal phalanx, and a 2-mm-diameter hole was drilled through the cortex until the medullary cavity was entered. This hole was used for collection of bone marrow samples from the proximal phalanx for use in another study.

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 MTCP joint was entered through the lateral collateral sesamoid ligament with a 23-gauge, 40-mm needle attached to a 2-mL syringe. The DIP joint was entered with a 21-gauge, 40-mm needle by use of the dorsomediopalmar, medial distopalmar, dorsolateral, and dorsomedial approaches, abaxial to the common digital extensor tendon with a 20-gauge, 40-mm needle attached to a 5-mL syringe. Both needles were kept in place during the 90 minutes of the procedure to minimize trauma to the synovial membrane from repeated punctures. The perfusate was manually infused at a rate of 2 mL/min by use of 10-mL syringes connected to an extension set through a 3-way stopcock. 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 MTCP and DIP joints and blood samples (volume, 2 mL) from a jugular vein were obtained 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.

After collection of samples at 90 minutes, the cannulated screw was removed and the skin incision was sutured with nonabsorbable polyamide 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 at 4, 8, 12, and 24 hours after beginning the infusion. To facilitate the procedure, horses were sedated with detomidine (0.01 mg/kg, IV). The MTCP joint was entered with a 21-gauge, 40-mm needle by use of the following approaches in the following order: lateral distopalmar, medial distopalmar, dorsolateral, and dorsomedial.

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

Clinical observations after IORPL—Horses were evaluated prior to the procedure and during the ensuing 7 days by the same investigator who was unaware of which treatment was performed on each limb. Clinical, lameness, and neurologic examinations were performed daily. Clinical observations regarding the perfused limbs were made and scored on the basis of a numeric scale, as previously described.1 These variables included lameness (scored on a scale from 0 [no lameness] to 4 [nonweight-bearing]); MTCP joint effusion (scored on a scale from 0 [no effusion observed or palpated] to 3 [severe or prominent effusion in both the lateral and dor- sal joint pouches]); palpable heat (scored on a scale from 0 [negative] to 3 [severe]), and sensitivity to digital pressure (scored on a scale from 0 [no sensitivity] to 3 [severe]); and signs of inflammation, discharge, or sensitivity to palpation at the infusion site on the third metacarpal bone (scored on a scale from 0 [negative] to 3 [severe]). Renal function variables (eg, plasma urea and creatinine concentrations) were measured prior to perfusion and on days 1, 2, 3, 5, and 7 after perfusion.

Sample processing and vancomycin assay—All blood and synovial fluid samples were collected into tubes containing heparin.1 Blood samples were centrifuged at 1,207 X 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 fluorescence polarization immunoassay, previously validated for use in equine samples.1

Pharmacokinetic analysis—After synovial fluid and plasma concentrations of vancomycin were measured, the disposition of vancomycin was analyzed via noncompartmental pharmacokinetic analysis by use of a software program.7 Variables determined included $\beta$ (minutes) calculated as the slope of the elimination phase of the concentration-versus-time curve), $t_{1/2}$ (minutes), AUC, (μg/min/mL), and MRT, (μg/min/mL). For synovial fluid samples obtained from the MTCP joint and plasma samples, variables were calculated for 24 hours (t = 24). For synovial fluid samples obtained from

References:
the DIP joint, variables were calculated for the 90 minutes studied in that joint (t = 90). Variables determined in synovial fluid samples from the MTCP joint up to 90 minutes were also calculated for comparison with synovial fluid samples obtained from the DIP joint.

**Statistical analysis**—Statistical analyses were performed by use of a computer program. Data were grouped and tested for normality distribution by use of the Kolmogorov-Smirnov test. Normally distributed data were analysed by use of ANOVA, whereas Wilcoxon signed rank and Mann-Whitney tests were used for nonparametric testing.

