Pharmacokinetics of florfenicol in serum and synovial fluid after regional intravenous perfusion in the distal portion of the hind limb of adult cows

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Objective—To define the pharmacokinetics of florfenicol in synovial fluid (SYNF) and serum from central venous (CV) and digital venous (DV) blood samples following regional IV perfusion (RIVP) of the distal portion of the hind limb in cows.

Animals—6 healthy adult cows.

Procedures—In each cow, IV catheters were placed in the dorsal common digital vein (DCDV) and the plantar vein of the lateral digit, and an indwelling catheter was placed in the metatarsophalangeal joint of the left hind limb. A pneumatic tourniquet was applied to the midmetatarsal region. Florfenicol (2.2 mg/kg) was administered into the DCDV. Samples of DV blood, SYNF, and CV (jugular) blood were collected after 0.25, 0.50, and 0.75 hours, and the tourniquet was removed; additional samples were collected at intervals for 24 hours after infusion. Florfenicol analysis was performed via high-performance liquid chromatography.

Results—In DV blood, CV blood, and SYNF, mean ± SD maximum florfenicol concentration was 714.79 ± 301.93 µg/mL, 5.90 ± 1.37 µg/mL, and 39.19 ± 29.42 µg/mL, respectively; area under the concentration versus time curve was 488.14 ± 272.53 h·µg·mL⁻¹, 23.10 ± 6.91 h·µg·mL⁻¹, and 113.82 ± 54.71 h·µg·mL⁻¹, respectively; and half-life was 4.09 ± 1.93 hours, 4.77 ± 0.67 hours, and 3.81 ± 0.81 hours, respectively.

Conclusions and Clinical Relevance—Following RIVP, high florfenicol concentrations were achieved in DV blood and SYNF, whereas the CV blood concentration remained low. In cattle, RIVP of florfenicol may be useful in the treatment of infectious processes involving the distal portion of limbs. (Am J Vet Res 2008;69:997–1004)
vitis, which are collectively known as deep digital sepsis. These conditions are among the most debilitating causes of lameness, often resulting in substantial loss of function or destruction of the animal.10 Treatment of deep digital sepsis generally requires surgical interventions (eg, debridement, drainage, and lavage) and stabilization of the afflicted tissues along with antimicrobial treatments. Antimicrobials may have to be administered for as long as 6 weeks in some instances.10,13–16 The expenses and labor associated with long-term antimicrobial administration may be impractical in many situations. In addition, systemic administration of antimicrobials may have limited efficacy because of the presence of necrotic material or inflammatory mediators, poor vascular perfusion, entrapment of bacteria in fibrin, and the presence of biofilm or glycolaxyl surrounding foreign material or surgical implants.17–20

Regional IV perfusion of antimicrobials in the distal portion of a limb may offer several advantages over systemic administration of those drugs in the treatment of deep digital sepsis. The advantages include development of high concentrations of the antimicrobial at the site of infection and, because of the more localized effects, low systemic drug exposure.20–22 Other potential advantages include decreased duration of antimicrobial treatment and reduced drug costs.

The successful use of RIVP in the treatment of orthopedic infections in horses has been reported.23,24 The dispositions of amikacin,25 gentamicin,26 ceftiofur,27 vancomycin,28 and enrofloxacin29 in horses following RIVP have been described. The concern regarding extended withdrawal periods for aminoglycosides limits the use of those drugs in food animals.30 Federal law prevents the extralabel use of enrofloxacin and vancomycin in food animals.31 However, the pharmacokinetics of cefazolin32 and ceftiofur33 delivered via RIVP in cattle have been described, and the RIVP of benzylpenicillin in cattle has been described in the European veterinary literature.34,35 Both of those reports refer to clinical studies and do not include assessments of the pharmacokinetics of the antimicrobials. Although concentration-dependent antimicrobials are ideally suited for RIVP, time-dependent antimicrobials may be effective as well.36 The concentration-dependent antimicrobials that are presently available to food animal practitioners include aminoglycosides and fluoroquinolones, but the use of such drugs in food animals is severely limited. Ceftiofur, a time-dependent antimicrobial, has been evaluated for use via RIVP in cattle.33 On the basis of the findings of that study, therapeutic cefotiofur concentrations are short-lived after tourniquet removal; consequently, an 8-hour dosing interval is required for maximum effectiveness of that drug.37 However, administration of RIVP every 8 hours is not practical in most clinical settings.

