Pharmacokinetics and tissue distribution of doxycycline after oral administration of single and multiple doses in horses

Jennifer L. Davis, DVM, MS; Jacklyn H. Salmon, BS; Mark G. Papich, DVM, MS

Objective—To determine pharmacokinetics, safety, and penetration into interstitial fluid (ISF), polymorphonuclear leukocytes (PMNLs), and aqueous humor of doxycycline after oral administration of single and multiple doses in horses.

Animals—6 adult horses.

Procedure—The effect of feeding on drug absorption was determined. Plasma samples were obtained after administration of single or multiple doses of doxycycline (20 mg/kg) via nasogastric tube. Additionally, ISF, PMNLs, and aqueous humor samples were obtained after the final administration. Horses were monitored for adverse reactions.

Results—Feeding decreased drug absorption. After multiple doses, mean ± SD time to maximum concentration was 1.63 ± 1.36 hours, maximum concentration was 1.74 ± 0.3 µg/mL, and elimination half-life was 12.07 ± 3.17 hours. Plasma protein binding was 81.76 ± 2.43%. The ISF concentrations correlated with the calculated percentage of non–protein-bound drug. Maximum concentration was 1277 ± 8.98 times as great in PMNLs, compared with plasma. Drug was detected in aqueous humor at 75% to 10% of plasma concentrations. One horse developed signs of acute colitis and required euthanasia.

Conclusions and Clinical Relevance—Results suggest that doxycycline is absorbed adequately for killing intracellular bacteria and bacteria with minimum inhibitory concentration ≤ 0.25 µg/mL. For bacteria with minimum inhibitory concentration of 0.5 to 1.0 µg/mL, a dosage of 20 mg/kg, PO, every 12 hours may be required; extreme caution should be exercised with the higher dosage until more safety data are available. (Am J Vet Res 2006;67:310–316)

Tetracycline antimicrobials have a long history of use in veterinary medicine as broad-spectrum agents active against many organisms of importance to equine medicine. Doxycycline is a structural isomer of tetracycline that is available in formulations for oral and parenteral administration. The oral formulation, although not approved for veterinary species, is generic and inexpensive. Intravenous administration of doxycycline to horses has caused collapse and sudden death, apparently secondary to cardiovascular effects. Oral administration of other tetracyclines has been associated with gastrointestinal problems such as changes in the bacterial flora and diarrhea. However, these problems, to date, have not been reported with oral administration of doxycycline in horses. In other species, doxycycline is bound to ingesta in the gastrointestinal tract, which may decrease its effect on the resident microflora. The oral formulation has been well tolerated and has been recommended for use at a dosage of 10 mg/kg, every 12 hours.

One of the most common uses of doxycycline in horses is treatment for ehrlichial infections, such as equine granulocytic ehrlichiosis caused by Anaplasma phagocytophilum (formerly Ehrlichia equi) and Potomac horse fever caused by Neorickettsia risticii (formerly Ehrlichia risticii). Because these organisms have a predilection for intracellular infection of WBCs (PMNLs for *A. phagocytophilum* and mononuclear cells for *N. risticii*), the intracellular concentrations of the drug may be more predictive of efficacy than plasma concentrations. Another important use of doxycycline in horses is for treatment for leptospirosis, which has been implicated in abortion outbreaks and recurrent uveitis. Results of a previous study indicate that doxycycline attains adequate concentrations in endometrial tissue. However, in another study, the drug was detectable in the vitreous humor but not in the aqueous humor. A study in humans revealed that ocular penetration is possible with higher doses and more frequent administration.

Orally administered doxycycline in horses does not result in plasma concentrations necessary for inhibition of some important pathogens. Research reveals that doxycycline is highly protein bound in plasma, which may limit its distribution. Low doses...
may limit the effective concentrations at the target site. Specific studies are necessary to determine the extent of protein binding in horses, the extracellular distribution, and intraocular and intracellular penetration. We hypothesized that intraocular penetration and intracellular penetration into leukocytes are possible in horses, but may require adjustment of the dosage regimens that are presently recommended.

