JOURNAL OF
Veterinary Pharmacology and Therapeutics

J. vet. Pharmacol. Therap. **33**, 376–382. doi: 10.1111/j.1365-2885.2009.01150.x.

Pharmacokinetics of an orally administered methylcellulose formulation of gallium maltolate in neonatal foals

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Chaffin, M. K., Fajt, V., Martens, R. J., Arnold, C. E., Cohen, N. D., O'Conor, M., Taylor, R. J., Bernstein, L. R. Pharmacokinetics of an orally administered methylcellulose formulation of gallium maltolate in neonatal foals. *J. vet. Pharmacol. Therap.* **33**, 376–382.

Gallium is a trivalent semi-metal with anti-microbial effects because of its incorporation into crucial iron-dependent reproductive enzyme systems. Gallium maltolate (GaM) provides significant gallium bioavailability to people and mice following oral administration and to neonatal foals following intragastric administration. To study the prophylactic and therapeutic effects of GaM against *Rhodococcus equi* pneumonia in foals, we developed a methylcellulose formulation of GaM (GaM-MCF) for oral administration to neonatal foals. Normal neonatal foals were studied. Six foals received 20 mg/kg and another six foals received 40 mg/kg of GaM-MCF orally. Serial serum samples were collected and serum gallium concentrations were determined using inductively coupled plasma mass spectroscopy. Gallium was rapidly absorbed (T_{max} of 4 h), and a mean C_{max} of 0.90 or 1.8 μg/mL was achieved in foals receiving 20 or 40 mg/kg respectively. Marked variability existed in C_{max} among foals: only half of the foals receiving 20 mg/kg attained serum concentrations of >0.7 μg/mL, a level suggested to be therapeutic against *R. equi* by previous studies. Mean elimination half-life was 32.8 or 32.4 h for foals receiving 20 or 40 mg/kg respectively. The results of this study suggest that at least 30 mg/kg orally every 24 h should be considered in future pharmacodynamic and efficacy studies.

(Paper received 26 June 2009; accepted for publication 6 October 2009)

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INTRODUCTION

Rhodococcus equi is a Gram-positive, intracellular pathogen that causes one of the most severe and devastating forms of foal pneumonia (Prescott, 1991; Cohen, 1994; Giguere & Prescott, 1997; Ainsworth *et al.*, 1998). The most common manifestation of *R. equi* infection is suppurative bronchopneumonia with extensive pyogranulomatous lesions in the lungs and mediastinal lymph nodes. Clinical signs include fever, cough, lethargy, nasal discharge, tachypnea and progressive respiratory distress (Prescott, 1991; Giguere & Prescott, 1997; Ainsworth *et al.*, 1998; Cohen *et al.*, 2000). Extrapulmonary lesions may include ulcerative enterocolitis, abdominal lymphadenopathy and abscessation, immune-mediated polysynovitis and uveitis, osteomyelitis and septic arthritis (Reuss *et al.*, 2009; Chaffin & Martens, 1997).

Rhodococcus equi pneumonia is principally a disease of young foals; clinical signs most frequently become apparent at 30–120 days of age. Older foals and adult horses, unless severely immunocompromised, are highly resistant to infection with *R. equi* (Giguere & Prescott, 1997). Infections with *R. equi* are insidious, and most foals become infected at a very young age. Foals are more susceptible to experimental infection with *R. equi* during the first 2 weeks of life (Martens *et al.*, 1989a,b), and most spontaneous infections occur very early in life (Horowitz *et al.*, 2001). The mechanism(s) responsible for increased susceptibility of neonatal foals remain(s) unknown, but immature immune defenses are thought to contribute (Kanaly *et al.*, 1996; Boyd *et al.*, 2003; Chaffin *et al.*, 2004; Breathnach *et al.*, 2006)

