Pharmacokinetics of Erythromycin Ethylsuccinate after Intragastric Administration to Healthy Foals*

Jeffrey Lakritz, DVM, PhD
W. David Wilson, BVMS, MS
Antoinette E. Marsh, PhD
Judy E. Mihalyi, BS

University of Missouri-Columbia
College of Veterinary Medicine
*Department of Veterinary Medicine and Surgery

University of California, Davis
School of Veterinary Medicine
bDepartment of Medicine and Epidemiology

Columbia, MO 65211
One Shields Avenue
Davis, CA 95616

ABSTRACT

Plasma concentrations and pharmacokinetics of erythromycin and related compounds were determined after administration of erythromycin ethylsuccinate to six healthy male foals 3 to 5 months of age. Hay was withheld from the foals overnight and erythromycin ethylsuccinate (25 mg/kg of body weight) was administered intragastrically. Plasma erythromycin concentrations were determined at specific times after drug administration by high-performance liquid chromatography assay. Maximum peak plasma concentrations, time to maximum concentrations, area under plasma concentration versus time curves, elimination half-life, and mean residence time were determined from concentration versus time curves. Maximum peak concentration of erythromycin A (0.45 ± 0.27 µg/ml) after administration of erythromycin ethylsuccinate was observed at 2.38 ± 1.54 hours after treatment. Concentrations of anhydroythromycin A were maximal at 2.2 ± 2.0 hours and reached a maximum of 2.6 ± 1.9 µg/ml. Plasma concentrations of the ester parent drug (erythromycin ethylsuccinate) were below the limit of quantitation (0.1 µg/ml) at all times except 2.5, 2.75, and 5.5 hours. Levels at those times ranged from 0.1 to 0.144 µg/ml. Erythromycin ethylsuccinate appears to be poorly absorbed after oral administration to fasted foals. Plasma concentrations of erythromycin A remained below 0.25 µg/ml (reported minimum inhibitory concentration for Rhodococcus equi) for less than 4 hours after intragastric administration of erythromycin ethylsuccinate, suggesting that the recommended dosage (25 mg/kg every 6 hours) would be suboptimal for treatment of R. equi infections.

INTRODUCTION

Pneumonia is a common cause of morbidity and mortality in foals in the United States, and Rhodococcus equi is one of the most common and economically important causes. The pneumatic process is often well advanced by the time clinical signs are recognized by owners because most cases of R. equi pneumonia
represent the chronic form of the disease. Therefore, the prognosis is guarded. The required course of antimicrobial treatment is prolonged and expensive, and the most common treatment regimen used is associated with important adverse effects.\(^2\) Erythromycin, a macrolide antibiotic, is suitable for treatment of pyogranulomatous lung inflammation such as that caused by *R. equi* because it can be administered orally, has wide tissue distribution, can reach high concentrations in lung tissue, and accumulates in neutrophils at concentrations higher than those achieved in plasma.\(^3\) *Rhodococcus equi* is susceptible to some erythromycin formulations that achieve adequate concentrations after oral or intragastric administration. When given in combination with rifampin, erythromycin has synergistic bactericidal activity against this organism.\(^3\)

Because no erythromycin formulations are approved for oral or intragastric administration to horses and because there have been no controlled trials evaluating optimal therapy, the pharmacokinetics of several different formulations of erythromycin have been studied in foals within the age range in which pneumonia commonly occurs.\(^1\) In these studies, concentrations of microbiologically active drug, as well as degradation products or other compounds that may enter the plasma of foals after oral or intragastric administration of erythromycin, have been studied. These studies were conducted in hopes of determining whether one formulation is pharmacokinetically superior to others based on plasma concentrations of active drug relative to established minimum inhibitory concentrations for commonly encountered foal pathogens. The purpose of the study reported here was to determine the pharmacokinetics of erythromycin ethylsuccinate and plasma concentrations of the inactive prodrug (erythromycin ethylsuccinate), the active molecule (erythromycin A), and the degradation product (anhydroerythromycin A) after intragastric administration to healthy foals. The results provide valuable information about the absorption of this formulation and its stability in the gut of foals. Other ester formulations (erythromycin estolate) are slowly hydrolyzed in foals, indicating greater stability of this ester formulation in young horses. Furthermore, previous studies examining erythromycin estolate have indicated that less anhydroerythromycin A is absorbed by foals, suggesting that the ester form is protected from gastric acid degradation.

## MATERIALS AND METHODS

### Foals

Six healthy foals (three quarter horse, two Thoroughbred, and one of mixed breeding), 3 and 5 months of age, were selected for the study. Foals were determined to be healthy based on results of physical examinations and complete blood cell counts (CBC). Body weights were recorded on the day before initiation of the study. On the night before dosing, each foal and its dam were placed in a stall with a small adjoining run. Mares and foals were fed routinely the night before the study, but on study days, hay was withheld until 1 hour after treatment. The foals were allowed to nurse their dams.

### Treatment

Erythromycin ethylsuccinate (Barre-National, Baltimore, MD) was obtained as a suspension and administered without modification after shaking thoroughly to ensure even suspension. Foals were then given erythromycin ethylsuccinate (25 mg/kg of body weight) via nasogastric tube.