Florfenicol is a phenolic antimicrobial that inhibits the 50S subunit of the bacterial ribosome.38 Florfenicol is approved for use in the treatment of bovine respiratory disease complex and interdigital necrobacillosis in cattle.39 Although labeled for IM or SC administration, florfenicol can be safely administered IV.40–44 Pharmacodynamic properties of florfenicol are not as well-known as those of other classes of antimicrobials, but recent research45 has revealed that the drug has concentration-dependent bactericidal activity. In the report of that study, florfenicol was described as a time-dependent antimicrobial with notable concentration dependency against several major pathogens in veterinary medicine46, the pathogens of concern for cattle included Mannheimia haemolytica, Pasteurella multocida, and Haemophilus (Histophilus) somnus. Although the concentration-dependent activity detected was not as marked as that associated with typical concentration-dependent antimicrobials, even limited concentration dependency may be beneficial in the application of florfenicol via RIVP in food animals.

The purpose of the study reported here was to define the pharmacokinetics of florfenicol in SYNF and serum from CV and DV blood samples following RIVP of the distal portion of the hind limb in cows. We hypothesized that RIVP of florfenicol would result in concentrations of florfenicol in SYNF and in serum from DV blood samples that would meet or exceed therapeutic targets for the antimicrobial.

Materials and Methods

Animals—Six adult mixed-breed beef cows were included in the study. The cows’ ages ranged from 6 to 10 years; weights ranged from 443 to 688 kg. The cows were each identified with an ear tag labeled with a single letter (A, D, E, F, G, or K). No clinical signs of lameness were evident at the beginning of the study. The cattle were owned by the veterinary teaching hospital for at least a year prior to this study and had not been treated with florfenicol during that time. Throughout the study, the cows were housed in stalls in the hospital and provided with free-choice grass hay and water ad libitum. The cows were evaluated for evidence of lameness by the principle investigator during the sampling period and daily for 1 week following completion of sample collections. The study was approved by the Institutional Animal Care and Use Committee of Oklahoma State University.

Catheter placement—The cows were sedated via IV administration of 25 mg of xylazine hydrochloride and restrained in lateral recumbency in a hydraulic tilt chute. The distal aspect of the left hind limb, beginning at the midmetatarsal region, was clipped and cleaned with chlorhexidine scrub solution. A rubber tourniquet was placed tightly around the midmetatarsal region. Anesthesia of the distal portion of the limb was accomplished by use of ring block anesthesia (2% lidocaine) applied around the midmetatarsal area. The digits were covered with a sterile glove, and the skin over the distal portion of the limb was prepared in sterile manner.

Venipuncture of the DCDV was performed by use of an 18-gauge, 2.5-cm needle. A sterile guide wire was placed through the needle into the vein, and the needle was removed. A stab incision was then made over the vein with a No. 15 scalpel blade while the wire was used as a guide. An 18-gauge, 4.8-cm catheter was then passed over the wire and into the vein. The wire was removed, and a T-port with injection cap was then placed on the catheter. The catheter and T-port were...
The metatarsophalangeal joint was catheterized with a 20-gauge epidural infusion catheter. Arthrocentesis was performed over the craniolateral aspect of the joint by use of an 18-gauge, 3.8-cm needle. An injection cap was placed on the needle, and 30 to 50 mL of sterile saline (0.9% NaCl) solution was infused into the joint space through a 19-gauge butterfly catheter\(^1\) that was placed in the injection cap. As the joint capsule was distended, the caudolateral aspect of the joint region was palpated to identify the joint pouch. A 0.5-cm stab incision was made over the joint pouch with a No. 15 blade, and a 19-gauge, 9-cm Tuohy needle was placed in the caudolateral joint pouch. This needle was placed in the stab incision and held at an angle of approximately 30° lateral to the median plane of the limb and 15° caudal to the dorsal plane of the limb. The needle was advanced until it entered the distended joint space. A curved 16-gauge, 10-cm needle was placed through the skin approximately 4 cm proximal to the stab incision, advanced under the skin, and allowed to exit through the stab incision. The purpose of this needle was to create a subcutaneous tunnel through which the catheter tubing could be placed. The catheter tubing was passed approximately 1.5 cm into the joint through the Tuohy needle. The Tuohy needle was removed, and the catheter tubing was shortened to the desired length. The free end of the tubing was passed through the 16-gauge needle, and the needle was removed. The tubing was pulled through the subcutaneous tunnel until it was no longer exposed at the stab incision. An injection cap was placed on the catheter tubing, and the catheter was secured to the skin by use of 2-0 nylon suture in a Chinese finger-cuff pattern. A light bandage was placed over the catheters for protection. Sedation was reversed when needed via IV administration of tolazoline\(^2\) (1 mg/kg). All catheters were placed at least 24 hours prior to beginning the study.

