Antimicrobial activity of gallium against virulent Rhodococcus equi in vitro and in vivo

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Rhodococcus equi, a facultative intracellular bacterium, causes severe pneumonia in foals. Evidence suggests that most foals become infected very early in life, when they have immature or ineffective innate immune responses. This study evaluated the antimicrobial activity of gallium against R. equi, as a potential chemoprophylactic and therapeutic agent. Rhodococcus equi was grown in media with various concentrations of gallium nitrate (GN), with and without excess iron. GN significantly inhibited growth and killed R. equi, and these effects were abolished with excess iron. Antimicrobial effects of Ga appear to be related to its interference with iron metabolism. Mice were treated orally with gallium maltolate (GaM), 10 or 50 mg/kg BW, or distilled H2O prior to and after experimental infection with R. equi. Six days post-infection, organs were harvested and R. equi concentrations assessed, and serum gallium concentrations determined. GaM was absorbed in a dose-dependent manner, and R. equi tissue burdens were greater in control mice than in all GaM-treated mice. GaM may aid in the control of disease by preventing development of overwhelming R. equi tissue burdens prior to the establishment of requisite innate and adaptive immune responses.

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INTRODUCTION

Infectious diseases are a major concern to the equine industry on a worldwide basis, and respiratory disease is a primary cause of disease and death in foals (Cohen, 1994). Rhodococcus equi, a gram-positive, soil-saprophytic, facultative-intracellular pathogen, is the causative agent of the most severe form of pneumonia in foals (Prescott, 1991; Hondalus, 1997). Rhodococcus equi is also an emerging opportunistic pathogen of immunosuppressed people, particularly AIDS patients (Takai et al., 1995). The bacterium, which shares many characteristics with Mycobacterium tuberculosis, is able to replicate within macrophages, eventually destroying them and producing granulomatous lesions (Giguere & Prescott, 1997).

Several characteristics of the R. equi–host interaction indicate its uniqueness among equine infectious diseases, and have marked effects on the prevention and control of the disease process. Rhodococcus equi foal pneumonia is endemic on some farms, but young foals residing on those farms are only sporadically affected even though exposure to the organism is widespread (Cohen et al., 2000). Passive transfer of maternal immunity to R. equi does not appear to be protective (Martens et al., 1991). Young foals are commonly affected by R. equi, whereas juvenile and adult horses rarely develop R. equi-induced disease (Prescott, 1991). Most foals with spontaneous disease appear to become infected very early in life (Horowitz et al., 2001), even though clinical signs of disease may not become apparent for several weeks or months (Prescott, 1991). A subpopulation of neonatal foals appears to have transiently ineffective or inefficient immune responses, which may render them more susceptible to infection with R. equi (Martens et al., 1988; Chaflin et al., 2004), and may help explain the age-related susceptibility to infection and sporadic distribution of clinical disease.

On the basis that some foals may not possess the requisite immunologic armamentarium early in life to control R. equi, and that there may not be sufficient time for foals to mount an adequate adaptive immune response before becoming infected, it may be most appropriate to develop prophylactic strategies (e.g. chemoprophylaxis or passive immunization) that provide immediate protection during the perinatal period. It is anticipated that such strategies may prevent infectious organisms from growing
to overwhelming numbers before innate immune responses sufficiently mature or specific adaptive immunity can be established.

Ferric iron (Fe$^{3+}$) is crucial for the survival and replication of most pathogens, thereby providing a potential target for prophylactic and therapeutic strategies. A variety of antimicrobial strategies that act by interfering with microbial acquisition and utilization of iron have been developed (Byrd & Horwitz, 1991; Weinberg, 1994; Bernstein, 1998). Gallium (Ga), a trivalent semi-metal that is chemically similar to Fe$^{3+}$, is taken up by some bacteria (Bernstein, 1998). The ability of gallium to inhibit growth of intracellular bacteria by interfering with bacterial iron metabolism was established in vitro by incorporating gallium nitrate (Ga(NO$_3$)$_3$; GN) in culture media with Mycobacterium spp. (Olakanmi et al., 2000). Those investigators further demonstrated that the antimicrobial effect was due to the Ga moiety of the GN, not the nitrate. A new formulation of gallium, gallium maltolate (Ga(C$_6$H$_5$O$_3$); GaM), which attains high bioavailability when administered orally to a variety of animal species, was recently developed by one of the authors (L.B.) (Bernstein et al., 2000). The purpose of this study was to investigate the potential use of gallium as a chemoprophylactic and chemotherapeutic agent for the control of R. equi infections.