### Blood Samples

A Teflon\(^6\) (E. I. du Pont de Nemours and Company, Wilmington, DE) -coated jugular...
catheter was placed for blood sampling. After first withdrawing and discarding 5 ml of blood from the intravenous catheter, blood was collected from each foal into tubes containing heparin as the anticoagulant immediately before treatment (Time 0) and then 10, 20, 30, 45, 60 minutes and 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 erythromycin ethylsuccinate. Immediately after collection, blood samples were centrifuged at 400 × g for 10 minutes, plasma was withdrawn, and duplicate aliquots were stored at −20°C until they could be analyzed by high-performance liquid chromatography (HPLC) assay. Samples were extracted and analyzed within 2 weeks of freezing.

**Determination of Parent Drug and Related Compounds**

Plasma concentrations of erythromycin A and anhydroerythromycin A were determined by use of a previously described HPLC assay. Briefly, 500-µl aliquots of thawed plasma were extracted by addition of 5 ml of methyl-tert-butyl ether followed by vortexing for 5 minutes and centrifugation at 1000 × g for 30 minutes at 4°C. The ether layer was removed, placed in conical tubes, and dried under nitrogen at room temperature (approximately 22°C). Dried samples were reconstituted in 500 µl of mobile phase and 50 µl was injected onto the chromatographic system for analysis. The HPLC system consisted of a pump, guard cell (+0.950 V), autosampler, 15-cm (length) × 4.6-mm (diameter) reverse-phase column (C18, particle size 5 µm) and guard column, and analytical electrode (E₁ = +0.75V; E₂ = +0.88V) connected to an electrochemical detector and computer-controlled data acquisition and analysis software. Chromatography was performed at room temperature, using a mobile phase that consisted of 40% 50 mM ammonium acetate (pH 6.5), 50% acetonitrile, and 10% methanol. Flow was maintained at 1.0 ml/min during use. The HPLC system used was the same as that used in previous studies, with the exception of the detector and electrode settings (E₁ = +0.75 V; E₂ = +0.88 V).

Standard curves were prepared by adding known concentrations of erythromycin A (erythromycin base, Lot L, USP Pharmacopeia, Rockville, MD), erythromycin ethylsuccinate (erythromycin ethylsuccinate, Lot F-2, USP Pharmacopeia), and anhydroerythromycin A (Anhydroerythromycin A, Abbott Laboratories, North Chicago, IL) to pooled plasma from clinically normal foals. Standard samples were extracted and analyzed in the same manner as unknown (test) samples. The limit of quantitation for erythromycin A, erythromycin ethylsuccinate, and anhydroerythromycin A by the methods in the laboratory conducting these studies are 0.025, 0.1, and 0.01 µg/ml, respectively.

**Pharmacokinetic Analyses**

Concentration × time profiles of erythromycin in plasma after oral administration of erythromycin ethylsuccinate to each foal were subjected to statistical moment analysis to determine each of the following parameters: area under the curve (AUC), area under the first moment of the curve (AUMC), mean residence time (MRT), maximum concentration in plasma (Cmax), time to maximum plasma concentration (Tmax), and elimination half-life (T1/2β) using a computer software program. Statistical moment analysis was used because these parameters can be determined from early time points, which are higher and relatively more accurately determined. Additionally, variables such as absorption, degradation, and/or first-pass effects may complicate the analysis of a drug administered extravascularly. Pharmacokinetic variables for each foal were used to determine the mean and standard deviation for AUC, AUMC, Cmax, and Tmax.
Mean residence time and $T_{1/2}$ were recorded as the median and median deviation.

**RESULTS**

Erythromycin ethylsuccinate was poorly absorbed after intragastric administration (Figure 1). The erythromycin A plasma concentration versus time curve indicates plasma levels of erythromycin were maintained above 0.25 $\mu$g/ml for approximately 2.75 hours, but in only one foal was the maximum plasma concentration near 1.0 $\mu$g/ml (Figure 2). The mean peak concentration of microbiologically active erythromycin A, based on computer-fitted data, was $0.45 \pm 0.27 \mu$g/ml (range = 0.26 to 0.95). Computer-fitted mean peak plasma concentration of anhydroerythromycin A ($2.6 \pm 1.9 \mu$g/ml; range=0.8 to 6.3) was achieved at $2.2 \pm 0.2$ (range=0.5 to 5.5) hours (Table 1). With the exception of three time points (2.5, 2.75, and 5.5 hours), plasma concentrations of parent erythromycin ethylsuccinate were below or near the limit of quantitation (0.1 $\mu$g/ml) of the assay method used. Plasma concentrations of erythromycin ethylsuccinate were too low to permit statistical moment analysis.