**Dosage calculation**—The dose of 2.2 mg of florfenicol/kg used in the study was determined by weighing the distal portion of a hind limb (distal half of metatarsus and foot) of a cow that had been euthanized for nonmusculoskeletal disease and calculating a dose for that weight (12-kg limb specimen obtained from a 545-kg cow) on the basis of 40 mg of florfenicol/kg. The dose of 2.2 mg/kg of whole body weight was chosen because it provided 2 to 3 times the upper limit of the label dose (40 mg/kg) for the weight of the distal portion of the limb. At that dose, the volume of florfenicol delivered was small.

**Florfenicol administration and sample collection**—Each cow was sedated via IV administration of xylazine (25 mg) and restrained in lateral recumbency on the hydraulic tilt chute. A pneumatic tourniquet\(^3\) was placed around the limb at the midmetatarsal level. A 3-mL blood sample was collected from the PVLD, and both IV catheters were flushed with 3 mL of saline solution containing heparin. A 3-mL CV blood sample was also collected from the left jugular vein via venipuncture. A 0.5-mL sample of SYNF was collected from the metatarsophalangeal joint via the indwelling catheter. These first samples served as the baseline (time 0 hour) samples. The tourniquet was inflated to 300 mm Hg. Florfenicol (2.2 mg/kg) was administered into the DCDV (time 0). The catheter was not flushed after florfenicol administration; because the catheter was not flushed, an additional 0.6 mL of florfenicol was added to the dose to account for the volume of the catheter. The volume of florfenicol administered ranged from 3.9 to 5.6 mL. Digital venous and CV blood samples (3 mL) were collected from the PVLD and jugular vein, respectively, and an SYNF sample (0.3 to 0.5 mL) was collected at 0.25, 0.50, and 0.75 hours after florfenicol administration. The tourniquet was removed, and the cow was allowed to stand. Sample collection was repeated at 1, 1.5, 2, 4, 8, 12, 18, and 24 hours after florfenicol administration. Prior to each sample collection, 3 mL of blood and 0.2 mL of SYNF were collected and discarded. Blood samples were collected in plain glass tubes, and SYNF samples were placed in 0.5-mL plain plastic cryotubes.\(^4\) Synovial fluid samples were labeled and placed on ice immediately after collection. Blood samples were allowed to clot at room temperature (approx 26.5°C), and serum was harvested via centrifugation. Serum was placed in 1.5-mL plain plastic cryotubes.\(^5\) Samples that could not be centrifuged within 2 hours of collection were refrigerated. All sera were harvested within 18 hours of collection. Synovial fluid samples that were contaminated with blood were centrifuged, and the supernatant was collected.\(^32,33\) Synovial fluid samples with visible blood contamination were from cow A only. Serum and synovial fluid samples were frozen at 20°C until analysis could be performed.\(^40\)

**Sample analysis**—The serum and synovial fluid samples were assayed for florfenicol via reverse-phase high-pressure liquid chromatography with UV detection. The laboratory used other published references\(^42–45\) as a guide but added some modifications to the procedure to generate an assay adapted for the fluids collected from the cows in this study. The high-pressure liquid chromatography system consisted of a quaternary pump and degasser,\(^6\) an automated sampler,\(^7\) a UV detector,\(^8\) Plasma extraction was accomplished with solid-phase hydrophilic-lipophilic balanced extraction cartridges\(^8\) that were conditioned with 1 mL of methanol followed by 1 mL of distilled water. After addition of 200 µL of a serum sample to the cartridge, it was washed with 1.0 mL of distilled water and methanol (95:5 mixture). The eluent was discarded. The final elution was achieved via addition of 1.0 mL of methanol into a clean glass tube. The eluate was evaporated in a hot water bath (45°C) for 20 to 25 minutes and reconstituted with 200 µL of mobile phase.

A reverse-phase, stable-bond C-8 column\(^9\) (4.6 mm X 15 cm) was heated to 40°C to achieve separation. The mobile phase consisted of 70% distilled water and 30% acetonitrile. The UV detector was set to a wavelength of 223 nm. The volume for each injection was 20 µL. Retention time for florfenicol was 4.5 to 5.0 minutes. Chromatograms were integrated with computer software.\(^7\) We were not aware of any active metabolites to be detected.
A stock solution of florfenicol was prepared by dissolving a pure analytical reference standard of florfenicol in acetonitrile at a concentration of 1 mg/mL; the stock solution was stored in a refrigerator. The analytical reference standard solution was used to make calibration standards and fortify quality-control samples. The 1 mg/mL stock solution was further diluted serially with distilled water to concentrations ranging from 1,000 to 3.91 µg/mL. Standard curves for serum analysis were prepared by fortifying 200 µL of pooled bovine serum with 20 µL of the diluted stock solutions to make 11 calibration standards (including zero concentration) of florfenicol. Concentrations in the calibration curve incorporated the range of 100 to 0.195 µg/mL. Unfortified cattle serum was used as a blank sample to verify that the assay contained no interfering compounds and to determine the background noise for the assay. The fortified calibration samples were processed and prepared exactly as described for the collected experimental samples. For each day’s assay run, fresh sets of calibration and blank samples were prepared. Calibration curves of peak height versus concentration were calculated by use of linear regression analysis. All calibration curves were linear with a value of $R^2 \geq 0.99$. Limit of quantification for florfenicol in bovine serum was 0.195 µg/mL, which was determined from the lowest point on a linear calibration curve that was accompanied by an acceptable signal-to-noise ratio. The laboratory used guidelines published by the United States Pharmacopeia (2006).