Currently, methods for control and prevention of *R. equi* pneumonia at endemic breeding farms are limited, and the only proven effective method of prevention, intravenous transfusion of hyperimmune plasma (Martens *et al.*, 1989a), is often prohibitively expensive, labour-intensive and not universally effective (Martens *et al.*, 1989b; Madigan *et al.*, 1991; Hurley & Begg, 1995; Becu *et al.*, 1997; Giguere & Prescott, 1997; Higuchi *et al.*, 1999; Cohen *et al.*, 2000). A novel approach to

prevention of *R. equi* pneumonia involves chemoprophylactic strategies employed during the first few weeks of life, when foals are most likely to become infected with *R. equi*. Studies of antimicrobial chemoprophylaxis at farms with recurrent pneumonia attributable to *R. equi* infections have shown conflicting results. In a randomized, controlled clinical trial of foals, born and raised at endemic equine breeding farms in the United States, azithromycin administered to foals during the first 2 weeks of life effectively reduced the cumulative incidence of *R. equi* pneumonia (Chaffin *et al.*, 2008). In contrast, a study from an endemic farm in Germany showed no significant effect of azithromycin chemopropylaxis on the prevalence of lung abscesses in foals (Venner *et al.*, 2007).

As a result of concerns regarding development of antimicrobial resistance with chemoprophylactic strategies that include commonly used therapeutic agents such as macrolides, alternative preventative strategies during the first few weeks of life may be advantageous. Pathogenic bacteria can commonly acquire all of the nutrients needed for multiplication from host tissues, except for ferric iron (Fe^{3+}). The tenacious binding of free iron by plasma proteins (e.g. transferrin, lactoferrin) is an innate anti-microbial defense mechanism. Recent studies showed that Fe^{3+} is crucial for survival and reproduction of *R. equi*, and that *R. equi* can acquire protein-bound iron, thereby negating this defense mechanism (Jordan *et al.*, 2003).

Strategies designed to exploit the iron dependency of various pathogenic organisms have proven effective in the management of a variety of bacterial diseases (Fettman *et al.*, 1987; Byrd & Horwitz, 1991; Weinberg, 1994; Bernstein, 1998). Gallium (Ga^{3+}) is a trivalent semi-metal that shares many chemical similarities with ferric iron (Fe^{3+}), which has prompted study of its use to exploit the iron dependency of bacteria (Bernstein, 2005, 1998; Oyebode *et al.*, 2000; Jordan *et al.*, 2003). Specifically, the ionic radius and chemical properties of Ga^{3+} are similar to those of Fe^{3+}, and Ga^{3+} is not distinguishable from Fe^{3+} in Fe^{3+}-dependent biological systems, including bacterial iron scavengers and transporters (Bernstein, 2005; Oyebode *et al.*, 2000). Gallium competes with Fe^{3+} for uptake by intracellular bacteria, resulting in bacterial acquisition of Ga^{3+} rather than Fe^{3+}. The antimicrobial effects of gallium are because of its incorporation into crucial Fe-dependent reproductive enzyme systems. Trivalent gallium, unlike Fe^{3+} is unable to undergo redox cycling, so the incorporation of Ga^{3+} in an Fe-dependent enzyme generally results in a loss of functionality of the enzyme, with subsequent bacterial stasis and/or death (Bernstein, 1998, 2005).

Gallium inhibits the growth of *Mycobacterium tuberculosis* and *M. avium* when the bacteria are located extracellularly or within human monocyte-derived macrophages (Oyebode *et al.*, 2000). Gallium inhibits *Pseudomonas aeruginosa* growth and biofilm formation *in vitro* and in murine lung infection models (Kaneko *et al.*, 2007). Gallium inhibits growth and kills *R. equi in vitro*; the mechanism of action involves interference with bacterial iron metabolism (Harrington *et al.*, 2006).

