Effects of prior feeding on pharmacokinetics and estimated bioavailability after oral administration of a single dose of microencapsulated erythromycin base in healthy foals

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Objective—To determine effects of prior feeding on pharmacokinetics and estimated bioavailability of orally administered microencapsulated erythromycin base (MEB) in healthy foals.

Animals—6 healthy foals, 3 to 5 months old.

Procedure—Foals were given 2 doses of MEB (25 mg/kg of body weight, PO). One dose was administered after food was withheld overnight, and the other was administered after foals had consumed hay. The study used a crossover design with a 2-week period between doses. Blood was collected via a jugular vein prior to and at specific times after drug administration. Concentrations of erythromycin A and anhydroerythromycin A in plasma were determined, using high-performance liquid chromatography. Results pharmacokinetic analysis of plasma concentration-time data for food-withheld and fed conditions were compared.

Results—Plasma concentrations of erythromycin A for foals were lower after feeding than concentrations when food was withheld. Area under the plasma concentration-time curve, maximum plasma concentration, and estimated bioavailability were greater in foals when food was withheld than when foals were fed. Anhydroerythromycin A was detected in plasma after administration of MEB in all foals.

Conclusions and Clinical Relevance—Foals should be given MEB before they are fed hay. Administration of MEB to foals from which food was withheld overnight apparently provides plasma concentrations of erythromycin A that exceed the minimum inhibitory concentration of Rhodococcus equi for approximately 5 hours. The dosage of 25 mg/kg every 8 hours, PO, appears appropriate. (Am J Vet Res 2000;61:1011–1015)

Erythromycin is used commonly in foals because of its antimicrobial activity against Rhodococcus equi and other gram-positive bacteria. Erythromycin and rifampin are synergistic in their in vitro antimicrobial activity against R equi.1 Improvement in the long-term outcome of foals with pulmonary infections caused by R equi has been reported after treatment with this drug combination.1-4 However, important adverse reactions have been reported during oral administration of erythromycin to foals.5-7 Degradation of erythromycin in the gastrointestinal tract or during the process of absorption has been observed after oral administration of erythromycin base and, to a lesser extent, after administration of erythromycin phosphate and erythromycin estolate to foals.6,7 This problem has been recognized in human medicine, leading to chemical modification of the erythromycin molecule or protection of the drug by addition of enteric coatings that result in less degradation prior to absorption.8 Despite low bioavailability, we observed that peak plasma concentrations of erythromycin were > 0.25 µg/ml (the minimum inhibitory concentration [MIC] for most clinical isolates of R equi from horses) for up to 4 to 6 hours after oral administration of crushed enteric-coated tablets containing erythromycin base to foals.5,9 In foals infected with R equi, treatment with crushed enteric-coated tablets containing erythromycin base is associated with resolution of infection despite low bioavailability and substantial degradation of this formulation after oral administration.

Microencapsulated erythromycin base (MEB) consists of small enteric-coated pellets of erythromycin base contained within gelatin capsules. The enteric coating is designed to protect the active drug from inactivation by gastric acid. In humans, the small size and enteric coating of the pellets results in rapid passage from the stomach to the small intestine, where the enteric coating is rapidly dissolved. In humans, performance of the pellets and, therefore, absorption of active drug is optimal after oral administration of the drug to patients from whom food has been withheld. In most studies,5,9 food was withheld from foals or horses prior to oral administration of erythromycin. However, in 1 report,10 a multiple-dose pharmacokinetic study was performed on adult horses that were fed during the study. Feeding prior to administration of the drug may have a substantial impact on absorption of erythromycin formulations. To our knowledge, this issue has not been addressed in foals.

The purpose of the study reported here was to examine the disposition of a MEB (delayed-release) formulation after oral administration to healthy foals from which food was withheld or that were previously
fed. We chose to use this formulation, because it is inexpensive and appears to be adequately absorbed in humans. Furthermore, anecdotal information from veterinary practitioners suggests it may be useful for treating foals with pneumonia attributable to *R equi*. Our hypothesis was that prior feeding of hay would result in substantially lower absorption, thus reducing plasma concentrations of active drug. To test this hypothesis, we determined plasma concentration-time profiles of erythromycin A and anhydroerythromycin A after oral administration of single doses of MEB to foals that were previously fed or from which food was withheld overnight. In addition, fractional absorption of erythromycin base was compared with data derived from another study in which erythromycin was administered IV.