MATERIALS AND METHODS

Culture study

**Bacteria and growth conditions**

Virulent, vapA-positive R. equi (ATCC 33701) were grown in brain heart infusion broth (Beckton-Dickinson, Cockeysville, MD, USA) (BHIB) with 10% newborn calf serum (Sigma Chemical Co, St Louis, MO, USA) for 48 h at 35 °C with rotation (Laboratory rotator, model 099A; Glas-Col, Terre Haute, IN, USA) (10 r.p.m.). Bacterial cells were pelleted by centrifugation at 1600 g for 10 min and washed three times with sterile phosphate-buffered saline (PBS; Gibco BRL, Frederick, MD, USA). The concentration of bacteria was determined spectrophotometrically at OD$_{600}$, adjusted to a concentration of approximately $1 \times 10^8$ CFU/mL with PBS, and stored in aliquots at −80 °C.

**Addition of gallium nitrate**

Citrates-buffered GN, in a 0.1 M sterile solution, was kindly provided by Gentia Inc. (Ganite; Gentia Inc., Berkeley Heights, NJ, USA). The concentrations of R. equi grown in MM-Fe containing GN at 50, 100, 150, and 200 µM (3.5, 7, 10.5, and 14 µg/mL) were determined at 0, 8, 24, and 48 h and compared with the concentrations of R. equi grown in MM.

**Addition of iron**

Excess iron, as FeCl$_3$·6H$_2$O, was added to MM-Fe containing GN to ascertain its effect on GN-induced growth suppression of R. equi. Concentrations of R. equi grown in: (i) MM; (ii) MM-Fe + 150 µM GN; and (iii) MM-Fe + 150 µM GN + 25 µM FeCl$_3$ were compared at 0, 8, 24, and 48 h incubation.

**Mouse study**

**Bacteria and growth conditions**

Virulent R. equi (ATCC 33701) were grown at 37 °C, with rotation (10 rpm), in BHIB, with 10% newborn calf serum. After 24 h incubation, 1.25 mL of the culture (containing approximately $1 \times 10^8$ R. equi/mL) were transferred to 25 mL fresh broth and incubated for 48 h under the same conditions. The bacteria were centrifuged at 1600 g for 10 min. The concentration of bacteria was determined spectrophotometrically at OD$_{600}$, adjusted to a concentration of approximately $1 \times 10^7$ CFU/mL with PBS, and stored in aliquots at −80 °C.

**Mice**

Eighteen, 5- to 6-week-old, female, BALB/c mice, weighing approximately 20 g, were obtained from a commercial source (Harlan Sprague Dawley, Inc., Indianapolis, IN, USA). GaM was kindly provided by Titan Pharmaceuticals, Inc. (South San Francisco, CA, USA). The mice were obtained in groups of six, for each of three independent studies. In each study, the six mice were separated into three treatment groups as follows: (i) distilled water (control); (ii) low-dose GaM (10 mg/kg); and (iii) high-dose GaM (50 mg/kg). Prior to initiation of the study, mice were acclimated for 10 days and fed a basal rodent diet containing 60 p.p.m. iron (Basal Test Diet #5755; Purina Mills, St Louis, MO, USA). Mice were administered 150 µL of distilled water (controls), or distilled water containing either 1.33 mg/mL GaM or 6.65 mg/mL GaM via oral gavage (Animal feeding needles, VWR) daily for 10 days. On the fourth treatment day, frozen aliquots of R. equi were thawed at 37 °C in a water bath and gently mixed. The mice were lightly anesthetized with methoxyfluorane (Pitman-Moore, Mundelein, IL, USA) in a closed chamber. and 200 µL of the bacterial suspension, containing approximately $2 \times 10^8$ CFU of R. equi, was injected into the peritoneal space with a 27 G × 5/8” needle (Monoject, VWR). The concentration of viable R. equi at the time of infection was determined by 10-fold serial dilutions cultured in duplicate on trypticase soy agar with 5% sheep red blood cells (Beckton-
determined by duplicate cultures of 10-fold serial dilutions on trypti
case soy agar with 5% sheep red blood cells. On the tenth day of treatmen
t (6 days post-infection), mice were gavaged with their respective doses of
distilled water or GaM 2 h prior to termination of the study, anesthetized
t with methoxyfluorane and killed by cardiac snip.