The purpose of the study reported here was to determine the pharmacokinetics of doxycycline after single and multiple administrations at a dosage of 20 mg/kg, PO, every 12 hours, in horses. Plasma protein binding and ISF, intraocular, and intracellular doxycycline concentrations were also determined. Previous reports of sudden death from IV administration of doxycycline in horses precluded an accompanying IV study to determine variables such as systemic clearance, volumes of distribution, and oral systemic availability; therefore, allometric principles were applied to the data to estimate these variables.

Materials and Methods

Pilot study—Initially, a pilot study was performed to determine the safety of orally administered doxycycline at 20 mg/kg and to measure the effects of feeding on doxycycline absorption in the horse. One horse was administered doxycycline hyclate at a dose of 20 mg/kg via nasogastric tube while being maintained on a normal ration of grain and being allowed access to free-choice timothy hay. Plasma samples were drawn at 0 (pretreatment), 15, and 30 minutes and 1, 2, 4, 8, 12, and 24 hours after administration. After a suitable washout period (>2 weeks), the same horse was given doxycycline hyclate (20 mg/kg) via nasogastric tube after feed was withheld for 12 hours. Plasma samples were drawn at 0, 15, 30, and 45 minutes and 1, 2, 3, 4, 6, 8, 12, 24, and 48 hours. Drug concentrations were analyzed by use of HPLC. The Cmax, Tmax, and AUC for fed versus nonfed conditions were compared. Because withholding feed yielded better results in the pilot study, food was withheld prior to drug administration in the remainder of the studies.

Horses—For the single- and multiple-dose studies, 6 adult horses donated for research purposes (3 males and 3 females) that weighed from 424 to 615 kg were used. The horses were determined to be healthy on the basis of results of a CBC, physical examination, and ophthalmic examination performed prior to inclusion in the study. The horses were housed in individual box stalls at least 12 hours prior to drug administration. Feed consisted of a commercially available 12% pelleted feed and grass hay. This study was approved by the Institutional Animal Care and Use Committee of North Carolina State University.

Drug administration—For all parts of this study, generic doxycycline hyclate tablets containing 500 mg of doxycycline base per tablet were used. The dose was 20 mg of doxycycline base/kg of body weight. The tablets were crushed, suspended in 500 mL of water, and administered via nasogastric tube; the tube was then flushed with 1.5 L of water to clear any drug residue from the tube. For the single-dose study, feed was withheld for 12 hours prior to drug administration and 2 hours after drug administration. For the multiple-dose study, doxycycline hyclate was administered at 12-hour intervals 5 times. Feed was withheld for 12 hours prior to the initial administration. Horses were fed their normal ration of grain and 1 flake of timothy hay 2 hours after each administration. Any feed that was not consumed was removed 2 hours later so that feed was withheld for at least 8 hours prior to the next administration.

Blood collection—For pharmacokinetic analysis, blood samples were collected with a 14-gauge, 6-inch jugular catheter at 0, 15, 30, and 45 minutes and 1, 2, 3, 4, 6, 8, 12, 24, and 48 hours after administration of the single dose and after the final administration of the multiple-dose study. The samples were immediately centrifuged at 600 × g, and the plasma was harvested and stored at −70°C until analysis.

PMNL isolation—Polymorphonuclear leukocytes were harvested from blood samples collected at 0 (prior to any drug administration), 1, 4, 8, and 24 hours after administration of the final dose in the multiple-dose study via density gradient centrifugation as described. Final cell counts were determined with a hemacytometer. The cell suspensions were centrifuged at 170 × g at 25°C for 7 minutes, decanted, and resuspended to a concentration of 20 × 10⁶ cells/mL. Cell suspensions were centrifuged at 240 × g at 25°C for 10 minutes, and the supernatant was discarded. The resulting cell pellet was stored at −70°C until analysis. To calculate the concentration of doxycycline in cells, a volume of 450 mL was assumed to be the mean volume of a PMNL. Ratios between the Cmax and AUC of the doxycycline concentration in the PMNL versus plasma were used to measure the extent of drug accumulation in the PMNLs.

ISF sampling—Intersitial fluid was sampled via a subcutaneously placed tissue probe at 1, 2, 4, 8, 12, and 24 hours after administration of the final dose in the multiple-dose study. Horses were sedated with xylazine hydrochloride (0.03 to 0.04 mg/kg, IV) to permit insertion of the probe. A 12 × 12-cm area of the neck was clipped and aseptically prepared, and skin and subcutaneous tissues were anesthetized with 2% lidocaine hydrochloride. The probe was inserted through an introducer needle and sutured in place. Collection of fluid was achieved via a sterile evacuated tube attached to the exposed end of the probe. Tissue probes were placed a minimum of 12 hours prior to the final administration and removed at the end of the experiment. Intersitial fluid was collected and stored prior to analysis at −70°C.

