Pharmacokinetics of difloxacin after intravenous, intramuscular, and intragastric administration to horses

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Objective—To study the pharmacokinetics of difloxacin (5 mg/kg) following IV, IM, and intragastric (IG) administration to healthy horses.

Animals—6 healthy mature horses.

Procedures—A crossover study design with 3 phases was used (15-day washout periods between treatments). An injectable formulation of difloxacin (5%) was administered IV and IM in single doses (5 mg/kg); for IG administration, an oral solution was prepared and administered via nasogastric tube. Blood samples were collected before and at intervals after each administration. A high-performance liquid chromatography assay with fluorescence detection was used to determine plasma difloxacin concentrations. Pharmacokinetic parameters of difloxacin were analyzed. Plasma creatine kinase activity was monitored to assess tissue damage.

Results—Difloxacin plasma concentration versus time data after IV administration were best described by a 2-compartment open model. The disposition of difloxacin following IM or IG administration was best described by a 1-compartment model. Mean half-life for difloxacin administered IV, IM, and IG was 2.66, 5.72, and 10.75 hours, respectively. Clearance after IV administration was 0.28 L/kg/h. After IM administration, the absolute mean ± SD bioavailability was 95.81 ± 3.11% and maximum plasma concentration (Cmax) was 0.732 ± 0.12 mg/L. After IG administration, the absolute bioavailability was 98.62 ± 10.60% and Cmax was 0.732 ± 0.06 mg/L. At 12 hours after IM administration, plasma creatine kinase activity had increased 7-fold, compared with the preinjection value.

Conclusions and Clinical Relevance—Data suggest that difloxacin is likely to be effective for treating susceptible bacterial infections in horses. (Am J Vet Res 2006;67:1076–1081)

Difloxacin is a fluoroquinolone carboxylic acid antimicrobial agent with high in vitro activity against a wide range of gram-positive and gram-negative aerobes and anaerobes,1 including most species of Klebsiella, Staphylococcus, Escherichia coli, Enterobacter, Campylobacter, Shigella, Proteus, Pasteurella, Mycoplasma, Rickettsia, and Chlamydia.2,3 As a member of the fluoroquinolone group, difloxacin acts on bacterial DNA topoisomerases II and IV.4 Fluoroquinolones are considered to have a concentration-dependent effect5; they also have characteristics such as a wide spectrum of bactericidal activity, a large volume of distribution, low plasma-protein binding, and relatively low MICs against target microorganisms.6,7 In recent years, there have been numerous investigations8-10 of various fluoroquinolones in veterinary species. In horses, pharmacokinetic studies with fluoroquinolones are scant, possibly because of concerns regarding fluoroquinolone-induced arthropathy in young horses.11,12 However, some fluoroquinolones, such as enrofloxacin, are being successfully used to treat infections in mature horses.13 Difloxacin pharmacokinetics have been reported in dogs,14 goats,15 rabbits,16 chickens and pigs,18 and mares.19 The purpose of the study reported here was to study the pharmacokinetics of difloxacin following IV, IM, and IG administration of a single dose of the antimicrobial (5 mg/kg) to healthy horses.

ABBREVIATIONS

| MIC | Minimal inhibitory concentration |
| IG | Intragastric |
| HPLC | High-performance liquid chromatography |
| RSD | Relative SD |
| t½ka | Absorption half-life (harmonic mean) |
| t½k1 | Disposition half-life associated with the initial slope (λ1) of a semilogarithmic concentration-time curve |
| t½k2 | Elimination half-life associated with the terminal slope (λ2) of a semilogarithmic concentration-time curve (harmonic mean) |
| AUC | Area under the concentration-time curve |
| MRT | Mean residence time |
| AUMC | Area under the first moment concentration-time curve |
| MAT | Mean absorption time |
| CI | Total body clearance of drug from the plasma |
| Vse | Apparent volume of distribution (area method) |
| Vss | Apparent volume of distribution at steady state |
| F | Proportion of the administered dose systematically available (bioavailability) |
| Cmax | Peak or maximum plasma concentration following IM and IG administration |
Materials and Methods

Animals—Six Spanish-breed adult horses that weighed 408 to 493 kg were used in the study. The horses were privately owned, and the owner consented to their use after being advised of potential problems and possible adverse effects of the study treatments. Horses were housed individually in boxes, received 3 meals daily, and had free access to water. The horses were determined to be clinically normal before the study on the basis of findings of physical examination and clinicopathologic analyses; they did not receive any drug treatment before the study. One month after completion of the study, the horses were examined to ensure that they were in good health. The study was approved by the Bioethics Committee of the University of Murcia.