Sample collection and analyses
Spleens, lungs, and livers were aseptically harvested and weighed. Each
organ was homogenized with a tissue grinder (Wheaton, Millville, NJ, USA) in 2 mL of sterile PBS and the
volume of homogenate was recorded. The R. equi concentration
(CFU/mL) of tissue homogenates was determined by duplicate
quantitative culture of 10-fold serial dilutions. The numbers of
bacteria per gram of tissue were determined by dividing the
product of bacterial concentration (CFU/mL) and homogenate
volume by the weight of the organ.

Following cardiac snip, whole blood was aspirated from the
thoracic cavity and serum was harvested and stored at −80 °C
for determination of gallium concentration. Samples were
thawed at 37 °C and diluted with 1% ultra-pure HNO₃
(Seastar Baseline; Seastar Chemicals Inc., Sidney, BC, Canada)
deionized water in preparation for gallium analysis by
inductively coupled plasma-mass spectroscopy (Model DRC 2;
Perkin Elmer, Foster City, CA, USA) (ICP/MS) using the
⁷¹Ga isotope and ¹⁰³Rh as an internal standard. Weighted linear
calibration was performed with a blank and four external
standards (0.2, 2.0, 20, and 200 µg/L). Data were acquired in
peak hopping mode, using the autolens feature and three
replicate reads per determination. Calibration and baseline
determinations were performed before and after the analytical
run.

Statistical analysis
Data for bacterial numbers were described by use of mean ± SD
of experiments conducted in triplicate. Data were examined both
as measured and because of skewness in the distribution of some
of the data and their variances, following natural logarithmic
transformation. For the in vitro culture experiments, the effects of

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>Mean cell density (CFU/mL)* after growth times (h) of</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>MM</td>
<td>3.62 × 10⁵</td>
</tr>
<tr>
<td>MM-Fe + 50 µM GN</td>
<td>3.62 × 10⁵</td>
</tr>
<tr>
<td>MM-Fe + 100 µM GN</td>
<td>3.62 × 10⁵</td>
</tr>
<tr>
<td>MM-Fe + 150 µM GN</td>
<td>3.62 × 10⁵</td>
</tr>
<tr>
<td>MM-Fe + 200 µM GN</td>
<td>3.62 × 10⁵</td>
</tr>
</tbody>
</table>

MM, minimal media (2.9 µM Fe); MM-Fe, minimal media without added iron (0.5 µM Fe); GN, gallium nitrate; ATCC, American Type Culture Collection.
*Cell densities determined by enumerating CFU in serial dilutions plated on trypticase soya agar with sheep RBC.
Averages of triplicate-independent experiments.

RESULTS

Gallium nitrate inhibits growth of R. equi in culture

The concentrations of R. equi at 0, 8, 24, and 48 h were
compared when cultivated in MM-Fe supplemented with
various concentrations of GN and in MM without GN
(Table 1). Significant effects of GN were not observed at 8 h.
At 24 and 48 h incubation, there were significant ($P < 0.05$)
decreases in concentrations of R. equi cultivated in media
containing 50, 100, 150, or 200 µM GN, compared with those
grown in MM. At 48 h, 50 µM GN produced a nearly 2-log
order decrease in R. equi concentration relative to control
levels, and 100, 150, or 200 µM GN produced a further
significant decrease (Table 1).