Aqueous humor—Aqueous humor samples were collected at either 2 or 4 hours after the final administration of doxycycline in the multiple-dose study. Samples were obtained from 3 horses at each time point. For humane collection of aqueous humor samples, the horses were sedated with detomidine (0.01 to 0.015 mg/kg, IV) and the eye was anesthetized by topical administration of 0.2 mL of anesthetic followed by auriculopalpebral, supraorbital, and retrobulbar nerve blocks performed with 2% lidocaine. The eye was cleansed with a dilute (10%) aqueous iodine solution and rinsed with sterile saline (0.9% NaCl) solution. A sterile 27-gauge, 0.5-inch needle was inserted into the anterior chamber at the dorsolateral or dorsoventral limbus, and 0.2 to 0.5 mL of aqueous humor was aspirated. Plasma samples were collected at times that corresponded with ocular fluid collection, and all samples were stored at −70°C prior to analysis.

Protein-binding assay—Protein binding was determined via ultrafiltration with a microcentrifugation system as described. Protein binding of doxycycline was determined according to the following formula:

\[
\text{Protein binding (\%)} = \frac{[\text{total}] - [\text{unbound}]}{[\text{total}]} \times 100
\]

where [total] is the drug concentration of bound and unbound doxycycline, prior to ultrafiltration, and [unbound] is the drug concentration collected in the filtrate.
The percentage of free drug was determined by subtracting the percentage protein bound from 100%.

**Drug analysis**—Doxycycline concentrations in all samples were determined via HPLC with UV detection. The HPLC apparatus consisted of a pump, autosampler, UV detector, and computer for data collection and analysis. A C8 reverse-phase column was used for separation. Doxycycline plasma samples and PMNL samples were prepared via a protein precipitation method validated and used in our laboratory. Tissue fluid and aqueous humor samples were analyzed directly via HPLC without extraction. Calibration curves were prepared daily in plasma, PBS solution, blank ISF, or aqueous humor for plasma, PMNLs, tissue fluid, and aqueous humor samples, respectively. The LOQ for this assay was 0.05 µg/mL for the plasma and PMNL samples and 0.01 µg/mL for the tissue fluid and aqueous humor samples.

A computer program was used to determine pharmacokinetic values. Drug concentrations after administration of the single dose were analyzed by use of noncompartmental pharmacodynamic models to determine drug disposition for each horse. Noncompartmental and compartmental analyses were performed on the plasma data obtained after multiple-dose administration. For compartmental analyses, doxycycline best fit a 1-compartment model described by the following equation:

\[ C = \frac{k_{01}FD}{V(k_{01} - k_{10})} \left( e^{-k_{10}t} - e^{-k_{01}t} \right) \]

where \( C \) is the plasma concentration, \( t \) is time, \( k_{01} \) is the oral absorption rate, \( k_{10} \) is the elimination rate constant, \( F \) is the fraction of drug absorbed, \( D \) is the non-IV dose, and \( V \) is the volume of distribution. This model assumes that there is first-order absorption and that \( k_{01} > k_{10} \). A weighting factor of \( 1/\sqrt{t} \) (\( y \) is the predicted plasma concentration) was added for the best fit.

Noncompartmental analyses were also performed on the data obtained from the ISF and PMNL samples. Mean plasma, tissue fluid, and PMNL concentrations; \( C_{\text{max}} \), \( t_{\text{max}} \); and \( \text{AUC} \) are reported. Tissue fluid-plasma, intraocular-plasma, and intracellular-plasma concentration ratios were calculated for protein-bound and unbound concentrations. An RA was calculated from the following equation:

\[ RA = 1/1 - e^{-\lambda \tau} \]

where \( \lambda \) is the slope of the elimination phase and \( \tau \) is the administration interval.