Results of noncompartmental statistical moment analysis for both erythromycin A and anhydroerythromycin A after intragastric administration of erythromycin ethylsuccinate are reported in Table 1. Mean AUC of erythromycin A determined after administration of
erythromycin ethylsuccinate was low (1.62 ± 1.23 µg × hr/ml; range = 0.3 to 3.5). Mean AUC for anhydroerythromycin A was higher (9.25 ± 6.0 µg × hr/ml; range = 1.6 to 16.0) than that observed for erythromycin A. Maximum plasma concentrations and \(T_{\text{max}}\) for both erythromycin A and anhydroerythromycin A varied considerably among foals (Table 1).

**DISCUSSION**

These data indicate that erythromycin ethylsuccinate is poorly absorbed after intragastric administration to foals that have been without access to hay overnight. Poor absorption of pro-drug results in low concentrations of both erythromycin ethylsuccinate and active erythromycin A in plasma. After intragastric administration of erythromycin ethylsuccinate (25 mg/kg), mean peak concentration of microbiologically active erythromycin A (0.45 ± 0.27 µg/ml) was similar to that found in fed adult horses given the same dose.8 However, peak concentrations of erythromycin A were lower than those reported for fasted foals given crushed, enteric-coated erythromycin base (1.1 ± 0.4 µg/ml; range = 0.6 to 1.6), erythromycin phosphate (2.9 ± 1.1 µg/ml; range = 1.6 to 4.0), microencapsulated erythromycin base (2.05 ± 1.3 µg/ml; range = 0.64 to 4.01), or erythromycin estolate (1.0 ± 0.82 µg/ml; range = 0.3 to 2.6) orally under the same experimental conditions as those used in this study.4–6 Similarly, concentrations of active erythromycin A remained detectable for a shorter duration after administration of erythromycin ethylsuccinate compared with administration of

![Figure 2. Individual plasma erythromycin A concentration versus time curves obtained after intragastric administration of erythromycin ethylsuccinate (25 mg/kg) to six healthy foals.](image-url)
these formulations listed above. Poor absorption resulted in concentrations of microbiologically active erythromycin A remaining above 0.25 µg/ml (the minimum inhibitory concentration of R. equi) for less than 4 hours after dosing with erythromycin ethylsuccinate. Care should be exercised when comparing plasma concentrations of a drug with variable absorption in different individuals and by different laboratories. However, with the exception of using different foals in some of these studies, all other parameters that may affect interpretation of the data (laboratory, equipment, technician) were the same.

The mean area under the anhydroerythromycin plasma concentration versus time curve was considerably larger than for erythromycin ethylsuccinate and erythromycin A, indicating that a substantial portion of the administered dose was degraded by gastric acid. Consequently, the oral bioavailability of the ethylsuccinate ester is similar to that of the estolate ester and is higher than that of erythromycin base in people. The observed elimination half-life of erythromycin ethylsuccinate is longer in comparison with crushed enteric-coated erythromycin base, erythromycin phosphate, or erythromycin estolate but is similar to microencapsulated erythromycin base when administered to comparable foals. In contrast, the elimination half-life of erythromycin ethylsuccinate in fasted foals is shorter than that observed in previously fed, adult horses. This suggests that foals are better capable of hydrolyzing this ester and differences in absorption or elimination of this drug exist in younger animals or absorption was delayed in adult horses resulting in longer elimination half-life. Erythromycin ethylsuccinate is not recommended for administration in young humans.

Comparison of the results of this study with those of previous studies of erythromycin formulations in foals under identical conditions in the authors’ laboratory indicates that the ethylsuccinate ester is considerably more susceptible
to acid degradation than either the estolate ester or microencapsulated erythromycin base when comparing peak plasma concentrations of anhydroerythromycin and the respective area under the plasma concentration versus time curves.\textsuperscript{4,6} Concentrations of anhydroerythromycin were considerably higher at all time points after administration of erythromycin ethylsuccinate compared with erythromycin estolate and microencapsulated base.\textsuperscript{4,6} It is possible (although unlikely) that absorption of erythromycin would have been enhanced if the experimental foals had been allowed to consume hay before dosing with erythromycin ethylsuccinate. Additional studies will be necessary to document the effect of feeding. However, plasma concentrations of active erythromycin in adult horses given erythromycin ethylsuccinate after feeding were similar to those reported in the hay-fasted foals in this study, suggesting that feeding is unlikely to enhance absorption.\textsuperscript{8} Furthermore, the foals used in this study were subsequently used in a study that demonstrated that feeding hay prior to dosing caused a substantial reduction in the bioavailability of microencapsulated erythromycin base.\textsuperscript{4}

Poor persistence of therapeutically effective concentrations of erythromycin A, combined with evidence of substantial degradation of erythromycin ethylsuccinate to anhydroerythromycin A followed by absorption of this inactive product, suggest that erythromycin ethylsuccinate will likely be less effective than equivalent doses of erythromycin estolate or microencapsulated erythromycin base for the treatment of \textit{R. equi} infections in foals. Recently published evidence that oral administration of erythromycin is associated with a significant risk of adverse reactions in foals and concern that some adverse reactions may be caused by degradation products suggest that the microencapsulated base or estolate formulations should be used in preference to erythromycin ethylsuccinate when treating foals with infections caused by erythromycin-susceptible bacteria.\textsuperscript{2}

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