Experimental design—A crossover study design was used; there were 3 phases (2 X 2 X 2) with 15-day washout periods between treatments. Each horse received each treatment; each treatment comprised a single dose of 5 mg/kg of difloxacin. A commercially available formulation of difloxacin (5%) was administered IV into the right jugular vein and IM into the right lower third region of the neck. For IG administration, an oral suspension was prepared from the tablet formulation of the drug by the Pharmacy Service of the Clinic Veterinary Hospital of the University of Murcia. The difloxacin oral suspension was compounded in a solution of simple syrup (sucrose to water, 2:1) and deionized water (60:40) at a difloxacin concentration of 100 mg/mL. Twenty to 25 mL (ie, 5 mg/kg) of suspension was administered through a nasogastric tube (19 mm in diameter; 3,000 mm in length) to each horse, followed by the administration of 300 mL of deionized water to clean the tube. The first daily meal was given 2 hours after beginning each experiment for all horses.

Blood samples were collected before (0 minutes) and at 5, 10, 15, 30, and 45 minutes and 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72, and 96 hours following IV, IM, and IG drug administration. Each blood sample (5 mL) was obtained through a 12-gauge catheter inserted in the left jugular vein of each horse by use of a 5-mL syringe containing heparin, after which the blood was placed in a tube. Within 30 minutes after collection, samples were centrifuged at 1,500 g for 15 minutes. Plasma was immediately removed, divided in 2 tubes, and stored at –45°C until assayed.

Analytical method—Plasma concentrations of difloxacin were measured by use of a modified HPLC method. The HPLC system was equipped with a quaternary pump, a fluorescence detector, and an autosampler. The aforementioned system was connected to a computer with a specific data program.

Difloxacin (pure substance) was used for quality controls. Ciprofloxacin was used as the internal standard. After the addition of 10 µL of the internal standard to 200 µL of plasma, 200 µL of acetonitrile was added. Plasma proteins were precipitated by shaking in an ultrasonic bath followed by centrifugation for 10 minutes at 1,600 X g. The supernatant was diluted 5-fold with 0.067M disodium hydrogen phosphate buffer (pH, 7.5) and transferred to HPLC autosampler vials. The HPLC separation was performed by use of a reverse-phase column with an injection volume of 25 µL. Autosampler vials and column temperature were set at 5°C. The mobile phase consisted of acetonitrile (40%) and tetrabutylammonium hydrogen sulphate solution (5 g/L; 60%) provided as an isocratic form with a flow rate of 1.0 mL/min. Difloxacin eluted at approximately 6.8 minutes. The fluorescence detection was performed at an excitation wavelength of 280 nm and an emission wavelength of 445 nm.

Method validation—Quality controls were prepared from a pool of blank horse plasma spiked with 7 concentrations of difloxacin from 5 to 2,000 µg/L. Plasma aliquots were stored at –45°C until assayed. Aliquots of quality controls were extracted as described, and 25 µL was injected into the chromatographic system. Standard curves were obtained via unweighted linear regression of the difloxacin peak areas versus known concentrations. Each point was established from the mean of 5 determinations. Correlation coefficients were > 0.98% for calibration curves.

The percentage recovery was determined by comparing the peak areas of the blank plasma samples spiked with different amounts of drug and treated as any sample with the peak areas of the same standards prepared in phosphate buffer. Each point was established from the mean of 5 deter-
minations. The mean ± SE recovery obtained was 98.2 ± 0.64%. The assay precision (expressed as the RSD) was assessed by expressing the SD of repeated samples used for calibration curves (RSD < 8%). Interday precision was estimated from the analysis of standard samples on 3 separate days (RSD < 10%). The limit of quantification of difloxacin in plasma was chosen as the concentration used for the lowest concentration level on the calibration curves and for which the RSD was < 15% (limit of quantification, 5 µg/L).