Gallium maltolate (GaM), a coordination complex of gallium and maltol, is absorbed rapidly following oral administration in people and laboratory animals, and is not associated with adverse effects (Bernstein *et al.*, 2000). GaM has shown promise as a novel anti-microbial agent. In a model of thermally injured mice with acute infection, GaM effectively reduced mortality and eradicated *Pseudomonas aeruginosa*, and reduced the colonization of *Staphylococcus aureus* and *Acinetobacter baumannii* in thermal wounds (DeLeon *et al.*, 2009). GaM effectively inhibits growth of *R. equi* in a dose-dependent manner in murine macrophage-like (J7741A.1) cells (Martens *et al.*, 2007a). In mice experimentally infected with *R. equi*, GaM is readily absorbed when administered orally, and prophylactic administration of GaM reduces the tissue burdens of *R. equi* (Harrington *et al.*, 2006).

The pharmacokinetic properties of GaM were recently reported for neonatal foals that received 20 mg/kg intragastrically (Martens *et al.*, 2007b). Gallium was rapidly absorbed and serum concentrations of gallium considered adequate for inhibiting growth of *R. equi* were achieved in most, but not all, foals studied. Administration of GaM to foals at breeding farms requires oral administration because frequent intragastric administration would be logistically difficult. Therefore, we developed a methylcellulose formulation of GaM (GaM-MCF) for oral administration to foals. The purpose of the study reported here was to evaluate pharmacokinetic parameters of GaM-MCF, when administered orally to neonatal foals at 20 and 40 mg/kg.

MATERIALS AND METHODS

Preparation of GaM-MCF

Gallium maltolate was prepared in a methylcellulose formulation at a concentration of 100 mg/mL, and in 500 mL batches. Deionized water, 350 mL, in a glass beaker, was warmed on a hot plate with stirring. Carboxymethylcellulose (Professional Compounding Centers of America, Inc., Houston, TX, USA) (CMC), 7.5 g, was slowly added to the warm water until all of the CMC had dissolved. GaM (Chiral Quest Inc., Monmouth Junction, NJ, USA) 50 g, simple syrup, (Humco, Texarkana, TX, USA) 100 mL and benzyol alcohol, (Professional Compounding Centers of America, Inc.) 5 mL, were added to the GaM mixture and blended in a food blender to dissipate GaM clumps and ensure uniformity. The GaM mixture was then added to the CMC solution and deionized water was added to make a total volume of 500 mL. The formulation was then mixed thoroughly and individual dosages of GaM-MCF were prepared in 35 mL syringes. Syringes (Tyco Healthcare Group LP, Mansfield, MA, USA) were sealed with a sterile rubber tip (Becton Dickson and Company, Franklin Lakes, NJ, USA) and stored in labelled containers at 40 °C.

Study foals

All experimental methods for this study were approved by the Institutional Animal Care and Use Committee at Texas A&M

University. Twelve clinically normal, neonatal Quarter Horse foals (six male and six female) were used for the study. Study foals were born via natural delivery in a box stall, and all foal births were attended. Inclusion criteria for the foals were a normal vaginal delivery, normal gestational length, normal physical examination, adequate passive transfer of immunity (immunoglobulin G concentration >800 mg/dL according to a commercially available assay (Snap Test; IDEXX Laboratories, Westbrook, ME, USA), normal findings on a complete blood count, fibrinogen concentration and serum biochemistry panel. Each foal was weighed immediately prior to administration of GaM-MCF for purposes of dose calculations. Foals were housed in a box stall with their dams during administration of GaM-MCF and collection of blood samples. *Ad libitum* access to suckling and water was allowed during the study.

Administration of GaM-MCF

The study was initiated when foals were 3–6 days (median, 3 days; mean, 3.5 days) of age. At the time of administration of GaM-MCF, foals weighed between 48 and 64 kg (median, 57.6 kg; mean, 58.6 kg). Six foals (three males and three females, randomly chosen) received a single dose of 20 mg/kg of GaM-MCF and the other six foals (three males and three females, randomly chosen) received a single dose of 40 mg/kg of GaM-MCF. The medication was administered slowly (over 15 sec) via catheter-tipped syringe into the back of the mouth and spread along the top of the tongue surface. Foals were observed daily for adverse reactions during the study.