For comparison of vancomycin and control treatments, clinical observations after IORLP were analyzed by use of the Wilcoxon signed rank test. Vancomycin concentrations were analyzed with a repeated-measures ANOVA test, with time as the unique factor for synovial fluid samples from the MTCP joint and plasma samples. 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 joint). When results of repeated-measures ANOVA were not significant, a Student t test was used for comparison of vancomycin concentrations in MTCP and DIP joints at each sampling time. Pharmacokinetic variables that were determined for synovial fluid from both joints until 90 minutes after IORLP began were compared with a Wilcoxon signed rank test. For all statistical methods, values of P < 0.05 were considered significant.

Results obtained after IORLP were statistically compared with those obtained after IVRLP, which have been previously reported. Clinical observations obtained in horses receiving vancomycin after IVRLP and IORLP were compared with a Mann-Whitney test, which was also used to compare clinical observations obtained after horses received the control treatment. Vancomycin concentrations in synovial fluid from each location (MTCP joint and DIP joint) and in plasma were compared between IVRLP and IORLP by means of repeated-measures ANOVA with 2 factors (time and route of administration), and differences were further analyzed with a Student t test. Pharmacokinetic variables obtained during IVRLP and IORLP were compared with a Mann-Whitney test. For all statistical methods, values of P < 0.05 were considered significant.

**Results**

None of the horses developed complications during IORLP. Results of clinical and neurologic examinations 7 days after the experiment were not significantly different between treatments. Plasma concentrations of urea and creatinine remained within reference ranges (20 to 50 mg/mL and 0.4 to 2.2 mg/dL, respectively). Minimal evidence of lameness (maximal mean ± SD, 0.25 ± 0.418 and 0.25 ± 0.258 for vancomycin and control treatments, respectively) and mild-to-moderate MTCP joint effusion (maximal mean ± SD, 1.25 ± 0.758 and 1.00 ± 0.837 for vancomycin and control treatments, respectively) and sensitivity to palpation (maximal mean ± SD, 0.50 ± 0.548 and 0.50 ± 0.548 for vancomycin and control treatments, respectively) were mild. At the injection site, mild soft tissue inflammation (no drainage and not associated with signs of pain), which resolved within 1 week without additional treatment, was observed in horses after both treatments (maximal mean ± SD inflammation value, 0.83 ± 0.816 for both vancomycin and control treatments). Surgical wounds at the lateral aspect of the third metacarpal bone healed without complications in horses after both treatments.

Vancomycin was not detected in plasma samples collected before perfusion, and there were no detectable concentrations in plasma until the tourniquet was released. The Cmax of vancomycin in plasma was reached 65 minutes after initiating IORLP (Table 1). For both joints, tourniquet release was associated with a change in the slope of the vancomycin concentration-versus-time curves; Cmax in synovial fluid was reached 45 minutes after initiating IORLP (Figure 1).

Time was a significant factor in the overall change in vancomycin concentrations in plasma and synovial fluid from MTCP and DIP joints. Time was also a significant factor for the sampling intervals from 45 minutes to 8 hours in plasma and for all sampling intervals in the MTCP joint, except for the following intervals: MTCP-45 to MTCP-65 (P = 0.193), MTCP-65 to MTCP-90 (P = 0.099), and MTCP-90 to MTCP-120 (P = 0.93). The overall change in synovial concentrations up to 90 minutes was not significantly different between joints (P = 0.062) or for any sampling interval (P > 0.05). The mean ± SD concentration of vancomycin in synovial fluid from the MTCP joint (47.33 ± 17.74 μg/mL) was higher than that in synovial fluid from the DIP joint (39.335 ± 17.74 μg/mL); however, this difference was not significant (P = 0.453).

Pharmacokinetic variables at the different locations were summarized (Table 2). Values for β and t1/2 were calculated for comparison with synovial fluid samples from the MTCP joint up to 90 minutes were also calculated for comparison with synovial fluid samples obtained from the DIP joint.