Assessment of tissue damage—Tissue damage was monitored through assessment of creatine kinase activity in plasma samples collected before and at 12, 24, 48, 72, and 96 hours after IM injection.1

Pharmacokinetic analysis—The concentration-time data obtained after each treatment in each horse was initially fitted to 1-, 2-, 3-, and 4-exponential equations by the retroprojection method.22 A pharmacokinetics computer program22 was used to obtain the best estimates of the parameters of these equations. The final curve fitting was extrapolated to time infinity by use of nonlinear regression analysis performed with the Gauss-Newton damping algorithm.23 The Akaike information criterion was used to determine the number of compartments used in the pharmacokinetic analysis and the most appropriate weighting for the data. The datum points were weighted with the inverse of the squared SD and the most appropriate weighting for the data. The absorption, disposition, fitted value. Pharmacokinetic parameters were obtained from the individual fitted equations.22 The absorption, disposition, and elimination half-lives were calculated as t½abs = ln2/ka, t½dis = ln2/kd, and t½elim = ln2/λz, respectively.

A noncompartmental model was used to determine the AUC by use of the linear trapezoidal rule with extrapolation to time infinity. The AUC, for calculating of AUC24-MIC25 was used to obtain the best estimates of the parameters. A pharmacokinetics computer program was used to obtain the best estimates of the parameters of these equations. The final curve fitting was extrapolated to time infinity by use of nonlinear regression analysis performed with the Gauss-Newton damping algorithm. The Akaike information criterion22 was used to determine the number of compartments used in the pharmacokinetic analysis and the most appropriate weighting for the data. The datum points were weighted with the inverse of the squared SD and the most appropriate weighting for the data. The absorption, disposition, and elimination half-lives were calculated as t½abs = ln2/ka, t½dis = ln2/kd, and t½elim = ln2/λz, respectively.

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The \( t_{1/2}\lambda \) for difloxacin administered via the IV, IM, and IG routes was 2.66, 5.72, and 10.75 hours, respectively. Clearance value after IV administration was 0.28 L/kg\( \cdot \)h. After IM administration of difloxacin, the mean \( \pm SD \) value of \( F \) was 95.81 \( \pm \) 3.11%. C\(_{\text{max}}\) was 14.31 \( \pm \) 0.12 mg/L, and \( t_{1/2\lambda} \) was 0.78 \( \pm \) 0.39 hours. After IG administration, \( F \) was 68.62 \( \pm \) 10.60%. C\(_{\text{max}}\) was 0.73 \( \pm \) 0.05 mg/L, and \( t_{1/2\lambda} \) was 0.44 \( \pm \) 0.18 hours. Statistical analysis revealed significant (\( P < 0.05 \)) differences in MRT and \( t_{1/2\lambda} \) between the IV and IM administrations; in \( t_{1/2\lambda} \), AUC, and MRT between the IV and IG administrations; and in \( F, \) MAT, and \( t_{1/2\lambda} \) between the IM and IG administrations of difloxacin.

After IM administration of difloxacin, plasma creatine kinase activity increased from the baseline value (134.17 \( \pm \) 28.97 U/L at time 0 minutes; reference range, 12 to 30 U/L). At 12 hours, creatine kinase activity was 678.5 \( \pm \) 68.92 U/L (7-fold increase from baseline). Values subsequently decreased (388.83 \( \pm \) 67.18 U/L, 322.67 \( \pm \) 63.33 U/L, and 249.33 \( \pm \) 44.02 U/L at 24, 48, and 72 hours, respectively) and subsequently approached (but did not attain) baseline value at 96 hours (183.83 \( \pm \) 22.54 U/L). Significant differences were identified between creatine kinase activity values at 12, 24, 48, and 72 hours and the 0-hour value (\( P < 0.05 \)); creatine kinase activity values at 12, 24, 48, and 72 hours were not significantly (\( P > 0.05 \)) different from the 96-hour value.