Sample collections

Blood samples were collected via jugular venipuncture at 0 (prior to drug administration) and 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h following administration of GaM-MCF. Samples were collected into glass test tubes without anti-coagulant. Serum was harvested by centrifugation at 400 ***g*** at 4 °C for 15 min. Serum samples were divided into three aliquots and frozen at −80 °C until drug analysis.

Determination of gallium concentrations

Serum gallium concentrations were determined in duplicate by inductively coupled plasma mass spectroscopy, as previously described (Martens *et al.*, 2007b). The inductively coupled plasma mass spectroscopy detection limit for gallium in serum was 0.5 ng/mL and the method blanks averaged 0.6 ng/mL, well below the 1.5 ng/mL limit of quantification. Analytical precision and accuracy were acceptable. The relative percent difference (range divided by the mean value) of nine duplicate pairs averaged 2%, whereas recovery of gallium added to eight blanks (spiked blanks per laboratory control samples) and eight samples (matrix spikes) averaged 107% and 100% respectively. Instrumental response was linear over the calibration range (0–20 ng/mL), with a correlation coefficient (R^2) of 0.9999 and a coefficient of variation of 1.4%.

Pharmacokinetic analysis

Noncompartmental and compartmental pharmacokinetic analyses of plasma gallium concentrations were performed using commercially available software (WINNONLIN Professional Version 5.0.1; Pharsight Corporation, Mountain View, CA, USA). For each foal, 1- and 2-compartment models were fit to serum concentration versus time data, and a combination of Akaike's information criterion (Yamaoka *et al.*, 1978) and visual analysis of plots of residuals was used to select the final model.

In the noncompartmental analysis, *AUC* (area under the time–concentration curve), λ_z (elimination rate constant for the terminal portion of the time–concentration curve), T_{max} (time of observed maximum plasma concentration), C_{max} (maximum observed plasma concentration), *AUMC* (area under the moment curve) and *MRT* (mean residence time) were calculated for serum gallium concentrations. Calculations were also made for T_{lag} (time to first measurable concentration) and $T_{1/2}$ (apparent elimination half-life, $0.693/\lambda_z$). To assess whether samples were taken for an adequate period of time after C_{max}, *AUC* observed and *AUC* extrapolated were compared as *AUC* % extrapolated.

In the compartmental analysis, microconstants (K01, K12, K21, and K10) were calculated, as were the half-lives associated with those rate constants. In addition, constants associated with the model equation were calculated, A, B, alpha and beta, as were C_{max}, T_{max} and T_{lag}.

The selected compartmental model for each of the 12 foals was used to simulate serum concentrations of gallium after multiple doses of 25 and 30 mg/kg q 24 h. Then, average concentrations from the 12 simulated concentrations at each time point and standard deviations were calculated.

RESULTS

Means and standard deviations of serum gallium concentrations following oral administration of GaM-MCF are presented in Table 1. For all 12 foals, gallium concentrations were detectable by the first sampling time point after administration (0.5 h). The time course of mean serum gallium concentrations are presented in Fig. 1.

Noncompartmental pharmacokinetic parameters are presented in Table 2. Mean C_{max} was 0.9 ± 0.5 μg/mL for foals receiving the 20 mg/kg dose and 1.8 ± 0.8 μg/mL for foals receiving the 40 mg/kg dose. Mean T_{max} was 3.5 ± 1.8 h for foals receiving the 20 mg/kg dose and 3.0 ± 1.1 h for foals receiving the 40 mg/kg dose, indicating rapid GaM absorption following oral administration. Of the foals receiving the 20 mg/kg dose of GaM-MCF, three of six foals (50%) achieved serum concentrations >0.7 μg/mL, and five of the six foals (83.3%) administered a 40 mg/kg dose of GaM, achieved concentrations >0.7 μg/mL. The elimination half-life was 32.8 ± 31.8 h and 32.4 ± 10.7 h for foals receiving the 20 and 40 mg/kg doses respectively.