The SYNF samples were prepared in the same manner as that of the serum samples, except for slight modification. Because SYNF is highly viscous, processing is difficult in extraction cartridges. Therefore, prior to processing, hyaluronidase (10 µL) was added to each sample followed by vortexing. The SYNF samples were then processed in the same manner as the sera. For the calibration samples, SYNF was collected from bovids that were examined by the college’s necropsy service for reasons other than musculoskeletal disease. According to each animal’s record, these cattle had not been treated with florfenicol. To prepare calibration samples, the SYNF was fortified with florfenicol in the same manner as the study serum samples. The calibration range was the same as that for the serum samples.

**Pharmacokinetic analysis**—Serum and SYNF concentrations of florfenicol after the injection into the DCDV were analyzed by use of a computer program. A noncompartmental analysis that does not assume any compartmental structure was used for the analysis because this was considered a locally (rather than systemically) administered injection and because calculation of compartmental parameters would have been subject to error. Calculation methods were derived from published methods.

For the noncompartmental analysis, the $AUC_{(0-\infty)}$ for serum or SYNF (defined by the limit of quantification) was calculated by use of the log-linear trapezoidal method. The $AUC_{(0-\infty)}$ was calculated by addition of the terminal portion of the curve (estimated from the relationship of $C_{\lambda}$, where $\lambda$ is the terminal rate constant of the curve, and $C_{\infty}$ is the last measured concentration) to the $AUC_{(0-\lambda)}$. The $AUC_{(0-\lambda)}$ (extrapolated by use of the trapezoidal rule) was calculated by use of the following equation:

$$AUC_{(0-\lambda)} = \left( \frac{[AUC_{(0-\lambda)} - AUC_{(0-C_t)}]}{AUC_{(0-\infty)}} \right) \times 100$$

Values for $C_{\max}$ and $T_{\max}$ were taken directly from the data. Half-lives were calculated from the terminal slope as follows: $t_{\frac{1}{2}} = \ln 2.0/(\text{terminal rate constant})$, where $\ln 2.0$ is the natural logarithm of 2.0. Traditional pharmacokinetic parameters, such as apparent volume of distribution and systemic clearance, were not calculated because the study involved regional administration of a drug and because those parameters describe whole-body effects.

**Statistical analysis**—Data were analyzed by use of computer software. The experimental design was a randomized complete-block design with repeated measures. The cow was considered the blocking variable, and location was considered the main unit factor. Time was the repeated-measures factor. Because of normality and heterogeneity of variance problems associated with the response variable, a natural logarithm(x+1) transformation was used to stabilize and normalize the data. Analysis of variance and an autoregressive period-1 covariance structure were used to model the intralocation covariances across time. If the test of simple effects yielded a significant result, pairwise $t$ tests were used to separate the means. A value of $P \leq 0.05$ was considered significant.

**Results**

Among the 6 cows, no lameness or other adverse effects were observed throughout the study period. A sample could not be collected from the PVLD for 1 cow at the 4-hour time point (cow D) and from another cow at the 18-hour time point (cow K; loss of catheter). The 24-hour DV blood sample for cow K was collected via venipuncture of the PVLD. Also, SYNF samples were not collected from 1 cow (cow A) at the 0.75- and 1-hour sample collection times because of difficulties with catheter function. Florfenicol was not detected in any of the samples collected at 0 hours or in the serum and synovial fluid samples used for calibration of the assay.

The concentration versus time profiles of florfenicol in DV blood, SYNF, and CV blood samples were calculated. Mean ± SD peak florfenicol concentrations in DV blood, SYNF, and CV blood samples were $714.8 \pm 301.9 \mu g/mL$, $379.2 \pm 294.4 \mu g/mL$, and $5.9 \pm 1.4 \mu g/mL$, respectively. At 0.25 hours after infusion, florfenicol concentration in DV blood samples was significantly higher than that in either SYNF or CV blood samples (Table 1). At 0.5 and 0.75 hours after infusion, concentrations in all sample types were significantly different. At 8 hours after infusion, no significant differences in florfenicol concentration were detected among sample types.