The AUC\(_{\text{cmax}}\) and C\(_{\text{max}}\) ratios were calculated for a range of MIC values (Table 2). The ratios obtained for AUC\(_{\text{cmax}}\) ranged from 3.52 to 146.7 hours (IM) and 25 to 104.3 hours (IG). In contrast, the C\(_{\text{max}}\) ratios ranged from 3 to 12.3 (IM) and 1.5 to 6.1 (IG).

### Discussion

The results obtained with respect to compartmental analysis (2 compartments) are similar to those reported for other fluoroquinolones in horses.\(^{5,15,20,27}\) Difloxacin had a relatively wide distribution in horses with a V\(_{\text{s}}\) of 1.02 L/kg, suggesting penetration through biological membranes and tissue distribution.

With regard to clearance, it is suggested that values of 0.21 to 0.48 L/kg\( \cdot \)h represent midrange clearance values in horses.\(^{28}\) The systemic clearance of difloxacin in our study (0.28 L/kg\( \cdot \)h) falls within this range of clearance values and is consistent with the clearance estimates for marbofloxacin (0.19 L/kg\( \cdot \)h\(^{26}\) and 0.25 L/kg\( \cdot \)h\(^{29}\)) and enrofloxacin (0.14 L/kg\( \cdot \)h\(^{25}\) and 0.51 L/kg\( \cdot \)h\(^{30}\)). The \( t_{1/2\lambda} \) (2.66 hours) after IV administration of difloxacin was shorter than values derived for enrofloxacin (6.09,\(^{26} 4.4,\(^{27}\) and 6.7 hours),\(^{26}\) norfloxacin (6.45 hours),\(^{30}\) marbofloxacin (4.76,\(^{27}\) and 7.56 hours),\(^{29}\) and ciprofloxacin (6.45 hours)\(^{31}\) in horses. The difference in \( t_{1/2\lambda} \) for marbofloxacin between the 2 published studies\(^{26,29}\) could be attributable to sample collection times (until 48 hours and 72 hours) after administration of the antimicrobial and the model fitted to the data (3 compartments\(^{29}\) vs 2 compartments\(^{25}\)).

In another investigation\(^{19}\) in mares, the \( t_{1/2\lambda} \) of difloxacin was 8.75 hours after 5 repeated IG doses of difloxacin, which is shorter than the value determined in our study. However, in that study in mares, the use of a repeated-dose design, 2-compartment model fit, microbiological assay, and a limit of quantification of 30 \( \mu \)g/L (3 \( \mu \)g/L in our study) could have been responsible for the shorter \( t_{1/2\lambda} \) prediction. With respect to \( t_{1/2\lambda} \) of difloxacin in other animal species, the \( t_{1/2\lambda} \) value obtained in our study is also shorter than values reported in rabbits (3.25 hours),\(^{27}\) goats (3.25 hours),\(^{27}\) and pigs and chickens (7.92 and 4.10 hours, respectively).\(^{27}\)

In the present study, the \( t_{1/2\lambda} \) values estimated following IM and IG administration of difloxacin in horses were 5.72 and 10.75 hours, respectively. These values were significantly longer than that determined following IV injection. Therefore, in both instances, a flip-flop effect (in which the half-life associated with the extravascular route is longer than that associated with the intravascular route) was apparent. The \( t_{1/2\lambda} \) of difloxacin after IG administration in horses was longer than that in dogs\(^{3}\) after a single oral dose of 5 mg/kg (6.94 hours). However, the \( t_{1/2\lambda} \) value (IG) in horses was significantly shorter than the value determined in humans (25.7 hours).\(^{1}\) In the horses of the present study, difloxacin was well absorbed following IM and IG administration (absolute bioavailability [\( F \)] of 95.81% and 68.62%, respectively). The intersubject variability estimates (coefficients of variation) after both IM and IG administration were \( \leq 15\% \) for AUC, C\(_{\text{max}}\), and F. If similarly low levels of intersubject variability are present under clinical conditions, this can be highly advantageous because it would help to avoid underexposure to the drug, which can result in treatment failure and the emergence of resistant microbial strains, or overexposure, which is associated with a corresponding increased risk of adverse effects.\(^{20,32}\)