**Allometric analysis**—The allometric equation used was \( Y = a (BW)^b \), where \( Y \) is the calculated systemic clearance, \( a \) is an allometric constant (the y-axis intercept for an animal of 1 kg), \( BW \) is body weight in kilograms, and \( b \) is the allometric exponent. Principles of allometric scaling have already been established for doxycycline in animals for a wide range of body weights. To determine systemic clearance \( (Y) \) for horses, a value of \( b = 0.75 \) was used, which corresponds to values from other species determined for doxycycline and body weight used was the mean weight of the horses in this study. On the basis of these results, the AUC for a dose administered IV was determined with the equation \( \text{AUC} = \text{Dose}/Y \). Oral bioavailability was then calculated as \( \text{AUC}_{\text{oral}}/\text{AUC}_{\text{IV}} \).

**Results**

**Pilot study**—Feeding resulted in lower \( C_{\text{max}} \), longer \( T_{\text{max}} \), shorter \( t_{1/2} \), steeper \( \lambda \), and smaller AUC, compared with that in horses for which feed was withheld (Table 1).

**Single-dose study**—The plasma concentration-versus-time curve for doxycycline after administration of a single dose was determined (Figure 1). After oral administration, doxycycline was detected in 5 of 6 horses at 15 minutes and in all horses at 30 minutes. Drug was still detected in the plasma at 24 hours in all horses, but was not detected in any of the horses at 48 hours. Mean \( T_{\text{max}} \) was 1.54 ± 1.3 hours, although 3 of the 6 horses had a second peak at 3 to 4 hours (Table 2). One horse had signs of mild discomfort (increased respiration and sternal recumbency) for approximately 1 hour after drug administration. This resolved without treatment.

**Multiple-dose study**—The concentration-versus-time curves for doxycycline in the plasma, ISF, and PMNLs after multiple oral administrations were determined (Figure 2). Mean \( T_{\text{max}} \) was similar to the single-dose study (1.63 ± 1.36 hours [Table 2]), and 3 of 6 horses again had a secondary peak at 3 to 4 hours.

Doxycycline was detected in all ISF samples (Table 3). The ultrafiltration devices were 46 cm long and held 160 µL of fluid. Mean collection rate was 1.13 ± 0.2 µL/min; therefore, a 141-minute (2.35-hour) lag time was used to adjust the ISF concentrations to the correct time reference. Results of the in vitro plasma protein-binding assays revealed that doxycycline was less highly protein bound in horses than other species, with mean percentage protein binding of 81.76 ± 2.43% (free drug, 18.24%). The \( C_{\text{max}} \text{ISF}/C_{\text{max}} \text{plasma} \) and \( \text{AUC}_{\text{ISF}}/\text{AUC}_{\text{plasma}} \) ratios were 17.88 ± 3.49% and 24.84 ± 7.54%, respectively. Substantial drug accumulation was detected in the plasma in the multiple-dose study (RA, 1.96). When an administration interval of 24 hours was used, the calculated RA was 1.32.

Mean half-life of doxycycline in the PMNLs was similar to the mean half-life in plasma (13.01 ± 9.2 hours vs 12.07 ± 3.17 hours, respectively). Maximum PMNL concentrations occurred later than maximum plasma concentration (3.33 ± 1.03 hours vs 1.63 ± 1.36 hours, respectively). The \( C_{\text{max}} \) and AUC of doxycycline in equine PMNLs were 17.27 ± 8.98 and 14.58 ± 6.64 times as high, compared with plasma \( C_{\text{max}} \) and AUC, respectively.

Doxycycline was detected in the aqueous humor samples from all 6 horses. Mean aqueous humor concentrations were 0.11 ± 0.01 µg/mL at 2 hours and 0.995 ± 0.016 µg/mL at 4 hours. These concentrations represented 7.5 ± 0.3% and 10.9 ± 1.8% of the corresponding plasma concentrations, respectively.