Excess iron inhibits GN-induced growth suppression

To determine whether the inhibitory effect of GN on growth of
R. equi was caused by interference with, or disruption of, iron
metabolism, iron-override experiments were conducted. Concentra-
tions of R. equi grown in MM and in MM-Fe + 150 µM
GN + 25 µM FeCl₃ were significantly ($P ≤ 0.001$) greater than
concentrations of R. equi grown in MM-Fe + 150 µM GN at 24 h
and 48 h (Table 2).

Status of mice

One mouse in the low-dose treatment group died on the fourth
day of the study, shortly after receiving its fourth dose of GaM
and being infected with R. equi. Gross lesions were not apparent
in this mouse and postmortem autolysis precluded accurate

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microscopic evaluation. None of the other mice exhibited clinical signs of disease or gross lesions.

**Gallium maltolate absorption in mice**

Serum samples were collected 2 h after the final treatment from all 17 mice that completed the study. However, a laboratory accident resulted in the loss of two serum samples from each experimental group. Consequently, data on serum gallium concentrations are only available for four control, three low-dose GaM, and four high-dose GaM-treated mice. GaM was absorbed in a dose-dependent manner (Fig. 1). After logarithmically transforming the data, the mean concentrations of gallium in sera analyzed by ICP-MS were significantly ($P \leq 0.05$) greater in low-dose GaM-treated mice (mean, 110.5 μg/mL; SE, 0.0316) than in control mice (mean, 0.870 μg/mL; SE, 0.000178), and were significantly ($P \leq 0.05$) greater in high-dose GaM-treated mice (mean, 559.3 μg/mL; SE, 0.136) than in either low-dose or control mice.

**Gallium maltolate suppresses growth of R. equi in mice**

Median tissue concentrations of *R. equi* were greater in control mice than in GaM-treated mice, and similar between mice treated with low-dose GaM (10 mg/kg BW) and high-dose GaM (50 mg/kg BW) (Table 3). Although not statistically significant, there was a clear trend toward an inverse relationship between serum gallium concentrations and *R. equi* tissue concentrations (Fig. 2).

**DISCUSSION**

*Rhodococcus equi*, an intracellular pathogen of macrophages, causes one of the most severe and devastating forms of foal pneumonia. Epidemiologic evidence suggests that most foals with spontaneous *R. equi* pneumonia become infected at a very early age, often in the first few months of life. This suggests that *R. equi* may be acquired horizontally through close social contact or contact with contaminated equipment or feed. The high prevalence of *R. equi* in wild animals, such as horses and donkeys, suggests that these animals may serve as reservoirs of infection. In addition, *R. equi* is commonly isolated from the environment, including soil, water, and manure. The role of the environment in the transmission of *R. equi* is not fully understood, but it is likely that *R. equi* can survive for extended periods in the environment, particularly in moist, humid conditions. This suggests that efforts to control *R. equi* should focus on reducing transmission to neonates, as well as on reducing the environmental reservoir of infection.

**Table 2. Reversal of gallium nitrate-induced growth suppression and death of virulent Rhodococcus equi (ATCC 33701) with excess iron**

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>Mean cell density (CFU/mL) after growth times (h) of 0 8 24 48</th>
</tr>
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<tbody>
<tr>
<td>MM</td>
<td>3.62 x 10^5 3.62 x 10^5 3.55 x 10^5 5.21 x 10^5</td>
</tr>
<tr>
<td>MM-Fe + 150 μM GN</td>
<td>3.62 x 10^5 1.58 x 10^6 1.70 x 10^6 6.89 x 10^5</td>
</tr>
<tr>
<td>MM-Fe + 150 μM GN + 25 μM FeCl₃</td>
<td>3.62 x 10^5 1.05 x 10^7 3.90 x 10^8 5.84 x 10^8</td>
</tr>
</tbody>
</table>

See Table 1 for key.