Variability in difloxacin pharmacokinetics was somewhat smaller following IM administration, compared with IG administration of the antimicrobial. However, it is widely known that horses have poor tolerance to irritant drugs that are administered IM. One study\(^{27}\) in horses reported tissue reactions (swelling and tenderness) after IM administration of enrofloxacin and serum creatine kinase activity that increased 10-fold, compared with the preinjection value. In another study\(^{15}\) in horses, IM injection of marbofloxacin resulted in an 8-fold increase in serum creatine kinase activity after 24 hours. In our study, IM administration of difloxacin increased plasma creatine kinase activity as much as 7-fold (with respect to the baseline value) at
12 hours, suggesting a similar tolerance of horses to other fluoroquinolones. However, this tissue irritation does not appear to affect bioavailability after IM administration (F, 95.81%). Nevertheless, additional studies with repeated IM injections are needed to evaluate the magnitude of tissue damage likely to develop in clinical practice situations.

With respect to oral tolerability of fluoroquinolone administration, Gardner et al.11 reported gastrointestinal adverse effects (diarrhea) in horses after 3 oral doses of moxifloxacin and concluded that administration of this drug should be avoided in this species. In our study, difloxacin administered IG appeared to be well tolerated (without evidence of diarrhea or other adverse events). However, as for IM administration, repeated-dose experiments are needed to confirm the safety of IG administration of difloxacin in horses in clinical practice.

The parameters most commonly correlated with clinical outcome of antimicrobial administration include the ratio of peak plasma concentration to MIC (Cmax/MIC), the ratio of the 24-hour AUC at steady-state to MIC (AUC24/MIC), and the duration of time that plasma concentration exceeds the MIC. For a concentration-dependent drug such as difloxacin, clinical response usually correlates with AUC24/MIC and Cmax/MIC ratios and high ratios of the latter have also been associated with a lower incidence of resistance development.13,14 Investigation of animal models involving different quinolones has revealed that an AUC24/MIC ratio of approximately 100 hours or Cmax/MIC ratio of 10 should be achieved to give maximum clinical and antimicrobial efficacy,15 particularly when treating gram-negative organisms. In our study, AUC24/MIC (AUC from 0 hours to infinity) is equal to the steady-state value of AUC24 and Cmax values reflect those determined after the first dose. It is important to consider that in clinical practice situations, repeated doses will be associated with higher Cmax values. It is also important to consider the magnitude of plasma protein binding. Although plasma protein binding of difloxacin in horses is unknown, values of this parameter in other animal species were 21.45% in rabbits,2 13.79% in goats,1 and 42% in humans.1 Therefore, it is likely that ratios estimated with total plasma difloxacin concentrations in horses have only minimal bias, compared with ratios corrected for plasma protein binding.

The MIC values of difloxacin against equine isolates have been recently reported.15 In that study, the investigators evaluated difloxacin activity against 174 equine isolates. Most gram-negative bacterial isolates examined were susceptible to difloxacin, whereas gram-positive bacterial susceptibility to difloxacin was variable. In the present study, the AUC24/MIC and Cmax/MIC ratios were calculated for a range of MIC values. From these ratios, difloxacin appears to have optimum ratios against bacteria with MIC of 0.25 mg/L mainly for the IM route.

It is necessary to interpret the clinical implications of these benchmark ratios with caution because clinical cures have been associated with fluoroquinolone doses based on AUC24/MIC ratios that are less than the ideal.16 The therapeutic effects of antimicrobial agents reflect a complex array of variables including microbial susceptibility, host pharmacokinetics, physiology of the disease condition, site of the infection, pathogen virulence factors, host immune status, duration of infection (acute or chronic), and anti-inflammatory activities of the drugs. Therefore, it would be helpful to have the results of studies that explore the target AUC24/MIC and Cmax/MIC ratios needed to maximize the likelihood of achieving the desired therapeutic outcome when difloxacin is administered to horses.

In the present study, the systemic difloxacin exposure achieved in horses following either IG or IM administration is consistent with the predicted blood concentrations needed for a positive therapeutic outcome for many equine infections. However, additional studies are needed to ascertain the actual blood concentrations associated with effectiveness against specific equine pathogens and the tolerability associated with these 2 routes of administration when doses are repeatedly administered.

References