**Materials and Methods**

Foals—Six clinically healthy male foals (Quarter Horse, Thoroughbred, and Quarter Horse cross) that were 3 to 5 months old were selected for the study. Foals were determined to be healthy prior to initiation of the study on the basis of results of physical examination, CBC, and serum biochemical analysis and during the study on the basis of results of daily physical examination. Body weight was recorded for each foal on the day prior to initiation of the study. Foals were housed with their dams in separate stalls with a small adjoining paddock. All horses were fed alfalfa hay and oat hay ad libitum. Using a crossover design, 3 foals were administered MEB (25 mg/kg of body weight, PO) after food was withheld overnight (food-withhold trial). Two weeks later, foals were administered MEB after food was withheld overnight but they had been allowed to consume hay for 1 hour before administration of drug (fed trial). Order of the trials was reversed for the remaining 3 foals. Each mare-foal pair was fed approximately 9.1 kg of hay each morning at 6 AM and received another 9.1 kg at 2 PM. The amount consumed specifically by each foal was not determined; however, all foals were observed to consume hay prior to initiation of the trial in which foals were fed prior to administration of drug.

**Procedure**—An indwelling catheter was placed in a jugular vein (day 1). Foals then were given MEB (25 mg/kg, PO), using an oral dose syringe. The MEB capsules were opened and each of the pellets suspended in corn syrup immediately prior to administration as a paste. For each blood sample, 5 ml of blood was obtained from the catheter and discarded, and 10 ml of blood then was collected and placed into heparinized tubes. Samples were collected 0, 10, 20, 30, and 45 minutes and 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 10, 12, and 24 hours after administration of the drug. Immediately after collection, blood samples were centrifuged at 400 X 1000 for 10 minutes, plasma was harvested, and duplicate aliquots were stored frozen in cryovials at −20 C. All samples were analyzed within 2 weeks after collection.

**Assay of compounds**—Erythromycin and anhydroerythromycin were recovered from plasma samples, using validated methods. Extracted samples were reconstituted in mobile phase, and 20 µl was injected for analysis, using high-pressure liquid chromatography (HPLC). Standard curves (0.025, 0.05, 0.10, 0.125, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, 10.0, 20.0 µg/ml) were prepared by spiking pooled plasma from clinically normal foals with known concentrations of erythromycin and anhydroerythromycin A stock solutions (0.01, 0.1, and 1.0 mg/ml) added to blank plasma for analysis. These standards were extracted and analyzed in the same manner as test samples, and a new standard curve was prepared for each set of test samples. The HPLC system consisted of a pump, guard cell (+0.950 V), autosampler, 15-cm, 4.6-mm diameter reverse-phase column (C18, 5-µm particle size) and guard column, and analytical electrode (E1 = +0.60 V; E2 = +0.88 V) connected to an electrochemical detector and computer-controlled data acquisition and analysis system. Chromatography was performed at ambient temperature (22 C), using a mobile phase that consisted of 60% 50 mM ammonium acetate (pH 6.5) in 30% acetonitrile and 10% methanol. Flow was maintained at a rate of 1.0 ml/min during use. The minimum detectable quantity of erythromycin or anhydroerythromycin was 0.025 µg/ml.

**Pharmacokinetic analysis**—Concentration-time profiles of erythromycin in plasma after oral administration of microencapsulated erythromycin base to each foal were subject to statistical moment analysis. Pharmacokinetic variables determined from these data included area under the plasma concentration-time curve (AUC), area under the first moment of the curve (AUMC), mean residence time (MRT), maximum concentration in plasma (Cpmax), time to maximum plasma concentration (T max), and terminal half-life (T β/2). Concentration-time data for each foal were analyzed, using a microcomputer pharmacokinetics program, and the appropriate model was chosen on the basis of goodness-of-fit variables from the concentration-time data for each foal. Data were weighted in the following manner:

\[ C = 1/C_{obs} \]

where \( C \) = concentration of drug and \( C_{obs} \) = concentration observed, because variances in data were of approximately equal proportion.