The mean pharmacokinetic parameters of florfenicol in DV blood, SYNF, and CV blood samples were calculated (Table 2). Values of pharmacokinetic parameters derived from DV blood, SYNF, and CV blood samples were calculated (Table 2). Values of pharmacokinetic parameters derived from DV blood, SYNF, and CV blood samples for individual cows varied considerably. For
example, 2 of the cows (cows A and F) had lower values for C\textsubscript{max}, AUC\textsubscript{0→∞}, and AUC\textsubscript{0→∞} relative to findings in other cows.

Discussion

Regional limb perfusion with antimicrobial agents for the treatment of chronic osteomyelitis in humans was reported in the late 1950s and early 1960s.\textsuperscript{49} Regional IV perfusion is easily performed on the distal portion of a limb by placing a tourniquet on the limb and infusing the antimicrobial distal to the tourniquet. Affected areas that are more proximal on the limb may be isolated by placing tourniquets proximal and distal to the area to be infused. Tourniquet application occludes venous drainage from the limb, resulting in an increase in intravascular pressure; the increased intravascular pressure and the concentration gradient created by infusion of the antimicrobial promote diffusion of the drug into the surrounding tissues.\textsuperscript{50} The antimicrobial agent may be infused into any accessible vein in the target area—the DCDV is the most easily accessible and commonly used vein for RIVP in the distal portion of the limbs of cattle.

In the present study, the metatarsophalangeal joint was catheterized to facilitate the collection of multiple SYNF samples over a period of time. During the placement of these catheters, the joint space was distended with sterile saline solution to facilitate identification of the caudalateral joint pouch and ensure correct placement of the catheter. Catheter placement was performed in an aseptic manner to minimize the risk of infection. Distention of the joint capsule could potentially result in inflammatory changes in the synovial membrane if the distention continued for a long period of time. Also, the acidic pH of physiologic saline solution could result in changes in the synovial membrane if it is not removed from the joint space. In the cows of our study, the distention was immediately relieved once the catheter was in place and was unlikely to result in notable changes in the joint. All catheters, including venous catheters, were placed at least 24 hours prior to the beginning of the study.

In horses, antimicrobials delivered via RIVP are often diluted to a volume of 60 mL.\textsuperscript{51} It is thought that a larger infusion volume results in increased intravascular pressure and better diffusion of the antimicrobial into the tissues.\textsuperscript{52} In the present study, florfenicol was not diluted because of the drug's poor solubility in water and we chose not to infuse high volumes of an organic solvent diluent into these tissues. In aqueous solution, the solubility of florfenicol is reported to be 2 mg/mL.\textsuperscript{53} The doses administered in our study would have had to be diluted in several liters of fluid to achieve solubilization, which is not practical for RIVP.

Because of the poor solubility of florfenicol in water, flushing a catheter with saline solution that contains heparin following florfenicol administration will result in precipitation of the florfenicol and obstruction of the catheter. In the authors’ clinical experience, injection of 1 mL of the patient’s own serum before and after the administration of florfenicol is an effective means of ensuring drug delivery through the catheter. Dilution of florfenicol with sterile water combined with a solubilizing agent, dimethyl formamide, has been described.\textsuperscript{52,53} However, that compound is a carcinogen\textsuperscript{54} and causes birth defects\textsuperscript{55}; therefore, it cannot be used in food animals.

Florfenicol is stable in physiologic fluids, which reduces the potential effects of sample handling on drug concentrations. Florfenicol recovery rates > 99% have been achieved in plasma and CSF that were stored at room temperature for 24 hours.\textsuperscript{56} In plasma samples that underwent 3 freeze-thaw cycles at –20°C over a 2-month period, florfenicol remained stable.\textsuperscript{57}

In the present study, administration of florfenicol via RIVP of the distal portion of the limb of cows resulted in high drug concentrations in DV blood and in SYNF collected from the metatarsophalangeal joint. There were some differences in drug concentrations in the 3 sample types among the study cows. In 2 cows, florfenicol concentrations in DV blood and SYNF samples were lower and the concentration in CV blood was higher than findings in corresponding samples collected from the other 4 cows. These data suggest that the tourniquet may not have provided enough venous occlusion in the limbs of those 2 cows, thereby allowing more of the administered dose to reach the systemic circulation prior to tourniquet removal. As a result, the comparatively lower concentrations of florfenicol in the vasculature distal to the tourniquet led to a lower concentration gradient for drug diffusion into the joint.