A two-compartment model, Model 12 in WinNonLin, appeared to be the best fit for the pharmacokinetic data.

Table 1. Means and standard deviations of serum concentrations of gallium (μg/mL) after oral administration of 20 and 40 mg/kg of gallium maltolate in a methylcellulose formulation to normal neonatal foals (six foals at each dose)

Dose	20 mg/kg		40 mg/kg	
Time	Mean	SD	Mean	SD
0	0.005	0.006	0.007	0.013
0.5	0.321	0.207	0.388	0.367
1	0.608	0.340	1.170	0.954
2	0.789	0.512	1.653	0.827
4	0.840	0.569	1.541	0.573
6	0.750	0.515	1.305	0.426
8	0.750	0.575	1.138	0.410
12	0.573	0.419	0.924	0.411
24	0.358	0.267	0.479	0.228
48	0.146	0.100	0.194	0.147
72	0.071	0.038	0.113	0.080
96	0.049	0.023	0.086	0.058

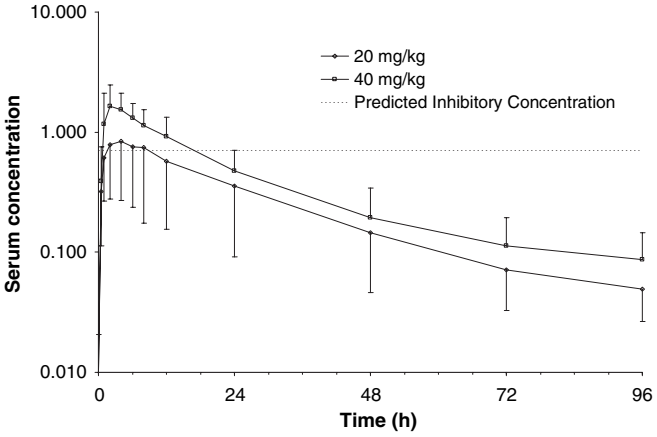

Fig. 1. Mean serum concentrations (μg/mL) of gallium after oral administration of 20 and 40 mg/kg gallium maltolate in methylcellulose gel formulation to 12 neonatal foals (six foals at each dose) (Error bars are standard deviations).

The equation for this model is, where C_t is plasma concentration at time (t) and A is the zero time intercept for the distribution phase, B is the zero time intercept for the elimination phase and C is the zero time intercept for the absorption phase:

$$C_t = A^{(-\alpha \cdot t)} + B^{(-\beta \cdot t)} + C^{(-K01 \cdot t)}$$

Compartmental pharmacokinetic parameters are presented in Table 2. In the compartmental modelling, one foal, Foal number 6, had parameters, including AUC and K_{21} that were markedly different from the other five foals that received the 20 mg/kg dose. We therefore compared the parameter means before and after removal of this foal from the database (see Table 2).

The results of simulating serum concentrations after multiple doses of 30 mg/kg q 24 h are presented in Fig. 2. The curve corresponds to average serum concentrations at each time point and one standard deviation below the average concentrations; this allows for predicting serum concentrations in a population of animals. Simulated serum concentrations after multiple 25 mg/kg doses 24 h apart did not consistently achieve the inhibitory concentration of 0.7 μg/mL (data not shown).

DISCUSSION

The report represents the first description of pharmacokinetic parameters of GaM when administered orally, in the form of GaM-MCF, to neonatal foals. It has been suggested that GaM administered orally to foals may be beneficial for treatment or prevention of *R. equi* pneumonia among foals at endemic breeding farms (Martens *et al.*, 2007b). It is essential to understand the pharmacokinetic disposition of a drug to use it effectively in a clinical setting. A previous report described the pharmacokinetic properties of GaM dissolved in water, when administered intragastrically at a dose of 20 mg/kg to neonatal foals (Martens *et al.*, 2007b). In that report, gallium was rapidly absorbed and serum concentrations considered therapeutic were achieved in five of six foals studied. Frequent administration of GaM to foals at breeding farms requires that the drug be administered orally instead of intragastrically; thus, we developed the GaM-MCF to facilitate administration of the medication orally, via syringe, to foals. It is important to note here that the compounding of drug product from bulk drugs is technically an adulterated drug in the United States, as the gallium used in this study is not an approved product.