The most appropriate model was chosen by examining values of the coefficient of determination (r²) and the lowest weighted sum of squares. The Cpmax was determined from concentration-time profiles for each foal, using second-order interpolating polynomial regression analysis. The maximum of this polynomial was used as Cpmax. The T max was determined as the time corresponding to Cpmax. The AUC was calculated from time zero to the last time for the last measured concentration, using the linear trapezoidal rule. Area from the last measured concentration of drug in plasma to infinity was estimated by the computer program, using the following equation:

\[ \text{Area } \text{Cp}^{\infty} = \text{Cp}^{-\beta/2} \]

where \( \text{Cp}^{\infty} \) represents the concentration in plasma at the last time point, and \( \beta \) is an estimate of the elimination rate constant. Plasma concentration-time data for anhydroerythromycin A determined after oral administration of MEB were used to determine the AUC anhy by use of trapezoidal approximations.

Area under the first moment of the curve was calculated by the computer program in accordance with the linear trapezoidal rule, using the following equation:

\[ \text{AUMC} = \int_{0}^{\infty} \text{Cp} \cdot dt \]

where \( \text{Cp} \) is the plasma concentration at time t for each corresponding datapoint (C t, t). The plasma concentration-time values for anhydroerythromycin detected in the plasma of foals after oral administration of a single dose of MEB were subjected to noncompartmental statistical moment analysis, and the AUC was estimated, using the trapezoidal rule with extrapolation to infinity. The AUMC was extrapolated to infinity by the computer program, using the following equation:
Mean residence time was calculated by assuming elimination was only from the central compartment, and clearance was independent of the site of elimination, using the following equation:

\[ \text{MRT} = \frac{\text{AUMC}}{\text{AUC}} \]

where \( t_{\text{last}} \) is the last time point, \( C_{\text{plast}} \) is the last plasma concentration measurable, and \( \beta \) is the terminal elimination rate constant. Mean residence time was calculated by assuming elimination of anhydroerythromycin was estimated by dividing AUC_{oral} by total AUC_{iv}, determined by IV administration of erythromycin as a bolus for the foals in the study. The fraction of drug in plasma as anhydroerythromycin was estimated by determining AUC_{anhy} and comparing it to total AUC_{iv} (% F = AUC_{anhy}/AUC_{iv}) and correcting this value for differences in amount given (10 mg/kg, IV, vs 25 mg/kg, PO). Estimated F was used to compare mean AUC_{iv} from those previous studies, rather than determining the AUC_{iv} after IV administration of erythromycin as a bolus for the foals included in the study reported here, because there can be unpredictable adverse reactions to this drug when given rapidly IV. The fraction of drug in plasma as anhydroerythromycin was estimated by determining AUC_{anhy} and comparing it to total AUC_{iv} (% F = AUC_{anhy}/AUC_{iv}) and correcting this value for differences in amount given (10 mg/kg, IV, vs 25 mg/kg, PO). The AUC_{iv} determined for the 10 foals used to calculate estimated F was 5.899 \( \mu \)g/ml.

Statistical analysis—Statistical analysis was performed to determine whether there were significant differences in the observed plasma concentrations of erythromycin A and anhydroerythromycin A between foals when food was withheld and when they were fed before administration of drug. Briefly, data were tested for normality, using the Kolmogorov-Smirnov test. Data that failed the normality test were analyzed by use of a paired t-test. Specific times of the concentration-time data for the food-withheld and fed trials were compared. Data that failed the normality test were analyzed by use of the Wilcoxon signed-rank test. Values for AUC, AUMC, MRT, \( C_{\text{pmax}} \), \( T_{\text{pmax}} \), \( \beta \), and \( t_{1/2} \) determined in foals given MEB were analyzed for significant differences between feeding conditions, using a paired t-test. The AUC of the metabolite anhydroerythromycin was compared for food-withheld and fed trials, using a paired t-test. Significance was assigned for values of \( P < 0.05 \).

