Gallium Therapy: A Novel Metal-Based Antimicrobial Strategy for Potential Control of *Rhodococcus equi* Foal Pneumonia

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The semi-metallic element gallium interferes with *Rhodococcus equi* use of iron, which is crucial for its survival. It thereby suppresses *R. equi* growth and is bactericidal. Gallium is a potentially valuable antimicrobial agent for use in the prevention and treatment of *R. equi* foal pneumonia. Authors' addresses: College of Veterinary Medicine and Biomedical Science, Texas A&M University, College Station, TX 77843 (Martens, Harrington, Cohen, Chaffin, Taylor); College of Veterinary Medicine, Washington State University, Pullman, WA 99164 (Mealey); and Terrametrix, 285 Willow Road, Menlo Park, CA 94025 (Bernstein); e-mail: rmartens@cvm.tamu.edu.

1. Introduction

Rhodococcus equi causes one of the most severe and devastating forms of pneumonia in foals. Evidence suggests that most foals become infected at a very early age (i.e., within the first few days of life)[1] when they have immature or ineffective innate immune responses. Thus, strategies designed to prevent or ameliorate these early infections may provide effective disease control.

Recent studies have documented the necessity of iron acquisition for the survival of *R. equi* and determined that *R. equi* can acquire and use iron bound to transferrin and lactoferrin (primary endogenous iron transport proteins that protect against microbial infection by binding free iron needed by most bacteria).[1] Gallium (Ga), a trivalent semimetal that shares many similarities with ferric iron and functions as an iron mimic, has been used to control various microorganisms by exploiting their iron dependency.[2,3] Gallium, particularly when administered orally, readily binds to plasma transferrin and lactoferrin,[2,4] which subsequently concentrate in macrophages, the target cell of *R. equi*. On the basis that gallium is an iron mimic, it is incorporated into crucial iron-dependent enzyme systems of certain bacteria, causing inactivation of those enzymes and bacterial death.[2]

This study was designed to determine the following: (1) whether gallium inhibits growth and kills *R. equi* in culture, and if so, the mechanistic basis of that activity; (2) whether oral treatment of mice with gallium maltolate (GaM, a compound with high oral bioavailability in mice and various other mammalian species[4]) would provide protection against

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experimental infection with virulent *R. equi*; (3) bioavailability of enteral GaM in neonatal foals; and (4) safety of enteral GaM in neonatal foals.

2. Materials and Methods

Culture Study

Virulent *R. equi* (ATCC 33701+) was propagated in *R. equi* minimal essential medium,[1] with the following treatments: no additives (control); gallium nitrate at various concentrations; and gallium nitrate + excess iron. Bacterial concentrations were assessed by serial dilution plate counts at 0, 8, 24, and 48 h of incubation. Studies were conducted in triplicate, and data for bacterial numbers were described as mean ± SD. The effects of covariates of time and media on bacterial concentrations were analyzed using analysis of variance. $p \leq 0.05$ was considered statistically significant.

Mouse Study

Three groups of six mice were treated by oral gavage, as follows: distilled water (control); low-dose GaM (10 mg/kg body weight); and, high-dose GaM (50 mg/kg body weight). Mice were treated once daily for 10 days. On treatment day 4, all mice were experimentally infected with virulent *R. equi* (ATCC 33701+) by intraperitoneal injection. On treatment day 10, all mice were killed. Lungs, livers, and spleens were aseptically harvested and homogenized, and tissue concentrations of *R. equi* were assessed by serial dilution plate counts. Data for bacterial numbers and GaM concentrations were examined as measured and after natural logarithmic transformation because of skewness in the distribution of some of the data, and their variances described as mean ± SD. $p \leq 0.05$ was considered statistically significant.