Table 1—Mean ± SE florfenicol concentration (µg/mL) in samples of serum derived from DV blood, SYNF, and serum derived from CV blood following RIVP of florfenicol (2.2 mg/kg) in the distal portion of a hind limb in 6 adult cows. Florfenicol was administered into the DCDV. Samples were collected for analysis after 0.25, 0.50, and 0.75 hours, and the tourniquet was removed; additional samples were collected at intervals for 24 hours after infusion.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>DV blood</th>
<th>SYNF</th>
<th>CV blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>706.15 ± 120.84¹</td>
<td>8.58 ± 2.87¹</td>
<td>4.18 ± 1.20¹</td>
</tr>
<tr>
<td>0.50</td>
<td>586.43 ± 145.17¹</td>
<td>21.03 ± 4.61¹</td>
<td>4.22 ± 1.29¹</td>
</tr>
<tr>
<td>0.75</td>
<td>588.65 ± 167.59¹</td>
<td>24.33 ± 7.28¹</td>
<td>3.76 ± 1.0¹</td>
</tr>
<tr>
<td>1.00</td>
<td>17.41 ± 5.5¹</td>
<td>42.58 ± 13.83¹</td>
<td>4.87 ± 0.46¹</td>
</tr>
<tr>
<td>1.50</td>
<td>9.30 ± 2.39¹</td>
<td>27.54 ± 6.38¹</td>
<td>3.57 ± 0.27¹</td>
</tr>
<tr>
<td>2.00</td>
<td>5.69 ± 0.96¹</td>
<td>21.99 ± 4.88¹</td>
<td>2.95 ± 0.28¹</td>
</tr>
<tr>
<td>4.00</td>
<td>3.26 ± 0.58¹</td>
<td>6.83 ± 2.37¹</td>
<td>1.90 ± 0.21¹</td>
</tr>
<tr>
<td>8.00</td>
<td>1.49 ± 0.38¹</td>
<td>2.71 ± 0.70¹</td>
<td>0.83 ± 0.12¹</td>
</tr>
<tr>
<td>12.00</td>
<td>1.07 ± 0.67¹</td>
<td>1.01 ± 0.32¹</td>
<td>0.40 ± 0.07¹</td>
</tr>
<tr>
<td>18.00</td>
<td>0.38 ± 0.20¹</td>
<td>0.42 ± 0.10¹</td>
<td>0.19 ± 0.04¹</td>
</tr>
<tr>
<td>24.00</td>
<td>0.49 ± 0.25¹</td>
<td>0.25 ± 0.07¹</td>
<td>0.09 ± 0.02¹</td>
</tr>
</tbody>
</table>

¹ For a given time point, values with different superscript letters are significantly (P < 0.05) different.

Table 2—Mean ± SD pharmacokinetic parameters for florfenicol in samples of serum derived from DV blood, SYNF, and serum derived from CV blood following RIVP of florfenicol (2.2 mg/kg) in the distal portion of a hind limb in 6 adult cows.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DV blood</th>
<th>SYNF</th>
<th>CV blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elimination rate (1/h)</td>
<td>0.22 ± 0.15</td>
<td>0.19 ± 0.04</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>t\textsubscript{1/2} (h)</td>
<td>4.09 ± 1.93</td>
<td>3.81 ± 0.81</td>
<td>4.77 ± 0.67</td>
</tr>
<tr>
<td>t\textsubscript{max} (h)</td>
<td>0.29 ± 0.10</td>
<td>0.38 ± 0.38</td>
<td>0.83 ± 0.31</td>
</tr>
<tr>
<td>C\textsubscript{max} (µg/mL)</td>
<td>714.79 ± 301.93</td>
<td>39.19 ± 29.42</td>
<td>5.9 ± 1.37</td>
</tr>
<tr>
<td>C\textsubscript{AUC} (µg·h/mL)</td>
<td>485.47 ± 270.17</td>
<td>112.97 ± 54.76</td>
<td>22.57 ± 6.72</td>
</tr>
<tr>
<td>AUC\textsubscript{0→∞} (µg·h/mL)</td>
<td>488.14 ± 272.53</td>
<td>113.82 ± 54.71</td>
<td>23.1 ± 6.91</td>
</tr>
<tr>
<td>AUC\textsubscript{0→t} (%)</td>
<td>0.46 ± 0.41</td>
<td>1.01 ± 0.88</td>
<td>2.95 ± 0.62</td>
</tr>
</tbody>
</table>
The tourniquet pressure of 300 mm Hg was chosen for use in our study on the basis of the pressure that has been reported to be effective in horses\(^2\); however, the pressure that we used was lower than that reported in 1 study\(^3\) involving mature cattle. Although the present study was not intended to evaluate the effects of various tourniquet pressures, we chose the lower pressure because it was effective in horses and theoretically less likely to cause adverse effects. Although none of the regionally perfused drug should reach the systemic circulation prior to tourniquet removal, some leakage does occasionally occur. In a previous study\(^4\) by one of the authors, amikacin was detectable systemically prior to tourniquet removal in 1 of 3 horses in which the drug was administered via RIVP. Another difference between the study reported here and other published studies is that most other studies are performed in anesthetized horses, whereas the cows of the present study were restrained but conscious. Struggling against restraint could potentially affect the performance of the tourniquet. We cannot speculate on the potential effect of higher tourniquet pressures in our study without additional data.