Table 1—Vancomycin concentrations (μg/mL) measured in synovial fluid obtained from the MTCP and DIP joints of a forelimb and plasma (P) of 6 horses in which vancomycin (300 mg in 60 mL of saline [0.9% NaCl solution]) was administered via IORLP for 30 minutes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ± SD</th>
<th>95% confidence interval</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTCP-00</td>
<td>0.0 ± 0.0</td>
<td>0.0–0.0</td>
<td>0.0–0.0</td>
</tr>
<tr>
<td>MTCP-15</td>
<td>17.4 ± 10.7</td>
<td>7.9–26.8</td>
<td>4.9–36.3</td>
</tr>
<tr>
<td>MTCP-30</td>
<td>50.3 ± 26.5</td>
<td>30.2–70.4</td>
<td>29.1–91.3</td>
</tr>
<tr>
<td>MTCP-45</td>
<td>87.7 ± 40.7</td>
<td>52.0–123.5</td>
<td>39.3–143.2</td>
</tr>
<tr>
<td>MTCP-65</td>
<td>73.9 ± 37.4</td>
<td>38.1–109.7</td>
<td>43.7–145.5</td>
</tr>
<tr>
<td>MTCP-90</td>
<td>54.5 ± 24.7</td>
<td>27.9–81.1</td>
<td>28.0–82.4</td>
</tr>
<tr>
<td>DIP-00</td>
<td>0.0 ± 0.0</td>
<td>0.0–0.0</td>
<td>0.0–0.0</td>
</tr>
<tr>
<td>DIP-15</td>
<td>33.1 ± 18.0</td>
<td>9.4–56.9</td>
<td>9.7–49.3</td>
</tr>
<tr>
<td>DIP-30</td>
<td>56.3 ± 18.7</td>
<td>28.2–84.3</td>
<td>33.7–82.4</td>
</tr>
<tr>
<td>DIP-45</td>
<td>72.6 ± 33.1</td>
<td>35.9–109.3</td>
<td>36.6–128.8</td>
</tr>
<tr>
<td>DIP-65</td>
<td>42.5 ± 24.3</td>
<td>11.5–73.5</td>
<td>20.4–76.4</td>
</tr>
<tr>
<td>DIP-90</td>
<td>31.3 ± 19.0</td>
<td>3.1–59.5</td>
<td>17.5–60.8</td>
</tr>
</tbody>
</table>

| P-00 | 0.0 ± 0.0 | 0.0–0.0 | 0.0–0.0 |
| P-15 | 0.0 ± 0.0 | 0.0–0.0 | 0.0–0.0 |
| P-30 | 0.0 ± 0.0 | 0.0–0.0 | 0.0–0.0 |
| P-45 | 0.0 ± 0.0 | 0.0–0.0 | 0.0–0.0 |
| P-65 | 1.8 ± 0.6 | 0.9–2.6 | 0.9–2.7 |
| P-90 | 1.1 ± 0.5 | 0.6–1.6 | 0.6–2.1 |
| P-4h | 0.4 ± 0.4 | 0.1–0.8 | 0.1–0.8 |
| P-8h | 0.1 ± 0.2 | 0.0–0.3 | 0.0–0.3 |
| P-12h | 0.0 ± 0.0 | 0.0–0.0 | 0.0–0.0 |
| P-24h | 0.0 ± 0.0 | 0.0–0.0 | 0.0–0.0 |

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β could not be calculated for plasma or synovial fluid from the DIP joint because of the low concentrations and because the study ended 90 minutes after beginning the perfusion, respectively. When variables were compared between joints up to 90 minutes, a significant difference was detected for MRT90, but not for AUC90.

No significant differences in clinical observations were detected in horses receiving IVRLP and IORLP. For the MTCP joint, time was a significant factor in the change of the vancomycin concentration for both administration routes, but this change was not significantly different overall (P = 0.607) or for each sampling interval (P > 0.05). The mean ± SD concentration of vancomycin in synovial fluid from the MTCP joint after IORLP (34.391 ± 13.974 μg/mL) was higher than after IVRLP (25.910 ± 13.974 μg/mL); however, this difference was not significant (P = 0.318).