**Table 3. Median (range) of Rhodococcus equi tissue concentrations in mice experimentally infected with virulent R. equi (ATCC 33701) and treated with water or gallium maltolate (GaM) (CFU/g tissue)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver</th>
<th>Lung</th>
<th>Spleen</th>
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<tbody>
<tr>
<td>Control (water)</td>
<td>3575 (396–609 000)</td>
<td>2233 (0–76 100)</td>
<td>32 950 (9500–2 040 000)</td>
</tr>
<tr>
<td>GaM (10 mg/kg)</td>
<td>1989 (268–690)</td>
<td>303 (0–2940)</td>
<td>14 500 (1470–140 000)</td>
</tr>
<tr>
<td>GaM (50 mg/kg)</td>
<td>2830 (430–6290)</td>
<td>906 (0–2410)</td>
<td>28 650 (1110–82 600)</td>
</tr>
</tbody>
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young age (i.e. within the first few days of life) (Horowitz et al., 2001), before they have time to develop an effective adaptive immune response. During primary infection with many intracellular bacteria the host must rely on innate immune defenses to prevent organisms from growing to overwhelming numbers before specific adaptive immunity can be generated and expressed (Pedrosa et al., 2000; Bennouna et al., 2003). Thus, innate immunity is likely important for controlling early \textit{R. equi} infections. Evidence suggests that immaturity of innate immune responses and individual variability in those responses may contribute to increased susceptibility to infection by \textit{R. equi} (Boyd et al., 2003; Kohler et al., 2003; Chaffin et al., 2004). On these bases, strategies designed to work in conjunction with innate immune responses may prevent or control early infections, thereby providing the host additional time for immune system maturation and for development of an adequate adaptive immune response.

Despite an abundance of iron in host tissues and fluids, the amount of free iron available for use by invading organisms is minute (Ratledge & Dover, 2000). Iron withholding is an essential antimicrobial component of the innate immune complex. Iron-binding proteins (e.g. transferrin, lactoferrin) bind virtually all free iron, thereby rendering it unavailable to most microorganisms. Some pathogens, however, can acquire iron from these iron-binding proteins or via siderophores (Weinberg, 1995, 1998; Boelaert, 1996; Ratledge & Dover, 2000). Recent evidence has demonstrated the importance of iron for growth and survival of \textit{R. equi}, and the ability of \textit{R. equi} to acquire iron from transferrin and lactoferrin (Jordon et al., 2003). In foals, virulence of \textit{R. equi} is strongly associated with the presence of an 80–90 kb plasmid that encodes and mediates the expression of vapA, a 15–17 kDa virulence-associated protein (Takai et al., 1993; Martens et al., 2000). Although the exact function of vapA is unknown, there is compelling evidence of its potential role in iron acquisition and utilization by \textit{R. equi} (Jordon et al., 2003; Rahman et al., 2003; Ren & Prescott, 2003).

On the basis that gallium interferes with iron-dependent metabolic pathways in \textit{Mycobacterium} spp. and suppresses growth (Olakanmi et al., 2000), we hypothesized that gallium would suppress the growth of \textit{R. equi} in a similar manner. In this study we: (i) demonstrated the ability of GN to suppress growth of \textit{R. equi in vitro}; (ii) determined that these growth-suppressing effects were abrogated by the addition of excess iron, and thus were most likely due to interference with iron acquisition and utilization; (iii) documented that mice readily absorb orally administered GaM in a dose-dependent manner; and (iv) showed that mice that received oral GaM before, and for a short time after, experimental infection with \textit{R. equi} had lower concentrations of bacteria in vital organs than untreated control mice.