On the basis of the results of allometric analysis, the systemic clearance of doxycycline in horses was

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fed</th>
<th>Feed withheld</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{\text{max}} ) (h)</td>
<td>4.032</td>
<td>0.966</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (µg/mL)</td>
<td>4.459</td>
<td>10.671</td>
</tr>
<tr>
<td>( \lambda ) (h⁻¹)</td>
<td>0.123</td>
<td>0.042</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>5.047</td>
<td>16.542</td>
</tr>
</tbody>
</table>

---

Table 1—Pharmacokinetic variables in a horse administered doxycycline hyclate (20 mg/kg) PO after feeding or withholding of feed.
estimated to be 0.683 mL/kg/min. By use of this value, an estimated AUC for a dose of 20 mg/kg administered IV to a horse is 488 µg•h/mL. Compared with the AUC from a single orally administered dose, an estimated systemic bioavailability for doxycycline after oral administration, and should be considered for further study as a treatment of infections caused by bacteria susceptible to tetracyclines. It has a long half-life, making a once-daily administration regimen possible. Because plasma concentrations reported in a study of orally administered doxycycline in horses using a dosage of 10 mg/kg every 12 hours were low,

Discussion
Results of this study indicated that doxycycline is poorly absorbed in horses, although it reaches adequate plasma concentrations, has good tissue penetration after oral administration, and should be considered for further study as a treatment of infections caused by bacteria susceptible to tetracyclines. It has a long half-life, making a once-daily administration regimen possible. Because plasma concentrations reported in a study of orally administered doxycycline in horses using a dosage of 10 mg/kg every 12 hours were low,
compared with other tetracycline antimicrobials. Mean plasma concentrations achieved after administration of 5 consecutive doses in our study were approximately 4 times that reported in the previous study (1.74 ± 0.3 µg/mL vs 0.42 ± 0.05 µg/mL), although only twice the dosage was used. The most likely explanation for this is that the horses in the previous study were allowed free-choice access to hay and the horses in this study had food withheld for a minimum of 8 hours prior to each administration. In humans and other species, oral absorption of doxycycline is not substantially affected by food intake, presumably because of the low affinity of doxycycline for chelating anions, such as calcium and magnesium, compared with other tetracycline antimicrobials. However, in our small pilot study, feeding inhibited absorption and decreased plasma concentrations by almost half. We suspect that the high roughage content of equine diets may create a physical barrier that blocks drug absorption by the intestinal wall. Additionally, doxycycline has an isoelectric point and maximum lipid solubility at a pH of 5.5, indicating optimal absorption occurs in the duodenum. Feeding may delay gastric emptying and therefore delay absorption.

Because of risk of cardiovascular toxicity, we could not conduct studies of IV administration in these horses to establish rates for systemic clearance and volumes of distribution and determine oral systemic availability. To provide an estimate of IV pharmacokinetic values, one can use principles of allometric scaling to extrapolate an IV plasma curve from values determined in other animals. A previous report of allometric analysis used to determine pharmacokinetic values for doxycycline in several species, not including horses, revealed a good fit on the basis of plasma protein binding and free drug concentrations. Pharmacokinetic values of clearance and t1/2 have been determined for tetracycline. Extrapolation may be used in this study to achieve plasma doxycycline concentrations, based on the Cmax and the AUC, respectively. Steady-state conditions were confirmed by comparing the AUCl2±12 of the last dose to the AUCl0 of the single-dose study, which were approximately equal (13.33 vs 12.2 h·µg/mL).

In this study, doxycycline penetrated the intact blood-aqueous barrier. This is in direct contrast to the findings of Gilmour et al. In that study, doxycycline was not detected in the aqueous humor. There are several explanations for this discrepancy. A lower dose was administered in the other study (10 vs 20 mg/kg), resulting in lower plasma drug concentrations and less drug available for diffusion into the eye. That study also used a bioassay for drug detection, which is less specific and less sensitive than the HPLC method used in the present study. The LOQ for the bioassay was 0.15 µg/mL, which is higher than the concentrations found in the present study. Aqueous concentrations were from 7.5% to 10.9% of plasma concentrations at the times they were measured. This was similar to findings in humans (concentration in the noninflamed eye, 11% to 13% of plasma concentrations). Concentrations are higher when the eye is inflamed, and therapeutic concentrations are reached after administration of a single dose. Doxycycline has better penetration into ocular tissues than tetracycline. The authors of that study speculated that this was attributable to the increased lipophilicity of doxycycline, compared with tetracycline, which allowed for easier movement of the drug across the blood-ocular barrier. Doxycycline has been used in equine medicine for anterior uveitis in horses with suspected infections with *Leptospira* spp in this study. The development of diarrhea in one of the horses in this study was an unexpected event. Orally and parenterally administered oxytetracycline in horses has been associated with fatal diarrhea and increased or prolonged shedding of *Salmonella* organisms, although no cases of diarrhea after treatment with doxycycline have been reported. *Clostridium perfringens, Salmonella* spp, and coliform bacteria have been implicated as contributing factors in cases of colitis induced by oxytetracycline; in other cases, no etiologic agent was identified. Culture for *Salmonella* spp in feces and gastrointestinal tissues yielded negative results in the horse of this report, as did testing for
C perfringens toxin type B. Although positive results would increase the likelihood of antimicrobial-associated diarrhea in this case, negative results do not rule that diagnosis out.