For the DIP joint, time was a significant factor in the change of the concentration of vancomycin. This change was significantly different overall between administration routes (P = 0.041) and for each sampling interval except for the DIP-45 to DIP-65 sampling interval (P = 0.057). The mean ± SD concentration of vancomycin in synovial fluid of the DIP joint after IORLP (39.345 ± 25.017 μg/mL) was lower than after IVRLP (62.45 ± 25.017 μg/mL); however, the difference was not significant (P = 0.141). Concentrations of vancomycin in synovial fluid after IVRLP were significantly higher than after IORLP 65 (P = 0.035) and 90 (P = 0.039) minutes after initiating each perfusion.

Regarding vancomycin concentration in plasma, time was a significant factor, but no differences between routes were detected. Only sampling times from 30 minutes to 8 hours were included in the analysis because vancomycin was not detected in plasma at the other sampling times, and the concentrations of vancomycin at 12 hours did not follow a normal distribution.

Comparison of the pharmacologic parameters after IVRLP and IORLP indicated that the MRT90 in the DIP joint after IVRLP was significantly (P = 0.033) higher than after IORLP. No other significant differences were detected between administration routes.

**Discussion**

In the study reported here, adverse clinical effects were not detected after IORLP with vancomycin in horses, and synovial concentrations of vancomycin were greater than the MIC (4 μg/mL)9,10 for MRSA for approximately 24 hours in the MTCP joint. Clinical signs of vancomycin toxicosis were not observed. Although the safe range of vancomycin concentrations in equine plasma is not known, plasma concentrations of vancomycin obtained in our study were similar to those obtained after IVRLP and significantly lower than the toxic thresholds described in humans11,12 and dogs.13 Intravenous regional limb perfusion performed in our previous study and IORLP performed in the study reported here maintained plasma concentrations of vancomycin that were substantially lower than concentrations detected in 1 study after IV administration of vancomycin and may help to decrease the risk of systemic toxicosis when repeated administration of vancomycin is necessary for treatment of orthopedic infections.15

Although measuring additional renal parameters would have provided an extensive assessment of nephrotoxicosis,16-18 measurement of plasma urea and creatinine concentrations for 5 days accurately detects changes associated with nephrotoxicosis.16-20 Concurrent administration of phenylbutazone, a known nephrotoxin, could have confounded our conclusions regarding vancomycin-induced nephrotoxicosis. However, similar to findings in the IVRLP study,16 clinical signs of nephrotoxicosis were not observed in the study reported here, indicating the absence of apparent toxicity after unique administration of vancomycin by both IVRLP or IORLP.

![Figure 1—Mean concentrations of vancomycin (μg/mL) in synovial fluid samples obtained from the MTCP and DIP joints of 6 horses in which vancomycin (300 mg in 60 mL of saline [0.9% NaCl] solution) was administered via IORLP for 30 minutes. Black triangle represents time of tourniquet release; vertical bars represent SDs.](image)

![Table 2—Mean ± SD values for pharmacokinetic variables measured in plasma and synovial fluid obtained from the MTCP joint of 6 horses 24 hours after and synovial fluid samples obtained from the MTCP and DIP joints 90 minutes after initiation of IORLP with vancomycin (300 mg in 60 mL of saline [0.9% NaCl] solution).](table)

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Plasma</th>
<th>MTCP</th>
<th>DIP</th>
<th>P value*</th>
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<tbody>
<tr>
<td>β × 10⁻⁶ (min⁻¹)</td>
<td>0.8 ± 0</td>
<td>1.42 ± 0.29</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>590.23 ± 98.04</td>
<td>509.23 ± 98.04</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>AUC (μg·min/mL)</td>
<td>25,102.84 ± 4,872.62</td>
<td>16,859.27 ± 11,626.06</td>
<td>1,965.15 ± 1,162.06</td>
<td>1,965.15 ± 1,162.06</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>11,626.06 ± 11,626.06</td>
<td>1,965.15 ± 1,162.06</td>
<td>67.14 ± 1.88</td>
<td>47.50 ± 4.11</td>
</tr>
</tbody>
</table>

Intraosseous regional limb perfusion was performed for 30 minutes.