A variety of preventative and therapeutic strategies for bacterial infections have been developed that act by interfering with microbial iron acquisition and utilization (Byrd & Horwitz, 1991; Weinberg, 1994; Bernstein, 1998). Gallium is a semi-metal that accumulates in rapidly dividing tumor cells, areas of inflammation, macrophages, neutrophils, and some bacteria (Tsan, 1986; Bernstein, 1998). The antimicrobial effects of gallium are related to its ability to compete with iron for the binding sites of host iron-binding proteins (e.g. transferrin and lactoferrin) and siderophores, which are sources of iron for many intracellular pathogens (Emery, 1986; Tsan, 1986; Bernstein, 1998). Bacteria acquire gallium, instead of iron, from the host iron-binding proteins and incorporate it in metabolic pathways and enzymes that require Fe$^{3+}$. Many of these enzymes, such as ribonucleotide reductase, are crucial for cell cycle regulation and DNA synthesis. Because gallium, unlike iron, is unable to undergo redox cycling (i.e. reduction from trivalent to divalent state), these enzymes are inactivated and the net result is inhibition of DNA synthesis and bacterial replication, and ultimately cell death ( Bernstein, 1998).

Gallium utilizes both transferrin-dependent and -independent pathways to enter mammalian cells. When plasma concentrations of gallium exceed approximately 50 \(\mu\)M, transferrin is saturated, and the majority of the unbound gallium exists as gallate, Ga(OH)$_3$, which is rapidly excreted in the urine and can be nephrotoxic ( Bernstein, 1998). This most commonly occurs when gallium enters the bloodstream (via intravenous administration) at a rate that exceeds the binding capacity of transferrin. The risk of adverse effects can be minimized by oral or subcutaneous administration of gallium, as it will enter the bloodstream more slowly and follow the endogenous pathway of iron uptake.

Observations from a previous study suggested that the iron requirement of \textit{R. equi} is very low (Jordan et al., 2003). In the present study, \textit{R. equi} MM was used as the control media, and MM-Fe as the principal media in all GN studies, on the following bases: (i) growth characteristics of \textit{R. equi} in MM are well characterized (Boland & Meijer, 2000; Jordan et al., 2003); (ii) MM contains more iron than needed for optimal \textit{R. equi} growth (Jordon et al., 2003); (iii) iron concentration in MM-Fe more nearly reflects that available to bacteria \textit{in vivo} (Jordan et al., 2003); (iv) \textit{R. equi} growth rates are similar in MM and MM-Fe (Carnes, 2002); and (v) excessive iron interferes with antimicrobial effects of gallium (Olakanmi et al., 2000). The addition of various concentrations of GN to culture media inhibited the growth of \textit{R. equi} during the first 24 h of incubation. Higher concentrations of GN resulted in bacterial death by 48 h incubation, as shown by decreased CFU counts. GN thus appears to initially induce bacterial stasis, by inhibiting DNA synthesis and cell replication, which then results in cell death. The same pattern has been observed in mammalian cell cultures, where cells unable to replicate due to gallium exposure soon undergo apoptosis ( Bernstein, 1998). Olakanmi et al. (2000) similarly demonstrated a delayed bactericidal effect of GN on intracellular \textit{M. tuberculosis}.

The antimicrobial effects of gallium were completely abolished when media were supplemented with excess iron, providing evidence that GN interferes with iron-dependent mechanisms that are crucial to the growth and survival of \textit{R. equi}. Our findings were similar to those obtained in a previous study in which the \textit{in vitro} antimicrobial activity of GN for \textit{M. tuberculosis} and \textit{M. avium} was due to Ga interference with bacterial iron metabolism (Olakanmi et al., 2000). Our \textit{in vitro} studies
indicated that GN causes significant inhibition of growth and results in death of R. equi, but because of the concerns associated with toxicity, as well as the need for continuous monitoring and infusions (Bernstein, 1998), the parenteral administration of GN to horses would not be practical. Rather, oral administration would be far superior in terms of safety, cost, and ease of administration.

On the basis of the R. equi antimicrobial effects demonstrated in the in vitro aspect of this study, we investigated the ability of orally administered GaM to control R. equi in experimentally infected mice. GaM has high oral bioavailability and safety in humans and a variety of animal species (Bernstein et al., 2000), and is not associated with the gastrointestinal irritation caused by oral GN and gallium chloride (Fettman et al., 1987), or the renal toxicity caused by intravenous GN (Bernstein et al., 2000). GaM is available as a moderately water-soluble powder that is stable at room temperature, has a pleasant odor and little flavor, and is relatively inexpensive to produce.