Results

On the basis of examination of the value of \( r^2 \) and residuals, and Akaike information criterion, erythromycin concentration-time data fit most appropriately a 1-compartment open model described by a biexponential expression. Mean plasma concentration-time profiles observed after administration of MEB capsules to foals when food was withheld differed significantly from that observed for the foals when they were fed hay (Fig 1). Allowing access to hay prior to drug administration resulted in a reduction of the concentrations of erythromycin A detected in all foals at all time points. Plasma concentrations of erythromycin A differed significantly in foals during food-withheld and fed trials 45 minutes (\( P = 0.049 \)) and 1.5 (\( P = 0.019 \)), 1.75 (\( P = 0.013 \)), 2.25 (\( P = 0.035 \)), 2.5 (\( P = 0.019 \)), 3.0 (\( P = 0.041 \)), 3.25 (\( P = 0.011 \)), and 3.5 (\( P = 0.032 \)) hours after administration. Plasma concentrations were not significantly different at all other time points measured. Peak plasma concentration of erythromycin A when foals were allowed to consume hay prior to administration of drug ranged from 0.2 to 0.84 \( \mu \)g/ml (mean \( \pm \) SD, 0.38 \( \pm \) 0.32 \( \mu \)g/ml). Peak plasma concentration of erythromycin A after administration when food was withheld from foals ranged from 0.64 to 4.01 \( \mu \)g/ml (mean, 2.03 \( \pm \) 1.3 \( \mu \)g/ml; Table 1). Plasma concentrations of erythromycin detected in 1 foal when it was fed hay were near the lower limit of detec-
tion during the entire 24-hour period following administration of drug. The \( T_{\text{p max}} \) was not significantly different between foals when food was withheld and when they were fed (1.5 ± 0.28 vs 1.43 ± 0.83 hours, respectively).

Mean plasma concentration-time data for anhydroerythromycin A in foals when food was withheld was not significantly different from foals when they were fed hay (Fig 2). Feeding hay did not cause significant differences in plasma concentrations of this acid-degradation product.

Pharmacokinetic moment analysis of the plasma concentration-time data in foals when food was withheld prior to administration of drug and in foals when allowed access to hay prior to administration of drug revealed significant differences (Table 1). Values were significantly different for AUC (\( P = 0.016 \)), AUMC (\( P = 0.032 \)), and \( C_{\text{p max}} \) (\( P = 0.034 \)) between foals for food-withheld and fed trials. Estimates of oral F of MEB differed significantly (\( P = 0.024 \)) between foals when food was withheld and when they were fed hay prior to administration of drug. A reduction in F was observed from 26 ± 15.4% (range, 6.4 to 44%) in foals when food was withheld to 7.7 ± 6.8% (range, 0.95 to 17.6%) in foals when fed before administration of drug.

**Discussion**

Finding that prior hay feeding markedly reduced the absorption of erythromycin in foals is important. The \( C_{\text{p max}} \) of erythromycin A achieved in foals when allowed access to hay prior to administration of drug was as low as 25% of that observed in the same foals when food was withheld prior to administration of drug. This led to significantly lower AUC, AUMC, and estimated F when foals were fed. Mean plasma concentrations of active drug remained > 0.25 µg/ml (the MIC for most clinical isolates of \( R \) equi) for only 2 hours in foals allowed access to hay prior to administration of the drug, whereas when food was withheld from foals, this concentration was maintained or surpassed for almost 5 hours. The lower \( C_{\text{p max}} \), AUC, and F and shorter persistence of plasma concentrations > 0.25 µg/ml suggested that plasma and, therefore, tissue concentrations of the drug may be suboptimal for treatment of foals with infections caused by \( R \) equi. Analysis of our results suggested that administration of MEB should be performed at least 1 hour before a foal is fed hay to maximize absorption of active drug, maximize peak concentration, and prolong the duration that plasma concentrations of active erythromycin remain greater than the MIC of susceptible bacteria.