Regional IV administration of florfenicol into the DCDV resulted in high drug concentrations in the serum derived from DV blood samples. The concentration remained high until the tourniquet was removed, at which time the concentration decreased rapidly. Although the concentration of florfenicol decreased after tourniquet removal, the concentration remained \(>1\) µg/mL for 12 hours. Following administration of a single dose of 22 mg of florfenicol/kg in the jugular vein of veal calves, an initial drug concentration of 65.68 µg/mL has been reported.\(^4\) In another study,\(^5\) an initial florfenicol concentration of 39.7 µg/mL was detected following administration of a 20 mg/kg dose to feeder calves. Both of those studies investigated whole-body pharmacokinetics and did not involve regional drug administration or sample collection. In the present study, the initial florfenicol concentration in DV blood was 706.15 µg/mL following administration of a much smaller dose of the drug into the DCDV; this indicates that RIVP has the ability to attain much higher drug concentrations in the digital circulation, compared with peripheral concentrations achieved via central IV administration, even though lower doses (on a mg/kg basis) are administered.

In the study reported here, administration of florfenicol via RIVP in the distal portion of the hind limbs of cows resulted in high concentrations of florfenicol in the metatarsophalangeal joint. The mean peak SYNF concentration was 39.19 µg/mL, and \(T_{\text{max}}\) was 0.88 hours. This peak concentration occurred after removal of the tourniquet, which indicated that diffusion of florfenicol into the joint continued to occur after tourniquet removal. Florfenicol concentration in SYNF remained \(>1\) µg/mL for 12 hours and was 0.25 µg/mL at 24 hours after administration.

As expected, the peak florfenicol concentration in the DV blood samples was detected in the first sample collected after drug administration. The concentration slowly decreased from that time point until the tourniquet was removed, after which the concentration decreased rapidly. This decrease was expected because florfenicol diffuses from the vascular space into the surrounding tissues. The rapid decrease in DV blood concentration after tourniquet removal was expected and attributable to the release of the sequestered DV blood into the systemic circulation. Also, the florfenicol concentration in SYNF samples increased slowly from the time of administration until tourniquet removal. This increase was expected because florfenicol diffuses into the digital tissues as a result of the concentration gradient generated by the high concentrations of florfenicol in the vascular space. However, peak SYNF concentration was detected after removal of the tourniquet, a finding that was unexpected. It is possible that florfenicol in the periarticular tissues continued to diffuse into the SYNF after tourniquet removal.

The concentrations of florfenicol detected in the CV blood samples remained low throughout the study. Initial concentrations were higher than anticipated; those concentrations were probably caused by leakage of the drug beyond the region confined by the tourniquet location.

The terminal \(t_{\text{½}}\) for florfenicol determined in our study was slightly longer than values in other reports in the literature. Reported mean \(t_{\text{½}}\) values for florfenicol following IV administration include 2.87,\(^6\) 3.0,\(^6\) and 2.65\(^7\) hours. In the present study, the \(t_{\text{½}}\) of florfenicol in DV blood, SYNF, and CV blood samples was 4.09, 3.81, and 4.77 hours, respectively. Following IM administration, the \(t_{\text{½}}\) of florfenicol ranges from 12.5\(^8\) to 18.3\(^9\) hours. Because this is an extralabel use of florfenicol, a scientifically derived meat withdrawal time should be applied to IV administration of the drug. Following IM administration at a dose of 20 mg/kg, florfenicol has a meat withdrawal time of 28 days. Administration of florfenicol via RIVP as described in this report involved a lower systemic dose, compared with the label dose; furthermore, IV administration did not prolong the terminal \(t_{\text{½}}\) compared with that achieved with an IM injection. Therefore, on the basis of established label recommendations, we propose that such extralabel IV administration of florfenicol is unlikely to result in violative tissue residues.