Although pneumonia is the primary disease caused by R. equi in horses and people (Prescott, 1991), a laboratory animal model for pneumonia is lacking. The virulence status of R. equi isolates has been established by infecting mice either i.v. or i.p. and quantifying the concentrations of bacteria in their spleens, lungs, and livers (Takai et al., 1992; Giguere et al., 1999). The i.v. route of infection is more commonly reported; however, we elected to use the i.p. route in this study because of the relative ease and accuracy of i.p. inoculation and the report of induction of less severe disease (Takai et al., 1992). The finding in this study of lower concentrations of R. equi in the lungs than in spleens and livers is consistent with previous experimental systemic infections of mice with R. equi. As the lung is not the primary target organ in mice systemically infected with R. equi, information obtained from such mice may not be directly applicable to horses or people having lung infections, but our results do demonstrate the likely efficacy of oral GaM against R. equi infections in a mammalian host.

The mice in the low-dose GaM treatment group that died appeared bright and alert, but progressively lost body weight following the initial GaM treatments. On the basis that no other mice in the study showed evidence of illness, the death of this mouse was most likely precipitated by the gavage administration of GaM. Gavage is the traditional method of delivering small volumes of substances to the stomach of mice. The procedure, however, is not risk-free. It can cause mechanical damage to the oropharyngeal and esophageal tissues, thereby interfering with food consumption, and administered substances can overflow and transport contaminants into the lung causing irritation or infection (Craig & Elliott, 1999). Alternatively, i.p. inoculation of the R. equi could have resulted in trauma to abdominal organs. Mice were lightly anesthetized, positioned in dorsal recumbency and the abdominal wall elevated during inoculations in an effort to circumvent such complications. There was no evidence of major organ trauma at the time of necropsy.

Gallium, in the form of GaM, was absorbed in a dose-dependent manner following oral administration. Although the loss of two serum samples from each experimental group markedly reduced the statistical power of the resulting gallium concentration data, there was still a significant difference between all three treatment groups. These data on oral bioavailability are in agreement with previous observations in a variety of animal species (Bernstein et al., 2000). Serum concentrations of gallium are useful for assessing drug absorption and relative availability, but do not take into account gallium that has left the serum and entered other tissues, where gallium is known to concentrate (Bernstein, 1998). The capacity of gallium to accumulate in areas of inflammation and infection, macrophages, neutrophils, and some bacteria provides an even greater source with which to combat infectious agents (Tsan, 1986; Bernstein, 1998).

Data obtained from the mice indicate that the administration of GaM before, and for a short period after, experimental infection resulted in lower concentrations of R. equi in vital organs. The median concentrations of R. equi in the spleens, lungs, and livers of untreated control mice were approximately 12-, 16- and 42-fold greater, respectively, than those of mice treated with GaM. Although the bacterial burden reductions in GaM-treated mice are not statistically significant, they may very well be clinically relevant. The small number of animals per experimental group severely limited the study’s statistical power, and with more mice it is likely that the differences would have been significant. These findings are similar to those demonstrating the effects of GaM on the concentrations of M. tuberculosis in tissues of experimentally infected guinea pigs (L.S. Schlesinger, personal communication).

The studies reported here demonstrate that the processes by which R. equi acquire and utilize iron are disrupted by gallium, thereby suppressing growth and causing bacterial death. Gallium may serve as a tool for investigating the mechanisms of iron acquisition by R. equi and the role of such mechanisms in the pathogenesis of R. equi pneumonia. Furthermore, our results suggest that gallium may have prophylactic and therapeutic attributes that would be useful in the control of disease caused by R. equi. Additional studies are needed to evaluate the safety and bioavailability of oral GaM in neonatal foals, and to assess its therapeutic effectiveness against R. equi and other pathogens.

ACKNOWLEDGMENTS

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