*Results of Wilcoxon signed rank tests comparing variables between the MTCP and DIP joints. NA = Not applicable.
The irritant properties of vancomycin have been described; however, administration of vancomycin via IVRLP did not cause clinical signs of phlebitis or thrombophlebitis. The medullary cavity of bones contains an extensive net of sinusoid vessels, the structure of which is comparable to capillaries. Although histopathologic study of the bone marrow of the third metacarpal bone would have been more reliable for determining toxic changes than clinical evaluation, only mild clinical changes (mild soft tissue inflammation without evidence of discharge or signs of sensitivity) were evident over the infusion sites and differences in clinical observations in limbs receiving vancomycin and control treatments were not detected. This finding indicates that vancomycin administered via IOPRL was safe, although effects of repeated administration of vancomycin are not known. The concentration of vancomycin (5 mg/mL) and the infusion rate (10 mg/min) used in our study are biocompatible with endothelial cell function and minimized the likelihood of thrombophlebitis. Concurrent administration of phenylbutazone may have influenced the development or detection of vancomycin-induced inflammation and phlebitis; however, differences in clinical observations in limbs receiving vancomycin via IORLP were not detected. In micropigs, repeated administration of vancomycin into the medullary cavity of the tibia was safe and changes in the pharmacokinetics of vancomycin in plasma were not detected during a 6-month period, which would have been expected if vancomycin exerted toxic changes in the bone marrow.

The high pressures attained during intraosseous infusion (as much as +50 psi) precluded use of an IV infusion pump in our previous study. An angiographic injector would have been useful but not economically feasible. On the basis of results of an experimental and a clinical study, we chose to perform the IORLP manually. The infusion rate was the same as that applied for IVRLP and in all horses, the perfusate was administered by the same investigator (JLS) to minimize potential individual factors. Small-volume syringes (10 mL) permitted greater control of the infusion and voluntary opening and closing of the stopcock avoided perfusate reflux. Consequently, we believed that results from both techniques, IVRLP and IORLP, are comparable. A manual infusion technique offers advantages in experimental and clinical circumstances in which high-pressure injectors are not available.

The high variability of vancomycin concentrations in synovial fluid was comparable with results after IVRLP of vancomycin and results of other studies. To minimize individual effects, the Esmarch bandage and the tourniquet were applied by the same investigator (LMRM) in every horse. Leakage of perfusate to the systemic circulation under the tourniquet is unlikely to have occurred because of the absence of vancomycin in plasma before the tourniquet was released in any horse. Use of exsanguination and wide tourniquets reduces this risk.

In both joints, synovial fluid concentrations of vancomycin peaked 45 minutes after initiating IORLP, suggesting that vancomycin concentration was higher in synovial membrane capillaries than in synovial fluid from 30 to 45 minutes. As a result, vancomycin continued to diffuse into joints, reducing the concentration gradient. Similar findings were detected after IVRLP, which was expected because intraosseous infusion provides ready access to the systemic circulation. On the basis of those results, it would appear appropriate, when either IVRLP or IORLP is used, to maintain a tourniquet for a period of time after the infusion is completed.

After IVRLP, antimicrobials appear to diffuse faster and to a larger extent to the DIP joint than to the MTCP joint. Indeed, we obtained significantly higher vancomycin concentrations in the DIP joint, and MRT₉₀ was also significantly longer in the DIP joint after IVRLP than IORLP. In our previous study, we speculated that the Fick principle may provide an explanation for these findings. Considering both studies, the IV route provided significantly higher concentrations of vancomycin in the DIP joint than the intraosseous route, whereas the intraosseous route maintained higher concentrations in the MTCP joint for a longer duration than the IV route. Therefore, it is unlikely that those differences were associated with the synovial fluid volume and membrane surface area ratio within each joint. Because 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 ones. In another study, IVRLP into the saphenous vein caused higher amikacin concentrations in the tibiotarsal joint than those detected after IORLP into the distal portion of the tibia or proximal portion of the metatarsus. In that study, differences in the distances between the infusion site and the sampling location were suggested as the reason for the findings. Vancomycin concentrations in synovial fluid of the MTCP joint were similar after IVRLP and IORLP, although concentrations were greater than the MIC value (4 µg/mL) for MRSA for 24 hours after IORLP and for 20 hours after IVRLP. Although not significant, the peak synovial concentration of vancomycin was higher after IORLP than after IVRLP. In studies in pigs, plasma concentrations of vancomycin after systemic intratibial infusion were lower than those attained after systemic IV infusion. Therefore, the authors did not recommend the intraosseous route for systemic administration of vancomycin and considered that accumulation of vancomycin in bone tissue was possible. This may partially explain the differences in synovial fluid concentrations of vancomycin detected after IVRLP in our previous study and IORLP in the study reported here. Differences in plasma concentrations of vancomycin between administration routes were not detected; however, further studies would be necessary to discern this speculation.