Data from our study revealed that at an oral dose of 20 mg/kg of GaM-MCF to neonatal foals resulted in a mean gallium C_{max} of 0.90 μg/mL, which is slightly less than, but similar to the 1.1 μg/mL reported for GaM administered intragastrically at 20 mg/kg to neonatal foals (Martens *et al.*, 2007b). The slightly lower mean C_{max} achieved in our study could reflect that gallium was not completely swallowed by foals following oral administration, that bioavailability was slightly altered in association with the methylcellulose formulation, or that there is a considerable variability in absorption among foals. Our subjective evaluation was that all GaM was swallowed by treated foals and hence the explanation related to administration of inadequate dose seems unlikely.

For the purposes of our study, a serum gallium concentration of 0.7 μg/mL was considered therapeutic. In murine, macrophage-like (J774A.1) cells experimentally infected with virulent *R. equi*, a concentration of 0.7 μg/mL significantly reduced intracellular concentrations of *R. equi* (Martens *et al.*, 2007b). Furthermore, in a study of mice experimentally infected with virulent *R. equi*, it was concluded that serum gallium concentrations of 0.7 μg/mL would result in a 90% reduction in *R. equi* tissue burdens (Harrington *et al.*, 2006). Additional studies are needed to better elucidate the minimum inhibitory concentration of gallium for *R. equi* isolates; however, for purposes of interpreting the data in this study, a concentration of 0.7 μg/mL of gallium was considered therapeutic.

Table 2. Noncompartmental and compartmental pharmacokinetic parameters of gallium after oral administration of 20 and 40 mg/kg of a methycellulose formulation of gallium maltolate to 12 neonatal foals (six foals for each dose)

	20 mg/kg		Outlier removed		40 mg/kg	
Animal	Mean	SD	Mean	SD	Mean	SD
Noncompartmental analysis						
Lambda λ	0.03	0.01			0.02	0.01
$T_{1/2el}$ (h)	32.8	31.77			32.38	10.65
T_{lag} (h)	0	0			0	0
T_{max} (h)	3.5	1.76			3	1.1
C_{max} (μg/mL)	0.9	0.52			1.75	0.78
AUC_{last} (μg·mL/h)	24.06	16.28			37.07	18.28
AUC_{INF} (μg·mL/h)	26.55	15.81			40.8	20.82
AUC % extrapolated	12.15	17.75			8.83	2.06
MRT_{last} (h)	25.44	5.93			22.6	2.69
MRT_{INF} (h)	45.74	42.96			33.38	4.26
Compartmental analysis						
V/F (L/kg)	23.8	14.6	25.6	16.3	22.4	15.8
K_{01} (/h)	1.789	1.633	1.576	1.73	2.605	2.862
K_{10} (/h)	0.029	0.019	0.035	0.014	0.056	0.032
K_{12} (/h)	0.065	0.106	0.067	0.118	0.093	0.07
K_{21} (/h)	0.015	0.017	0.018	0.018	0.11	0.105
AUC (μg·mL/h)	191.3	378.63	37.01	25.64	70.52	78.53
K_{01} half-life (h)	0.69	0.45	0.78	0.44	0.58	0.55
K_{10} half-life (h)	144.21	299.32	22.04	6.14	25.16	33.58
Alpha	0.104	0.121	0.113	0.133	0.235	0.172
Beta	0.006	0.006	0.007	0.006	0.024	0.017
Alpha half-life (h)	11.67	5.72	11.56	6.39	5.12	3.75
Beta half-life (h)	3669.9	8490.94	204.05	170.18	561.55	1281.97
A	1.04	0.61	1.08	0.67	21.79	49.84
B	0.07	0.05	0.07	0.05	0.93	0.83
T_{max} (h)	3.17	1.73	3.5	1.72	2.38	0.98
C_{max} (h)	0.9	0.52	0.92	0.58	1.78	0.8
T_{lag} (h)	0.24	0.07	0.26	0.05	0.51	0.3