Most digital infections in cattle involve a mixed bacterial population, including Arcanobacterium pyogenes and Fusobacterium necrophorum.\(^1,8,37\) A study involving 445 bovids with infections of portions of the appendicular skeletal revealed that A pyogenes was the most common bacterial agent. Florfenicol is active against both of these organisms. A New Animal Drug Application for florfenicol (NADA-141-063)\(^39\) reports an MIC\(_{90}\) for F necrophorum of 0.25 µg/mL. In a study\(^40\) of 49 A pyogenes isolates from cattle and pigs, the MIC\(_{90}\) for florfenicol was 1.56 µg/mL. In another investigation\(^41\) of 16 A pyogenes isolates from white-tailed deer, the MIC\(_{90}\) for florfenicol was 0.5 µg/mL. In the present study, the florfenicol concentrations from the DV blood samples remained greater than the florfenicol MIC\(_{90}\) for A pyogenes for a minimum of 8 hours and greater than the florfenicol MIC\(_{90}\) for F necrophorum for a minimum of 18 hours. In the SYNF samples, the drug concentration remained greater than the florfenicol MIC\(_{90}\) for A pyogenes for a minimum of 8 hours and greater than...
the florfenicol MIC$_{90}$ for *F. necrophorum* for 24-hour study period.

*Mycoplasma bovis* is a common cause of infectious arthritis in calves, especially following an episode of respiratory disease. The florfenicol MIC$_{90}$ against *M. bovis* is 16 µg/mL, and the minimum mycoplasmal concentrations of florfenicol at which 50% and 90% of organisms are inhibited are 16 and 32 µg/mL, respectively. Only danofloxacin is more active than florfenicol against *M. bovis*. In our study, RIVP of florfenicol resulted in SYNF concentrations that exceeded the minimum mycoplasmal concentrations of florfenicol at which 50% and 90% of *M. bovis* are inhibited for 2 hours and 1 hour, respectively.

Although it is thought that concentration-dependent antimicrobial agents are best suited for use in RIVP, both time- and concentration-dependent antimicrobials have been used. Pharmacokinetics of antimicrobials that have both time- and concentration-dependent pharmacodynamic characteristics have been investigated, but the efficacies of these 2 types of antimicrobial agents have not been compared in a controlled study, to our knowledge. Florfenicol pharmacodynamic properties are not understood as well as those of agents in other antimicrobial classes. However, recent research has revealed that florfenicol has concentration-dependent bactericidal activity. Unfortunately, the organisms investigated in that study were respiratory tract pathogens and did not include *F. necrophorum* or *A. pyogenes*.

Results of the present study indicated that RIVP of florfenicol administered at a dose of 2.2 mg/kg resulted in high drug concentrations in both serum and SYNF samples collected from the distal portion of the limb in cattle; however, that method of administration resulted in low drug concentration in CV blood. The most likely pathogens encountered in cases of deep digital sepsis include *F. necrophorum* and *A. pyogenes*, and the concentrations of florfenicol achieved in the distal portion of the limbs in our study exceed the published MIC$_{90}$ of these pathogens by several-fold. In addition, the concentration of florfenicol in SYNF exceeded the MIC$_{90}$ of *F. necrophorum* for at least 24 hours. In cattle, RIVP of florfenicol is worth investigating as a potential treatment of deep digital sepsis.

a. Xylazine 20 injection (20 mg/mL), The Butler Co. Dublin, Ohio.
b. Lidocaine 2% injectable solution, The Butler Co. Dublin, Ohio.
c. Intravenous catheter (1.348 mm), BD Insyte, Franklin Lakes, NJ.
d. Non-DEHP T connector, Medex, Dublin, Ohio.
e. Prepierced reseal male adapter plug-short, Hospira, Forest Lake, Ill.
f. Periflex continuous epidural anesthesia set, B. Braun Medical, Bethlehem, Pa.
g. Surflo winged infusion set, 19-gauge, 0.75-inch thin-walled needle with 12-inch tubing, Terumo Medical, Somerset, NJ.
h. Tolazoline injectable solution (100 mg/mL), Lloyd Laboratories, Shenandoah, Iowa.
i. Portable tourniquet system, Delfi Medical Innovations Inc, Vancouver, BC, Canada.
j. Florfenicol injectable solution (300 mg/mL), Schering-Plough Animal Health, Omaha, Neb.
k. Microcentrifuge tube (0.5 mL), SCI Dynamics, Adelphia, NJ.
l. Microcentrifuge tubes (1.5 mL), SCI Dynamics, Adelphia, NJ.
m. Agilent 1000 series solvent delivery system, Agilent Technologies, Wilmington, Del.

References