As a time-dependent antimicrobial, vancomycin bactericidal activity is mainly determined by the surrogate parameter T > MIC (ie, time [T] that the concentration of the drug remains greater than the MIC). Isolates of Staphylococcus aureus with MIC values ≤ 4 µg/mL are considered to be susceptible to vancomycin. At the University of Guelph, 3 equine
MRSA isolates had MIC values ≤ 4 μg/mL. The concentration of vancomycin in the DIP joint remained ≥ 4 μg/mL during the 90 minutes of study and for 24 hours in the MTCP joint, which is longer than after IVRLP. Therefore, administration of vancomycin by use of the IORLP technique reported here may be preferable to the IVRLP when treating infections of the MTCP joint; however, significant differences in MTCP synovial fluid concentrations were not detected between IVRLP and IORLP; thus, further analyses would be needed. Antibacterial activity in the MTCP joint may be maintained for longer than 24 hours after administration of 300 mg of vancomycin via IORLP in healthy horses. Considering that after IORLP, MRT 90 in the MTCP joint was longer than in the DIP joint, higher antimicrobial activity would be expected in the MTCP joint. However, the elimination phase of vancomycin pharmacokinetics was not studied in the DIP joint. After IVRLP, concentrations were higher in the DIP joint than in the MTCP joint, although no significant differences were detected in any of the surrogate variables (t½ β, AUC 0→∞, or MRT 90). MRT 90 in the DIP joint was longer after IVRLP, indicating that the IV route of administration may be preferable when treating infections in the DIP joint. However, further studies would be necessary before making definitive conclusions, and clinicians must be cautious before extending these observations to other antimicrobials because particular differences in pharmacokinetics may lead to different results. Compared with systemic IV administration of vancomycin, IVRLP or IORLP with 300 mg of vancomycin maintained their targeted trough drug concentration in synovial fluid. The mean t½ β of vancomycin in synovial fluid after administration of vancomycin via IORLP was > 8 hours, which is similar to that in synovial fluid after IVRLP but higher than t½ β values detected in synovial fluid (1.2 ± 0.42 hours) and blood (3.69 ± 0.81 hours) after systemic IV administration of 3 doses of vancomycin (4.3 to 7.5 mg/kg).

Despite the time-dependent nature of vancomycin, increasing the Cmax has been correlated with augmentation of bacterial killing and has been associated with therapeutic efficacy and outcome in a mouse model. Consequently, a higher bactericidal activity of vancomycin and, potentially, a higher bacteriologic response rate may be observed after either IVRLP or IORLP (synovial Cmax/MIC > 20 and AUC 0→∞ > 17,000 μg·min/mL), than after IV administration of vancomycin (Cmax/MIC < 10 and AUC = 2,100 ± 354 μg·min/mL).

Concentrations of vancomycin attained in synovial fluid after IORLP similar to those after 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 μg/mL). Administration of vancomycin by regional limb perfusion would avoid exposing organisms to suboptimal concentrations of the antimicrobial, the most important risk factor for emergence of resistant strains. However, synovial fluid concentrations and pharmacokinetics of vancomycin described in our study may be different during sepsis, in which vascular drug permeability may be greatly affected. Further experimental studies in horses with infectious arthritis would be necessary to ascertain differences and determine the administration protocol (dose and interval) during orthopedic sepsis.

As recommended by the AVMA guidelines 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 as 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 against vancomycin would constitute a serious concern for both human and animal health.

References