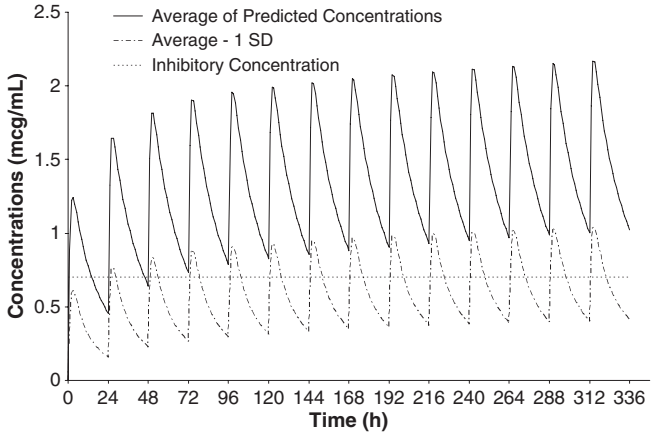

Fig. 2. Simulated serum gallium concentrations following administration of 30 mg/kg gallium maltolate every 24 h. Simulations are based on individual two-compartment models of pharmacokinetic parameters of 12 normal neonatal foals, which received a single dose of 20 mg/kg (six foals) or 40 mg/kg (six foals) of gallium maltolate orally. The average concentrations and standard deviations are based on the individual predicted concentration at each time point.

Although not evaluated in our study, another important consideration regarding the therapeutic effectiveness of gallium against *R. equi* is the concentration of gallium in infected tissues, particularly the target cell for *R. equi* (macrophage), rather than serum concentrations alone. Gallium exists in plasma primarily bound to transferrin and is preferentially taken up by phagocytic cells at sites of inflammation and granulomatous lesions and accumulates at these sites (Tsan, 1986). Gallium enters macrophages via transferrin-dependent and transferrin-independent mechanisms (Tsan, 1986; Begin *et al.*, 1986; Chitambar & Zivkovic, 1987). Concentrations of gallium are thought to be higher within macrophages and at sites of inflammation; such tissue concentrations probably better reflect antimicrobial effects against *R. equi* in affected foals. It was beyond the scope of this study to examine tissue concentrations of gallium, and thus future studies should examine the pharmacodynamic properties of gallium and determine distribution into various tissues, including lung tissue and macrophages.

Foals that received the higher dose (40 mg/kg) had a mean C_{max} of 1.7 μg/mL, which was roughly twice that achieved with the 20 mg/kg dose, suggesting that bioavailability is similar at the two dosages, and is not dose-dependent. There was marked variability in C_{max} among foals receiving both the 20 and 40 mg/kg doses in this study. Although we did not determine bioavailability, we assumed that some foals did not appear to absorb gallium as well as other foals. However, we did not determine concentrations of the gallium formulation prior to administration of the drug to the foals and hence it is possible

that the assumed and the actual concentrations were not the same. We do not expect that this is true, given that gallium is an element and not likely to deteriorate; on the other hand, mixing and measuring errors could have occurred.

Of the foals receiving 20 mg/kg GaM-MCF, only three achieved serum concentrations considered therapeutic, whereas five of the foals receiving the 40 mg/kg dose achieved therapeutic concentrations. Based upon these findings, it is unlikely that 20 mg/kg is an adequate oral dosage for control of *R. equi* infections in foals, and higher dosages should be considered in future studies. Simulations of serum concentrations (see Fig. 2) following multiple dosages of GaM-MCF at 25 and 30 mg/kg suggest that dosage regimens of at least 30 mg/kg PO q 24 h are more likely to achieve consistently therapeutic concentrations for prophylaxis or treatment of *R. equi* infections.