Bioavailability Study

Pharmacokinetic analysis of GaM was assessed in neonatal foals after intragastric administration to determine bioavailability and define an appropriate dosage regimen. Six Quarter Horse foals received GaM-distilled water solution (20 mg/kg body weight) by naso-gastric tube at 1 day of age. Serum samples were collected before dosing and sequentially through 48 h after administration, and assayed for gallium using inductively coupled plasma/mass spectroscopy (ICP/MS). Log serum Ga concentrations versus time data were subjected to computer-assisted linear regression to determine pharmacokinetic parameters including maximum serum concentration (C_{max}), time to reach maximum concentration (T_{max}), area under the concentration versus time curve (AUC), and mean residence time (MRT), and bioavailability was calculated. Descriptive statistics were determined using commercially available software, and all data are reported as mean ± SD.

Safety Study

Safety and serum kinetics of GaM were assessed in six neonatal Quarter Horse foals administered GaM (20 mg/kg, q 24 h) by naso-gastric tube at 1–5 days of age. Three age-matched foals (controls) were administered intra-gastric water, q 24 h, at 1–5 days of age. All foals were monitored (i.e., physical exam, body weight, complete blood count, serum biochemical profile, serum iron-binding capacity, and total serum iron) before the first treatment and daily for 7 days after treatment.

Serum samples were collected before and 2 h after GaM administration on each of 5 consecutive days, and at 12-h intervals after the final dose through day 7. Serum Ga concentrations were analyzed using ICP/MS. Differences between treatment and control groups were analyzed using either t test or Wilcoxin rank-sum test. Comparisons between groups and among times were conducted using generalized linear modeling, with time and treatment as main effects. $p \leq 0.05$ was considered statistically significant.

3. Results

Culture Study

Compared with control cultures, there were significantly lower concentrations of *R. equi* at 24 and 48 h when grown in media containing gallium nitrate, and the response was highly dose dependent.[5] The growth suppression and bactericidal effects of gallium nitrate were abolished when excess iron was added to the media.[5]

Mouse Study

GaM was absorbed in a dose-dependent manner, as evidenced by significantly greater serum Ga concentrations in low-dose GaM-treated mice than in controls and significantly greater concentrations in serum of high-dose GaM-treated mice than in either low-dose or control mice.[5] Concentrations of *R. equi* in lung, liver, and spleen tissues of mice treated with both high and low doses of GaM were similar. Median concentrations of *R. equi* in the spleens, lungs, and livers of untreated control mice were ~12-, 16-, and 42-fold greater, respectively, than those of mice treated with GaM.[5]

Bioavailability Study

Data indicated that a single enteral dose of GaM (20 mg/kg body weight) results in serum concentrations of Ga considered sufficient to suppress growth of *R. equi* (>700 ng/ml).

Safety Study

On the basis of the bioavailability data, safety and serum kinetics were assessed in neonatal foals after five daily intra-gastric doses of GaM. There were no significant differences between GaM-treated foals and control foals for any of the physical, hematologic, or serum biochemical parameters assessed.

An intra-gastric dose of GaM (20 mg/kg, q 24 h) achieved peak and prolonged serum Ga concentrations that should be sufficient to suppress growth and cause the death of *R. equi*.

4. Discussion

The antimicrobial activity of gallium against virulent *R. equi* is associated with its ability to interfere with the acquisition and use of iron by the bacterium. Although the bacterial burden reductions in GaM-treated mice were not statistically significant, they may very well be clinically relevant given the observed magnitude of effect and the low statistical power of the study. There were no apparent toxic effects after five consecutive daily administrations of enteral GaM in newborn foals. However, larger numbers of foals treated over longer periods of time will be needed to more fully characterize safety issues.

Gallium seems to have great potential for the prevention and control of disease in foals caused by *R. equi*. Short-term oral GaM therapy in newborn foals could provide protection against early infection with *R. equi.* This would provide additional time for maturation of requisite innate and adaptive immune functions and could substantially reduce the incidence of disease on *R. equi* endemic farms. In addition, gallium used alone or in conjunction with standard antibiotic protocols may be valuable for the treatment of established *R. equi* infections.

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