Antimicrobial-associated diarrhea has not been associated with doxycycline administration in humans or other species because it has minimal effect on gastrointestinal flora after repeated administration, especially compared with other antimicrobials such as clindamycin and erythromycin.78 Doxycycline also preserves the host's resistance to bacterial colonization, compared with clindamycin and erythromycin.78 Doxycycline had no effect on colonic motility in an in vitro model of rabbit and guinea pig colon.79 Development of diarrhea in the horse reported here may be attributed to the low oral bioavailability of doxycycline, which leads to an increased amount of drug in the intestine. In 2 previous reports,9,10 on doxycycline administration in horses, gastrointestinal problems were not associated with drug administration. Each of those studies used lower doses, and it is possible that the high dose used in our study increased the risk of a gastrointestinal adverse reaction.

Administration and dosage regimens were formulated on the basis of the calculated accumulation ratios that would maintain trough plasma concentrations greater than the reported MIC90 of common equine pathogens throughout the administration interval.80 For those bacteria with an MIC ≤ 0.25 μg/mL, a dose of 20 mg/kg, PO, every 24 hours will achieve this goal. This would include many susceptible Streptococcus spp, Staphylococcus spp, Pasteurella spp, Rhodococcus equi, Actinobacillus equuli, and most ehrlichial organisms.42 Doxycycline also preserves the host's resistance to bacterial colonization, compared with clindamycin and erythromycin. Doxycycline had no effect on colonic motility in an in vitro model of rabbit and guinea pig colon. Development of diarrhea in the horse reported here may be attributed to the low oral bioavailability of doxycycline, which leads to an increased amount of drug in the intestine. In 2 previous reports on doxycycline administration in horses, gastrointestinal problems were not associated with drug administration. Each of those studies used lower doses, and it is possible that the high dose used in our study increased the risk of a gastrointestinal adverse reaction.

Administration and dosage regimens were formulated on the basis of the calculated accumulation ratios that would maintain trough plasma concentrations greater than the reported MIC90 of common equine pathogens throughout the administration interval.80 For those bacteria with an MIC ≤ 0.25 μg/mL, a dose of 20 mg/kg, PO, every 24 hours will achieve this goal. This would include many susceptible Streptococcus spp, Staphylococcus spp, Pasteurella spp, Rhodococcus equi, Actinobacillus equuli, and most ehrlichial organisms.42 Those bacteria that reside within PMNLs would also be susceptible with this regimen because of the high degree of accumulation of doxycycline within these cells. For less susceptible bacteria with an MIC of 0.5 to 1.0 μg/mL, a dose of 20 mg/kg, PO, every 12 hours would be needed to maintain the trough in the necessary range. As revealed in this study, doxycycline is absorbed better if food is withheld for a minimum of 8 hours before and 2 hours after doxycycline administration, although this may not be possible in practice, particularly if twice-daily administration is used.

Although absorption after oral administration appears to be low, with doses used in this study, concentrations in cells and tissues are in a range that may give therapeutic results in horses. The value of determining plasma protein binding for studies with antimicrobials in horses was also apparent. The unbound drug in the plasma diffused through tissue capillaries and into the interstitial space. The ISF drug concentrations closely paralleled the unbound concentrations in plasma. Oral administration of doxycycline results in concentrations in PMNLs that are higher than in plasma and therefore may be well suited to treat horses with infections caused by susceptible intracellular organisms. Doxycycline penetrates an intact blood-aqueous barrier and may be useful in the treatment of several ocular diseases. Because of development of severe, acute colitis in 1 of the 6 horses used in this study, we recommend that further clinical and safety studies that use similar doses for longer intervals be performed on horses prior to using this administration regimen.

References