Our data demonstrated that the elimination half-life of gallium was similar for foals receiving the 20 and 40 mg/kg doses of GaM-MCF (32.8 and 32.4 h respectively) and was slightly longer than that previously reported for intragastrically administered GaM (26.6 h) (Martens *et al.*, 2007b). The finding that elimination half-life is similar for the two dosages provides evidence that mechanisms for elimination of gallium are not saturated at the higher concentrations achieved with the 40 mg/kg dose. Our data revealed a mean *MRT* of 45.7 and 33.4 h for foals receiving the 20 and 40 mg/kg doses respectively. A previous report (Martens *et al.*, 2007b) described similar values for *MRT* (39.5 h) for foals administered 20 mg/kg GaM intragastrically.

A 2-compartment model appeared to fit best the pharmacokinetic data from our study, based on AIC and visual analysis of the residual plots. One outlier, Foal 6, significantly affected the means of the parameters in the 2-compartment model. We can only speculate the reason for this difference, with the most likely explanation being delayed absorption in this foal, leading to an increased elimination rate. Population pharmacokinetic models might be used in further research to explain the reason behind these anomalous animals.

In healthy people, GaM is well tolerated when administered orally and has bioavailability of 25–57% (Bernstein *et al.*, 2000). Absorption and elimination kinetics appear to be approximately linear. Following a 500 mg dose to humans, a mean C_{max} of 0.6 μg/mL is achieved (Bernstein *et al.*, 2000). In healthy dogs, GaM administered orally results in 40% bioavailability (Bernstein *et al.*, 2000). In our study, we did not determine the bioavailability of GaM in neonatal foals because this calculation requires *AUC* data obtained following intravenous administration of the drug. Pharmacokinetic parameters following i.v. administration were not performed because a suitable intravenous formulation of GaM was not available. Nonetheless, bioavailability appears to be sufficient, at least at the 40 mg/kg dose, to achieve therapeutic gallium concentrations.

Data from our study show that gallium is rapidly absorbed following oral administration of GaM-MCF, and achieves serum concentrations which, on the basis of findings in mice and in murine macrophage-like cell lines, should be adequate to inhibit growth and kill *R. equi* for at least 24 h. We propose that prophylactic administration of GaM-MCF during the first few weeks of life may afford protection against early infection of foals with *R. equi*, thereby providing additional time for maturation of innate and adaptive immune functions and ultimately resulting in a lower incidence of *R. equi* pneumonia. Furthermore, GaM-MCF used alone or in conjunction with other anti-microbial agents may be valuable for the treatment of foals with pre- or sub-clinical or clinically apparent *R. equi* infections.

We used simulations of multiple doses based on the 2-compartment model to predict serum concentrations of gallium when administered at dosages other than 20 and 40 mg/kg. Based upon the finding that three of six foals administered 20 mg/kg GaM orally did not achieve serum concentrations above 0.7 μg/mL, it was apparent that higher dosages would probably be needed to achieve adequate bactericidal concentrations. Assessment of the simulated serum concentrations reveals that a dose of at least 30 mg/kg would better achieve adequate serum concentrations than would the 20 mg/kg dosage regimen.

To assess better the proper dosage of GaM for neonatal foals, additional studies are needed to determine minimum inhibitory concentrations for gallium against *R. equi*. Also, pharmacodynamic studies are needed to assess the relationship between serum concentrations and concentrations at the site of infection with *R. equi* and the time- and concentration-dependent relationship of GaM to its bactericidal capacity. Based on data reported in this study, we believe that future studies should consider a GaM-MCF dosage regimen of at least 30 mg/kg administered orally once daily for bactericidal effect against *R. equi* in neonatal foals.

ACKNOWLEDGMENTS

This study was supported by the Grayson Jockey Club Research Foundation and the Link Equine Research Endowment, Texas A&M University.

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