The \( C_{\text{p max}} \) of erythromycin A achieved in foals after administration of MEB during the food-withheld trial (range, 0.64 to 4.01 µg/ml; mean, 2.05 ± 1.3 µg/ml) was nearly twice that observed after administration of crushed enteric-coated film tabs containing erythromycin base (range, 0.6 to 1.6 µg/ml; mean, 1.1 ± 0.4 µg/ml) in foals involved in a study that used identical conditions. In another study, we reported that \( C_{\text{p max}} \) of erythromycin A after oral administration of erythromycin phosphate and erythromycin estolate were 2.9 ± 1.1 and 1.0 ± 0.8 µg/ml, respectively. Because a portion of the administered dose of erythromycin estolate was absorbed as estolate, resulting \( C_{\text{p max}} \) of potentially active erythromycin was approximately 3.0 ± 2.1 µg/ml. Analysis of these results suggests that administration of MEB achieves higher and more persistent plasma concentrations of erythromycin than is achieved with crushed enteric-coated tablets containing erythromycin base. Erythromycin phosphate and erythromycin estolate may be superior to both formulations of erythromycin base from the standpoint of peak plasma concentrations of the active drug that are achieved.

Estimates of F of MEB in foals after oral administration, determined by comparing the AUC reported here with the AUC in 10 foals after bolus IV administration of erythromycin lactobionate in another study, were 26% for foals when food was withheld and 7.7% for foals when fed. These estimates suggest that with-
holding food from foals and use of MEB results in improved F after oral administration, whereas prior feeding results in F similar to or lower than that observed for use of crushed enteric-coated tablets containing erythromycin base. The F of MEB after oral administration appears to be similar to that observed for erythromycin phosphate in foals from which food is withheld.

The estimated F of MEB reported here was limited, because we were not able to use these foals in a crossover study incorporating IV administration of erythromycin. Although less than optimal, this procedure was necessitated because of unpredictable adverse reactions during or immediately after rapid IV administration of erythromycin for pharmacokinetic studies. Of 16 foals in previous studies, 10 tolerated the IV injection sufficiently well to enable use of the data obtained from them. The other 6 were removed from further study. Animal health and welfare considerations have precluded use of the IV route in subsequent studies.

After oral administration of crushed enteric-coated tablets containing erythromycin base to foals, the parent drug is degraded to anhydroerythromycin A prior to or during absorption. This degradation also has been observed to a lesser extent after oral administration of erythromycin phosphate and erythromycin estolate. This finding is in agreement with observations in humans in which salts of erythromycin base and salts of esters (erythromycin ethylsuccinate, erythromycin estolate) reported are less likely to undergo degradation prior to absorption.

Although anhydroerythromycin was detected in plasma after administration of MEB, concentrations were lower than those observed in a study of foals receiving crushed enteric-coated tablets containing erythromycin base. This suggests that the MEB pellets provide at least partial protection from acid degradation during passage through the stomach. This also may explain the longer time to \( C_p_{\text{max}} \) observed with this formulation, compared with that observed by us previously when using crushed enteric-coated film tabs containing erythromycin base. Although feeding hay prior to oral administration of MEB resulted in less absorption of drug from the gastrointestinal tract, feeding had little effect on plasma concentrations or fractional absorption of anhydroerythromycin A that were generated. This suggests that acid degradation is not the major reason for reduced bioavailability of this formulation with prior hay feeding and that this formulation is suitably protected from the environment of the stomach after oral administration to foals.

It appears that MEB is more completely absorbed and achieves higher peak concentrations of erythromycin when administered to foals from which food is withheld, compared with concentrations for foals allowed to consume hay before oral administration of the drug. To improve absorption, we recommend that MEB be administered to foals before they are allowed access to hay (before feeding in the morning or afternoon). Although consumption of hay prior to administration reduces absorption, it does not substantially increase degradation of erythromycin. Administering MEB (25 mg/kg, PO) to foals from which food was withheld resulted in plasma concentrations that remained greater than the MIC for most clinical isolates of \( R\) equi for approximately 5 hours. Therefore, administration of MEB at a dosage of 25 mg/kg, PO, q 8 h, appears appropriate for treatment of foals with \( R\) equi infections.

References